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Functional interactions between p53 and the TFIIH complex are affected by tumour-associated mutations

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The p53 tumour suppressor is mutated in the majority of human tumours. p53's proposed role as the guardian of the genome is reflected in its multiple effects on transcription, genome stability, cell growth and survival. We show that p53 interacts both physically and functionally with the TFIIH complex. There are multiple protein–protein contacts, involving two regions of p53 and three subunits of TFIIH, ERCC2 (XPD), ERCC3 (XPB) and p62. p53 and its C-terminus (amino acids 320–393) inhibit both of the TFIIH helicases and *in vitro* transcription in the absence of TFIIH. Transcription inhibition is overcome by TFIIH. The N-terminal region of p53 (1–320), lacking the C-terminus, is inactive on its own, yet apparently affects the activity of the C-terminus in the native protein. Interestingly, mutant p53s that are frequently found in tumours are less efficient inhibitors of the helicases and transcription. We hypothesize that the interactions provide an immediate and direct link for p53 to the multiple functions of TFIIH in transcription, DNA repair and possibly the cell cycle.

Keywords: cell cycle control/DNA repair/transcription/tumour suppressor/xeroderma pigmentosum

Introduction

Cancer is believed to result from mutation in proto-oncogenes and tumour suppressor genes, a process that is favoured by defects in DNA repair (Bodmer *et al.*, 1994; Shibata *et al.*, 1994). p53 appears to be particularly important in maintaining DNA integrity (Lane, 1992, 1993). Mutations in the p53 tumour suppressor gene are the most common genetic alteration in human tumours (Hollstein *et al.*, 1991; Caron de Fromentel and Soussi, 1992; Harris, 1993). p53 mutations lead to genome instability, tumour susceptibility and transformation. p53 is not essential for cellular functions since p53 null mice are viable, but they develop tumours spontaneously early in life (Donehower *et al.*, 1992). p53 null cells display both genetic instability (Livinstone *et al.*, 1992; Yin *et al.*, 1992) and increased resistance to cell death following exposure to γ-radiation (Clarke *et al.*, 1993; Lowe *et al.*, 1993).

p53 is a transcription factor that regulates cell growth and apoptosis (Baker *et al.*, 1990; Younish-Rouach *et al.*, 1991; Shaw *et al.*, 1992; Yew and Berk, 1992). Transcriptional regulation appears to be essential for its functions. p53 downstream effectors include the cell cycle inhibitor $p21^{WAF1}$ (El-Deiry *et al.*, 1993) and the apoptosis-promoting factor bax (Miyashita and Reed, 1995). Tumour-associated mutations generally alter amino acids that are essential for DNA binding (Cho *et al.*, 1994), thereby affecting its transcriptional properties. However, there are effects of p53 that do not require transcription of effector genes (Wang *et al.*, 1989; Momand *et al.*, 1992; Caelles *et al.*, 1994; Cox *et al.*, 1995; Dubs-Poterzman *et al.*, 1995; Haupt *et al.*, 1995). The transactivation domain of p53 resides in the N-terminus (Fields and Jang, 1990), and the specific DNA binding 'core' domain is in the centre (Bargonetti *et al.*, 1993; Pavlevitch *et al.*, 1993; Wang *et al.*, 1993). The C-terminal region is involved in many functions. It mediates tetramerization (Stürzbecher *et al.*, 1992; Clore *et al.*, 1995; Jeffrey *et al.*, 1995), regulates specific DNA binding of the full-length protein (Hupp *et al.*, 1992, 1995; Jayaraman and Prives, 1995), binds to damaged DNA (Lee *et al.*, 1995; Reed *et al.*, 1995), re-anneals DNA and RNA (Bakalkin *et al.*, 1994; Wu *et al.*, 1995), inhibits transcription in transfection assays (Shaulian *et al.*, 1995) and has transforming potential (Shaulian *et al.*, 1992; Reed *et al.*, 1993).

TFIIH is a complex factor that has roles in RNA polymerase II (pol II) transcription (Gerard *et al.*, 1991; Flores *et al.*, 1992), DNA repair (van Vuuren *et al.*, 1994) and possibly the cell cycle (Seroz *et al.*, 1995). TFIIH forms part of the transcription initiation complex and, with TFIIE, directs promoter clearance by pol II (Goodrich and Tjian, 1994; Maxon *et al.*, 1994). TFIIH is also a component of the pol II holoenzyme that is competent for specific initiation (Ossipow *et al.*, 1995), but it is absent from the elongating pol II complex (Zawel *et al.*, 1995). TFIIH contains two helicases, ERCC2 and ERCC3, that are essential components of the cellular DNA repair machinery (Feaver *et al.*, 1993; Schaeffer *et al.*, 1993, 1994; Drapkin *et al.*, 1994). Their genes are mutated in subgroups of xeroderma pigmentosum (XP-D and -B, respectively), a human syndrome with defects in nucleotide excision repair (NER) and susceptibility to cancer (Weeda *et al.*, 1990; Flejter *et al.* 1992). TFIIH also contains a kinase, composed of MO15 (CDK7) and cyclin H (Feaver *et al.*, 1994; Roy *et al.*, 1994; Shiekhattar *et al.*, 1995). TFIIH phosphorylates pol II and may thereby regulate transcription initiation (Lu *et al.*, 1992; Akoulitchev *et al.*, 1995). CDK7 and cyclin H are also implicated in activation

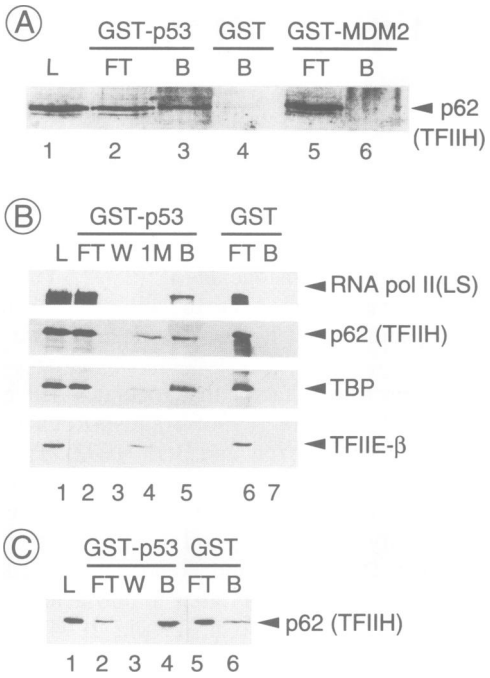

Fig. 1. p53 binds TFIIH *in vitro*. HeLa cell protein fractions (Gerard *et al.*, 1991; Fisher *et al.*, 1992) were adjusted to 150 mM KCl and applied to 50 µl mini-columns containing 5–10 µg of GST–p53, GST–MDM2 or GST. The mini-columns were washed with 0.6 ml of the binding buffer. One-tenth of the load (L) and equivalent amounts of the flow through (FT), wash (W) and 1/3 of the bound (B) proteins were analysed on SDS–PAGE and by Western blotting. (**A**) TFIIH binds to GST–p53 and not to GST–MDM2 or GST. The 0.6 M heparin–Sepharose fraction (50 µg protein) was analysed, and the blots were probed with the p62 subunit antibody. (**B**) Salt stability of interactions with GST–p53. As in (A) except that the columns were washed sequentially with buffers containing 150 mM and 1 M KCl, and the blots probed with antibodies against the large subunit of pol II [RNA pol II (LS)], TFIIH p62, TBP and TFIIE-β. (**C**) Purified TFIIH in a fraction lacking TFIID binds to GST–p53. Five µg of the heparin–5PW fraction was applied to mini-columns and the Western blot was probed with p62 mAbs. The arrows point to the specific bands.

of cyclin-dependent kinases (CDKs) that are required for cell cycle progression (Fisher and Morgan, 1994).

We report that p53 interacts physically and functionally with the TFIIH complex. Direct physical interactions with TFIIH provide an immediate means by which p53 might regulate cellular functions without recourse to transcription of effector genes, that might even have been damaged by the genotoxic agents that induce p53 (Kastan *et al.*, 1991).

Results

TFIIH interacts with p53

We found that TFIIH interacts specifically with p53 (Figure 1A). Crude HeLa cell extracts (0.6 M heparin–ultrogel fraction, Gerard *et al.*, 1991) were applied to mini-columns containing glutathione *S*-transferase (GST) proteins bound to glutathione–Sepharose and fractions were tested for the presence of TFIIH by Western blotting with the p62 subunit monoclonal antibody (mAb) (3c9, Fisher *et al.*, 1992). TFIIH bound to GST–p53 (lanes 1–3), but not to either GST alone (lane 4) or GST–MDM2 (lanes 5 and 6), indicating that p53 has a specific affinity for TFIIH. Similar results were obtained with antibodies against the ERCC3 and ERCC2 subunits of TFIIH (not shown). TFIIH interacts strongly with p53, since most of it remains bound after a 1 M KCl wash, similarly to TATA box binding protein (TBP) which is known to interact with p53 (compare lanes 4 and 5, Figure 1B; Seto *et al.*, 1992; Liu *et al.*, 1993; Truant *et al.*, 1993; Horikoshi *et al.*, 1995). Pol II and TFIIE were also specifically retained on the p53 column.

TFIIH may associate with p53 directly, or as a complex with other factors in the crude extract. TFIIE is absolutely required for TFIIH to bind to the pre-initiation complex (Flores *et al.*, 1992; Maxon *et al.*, 1994; Ohkuma and Roeder, 1994). However, TFIIE is apparently not essential for the association of TFIIH with p53, since it was quantitatively eluted from the column by 1 M KCl, whereas most of the TFIIH was retained (Figure 1B, lanes 4 and 5). TFIIH in an extensively purified fraction (heparin HPLC, Gerard *et al.*, 1991) still bound specifically to the p53 column (Figure 1C), indicating that TFIIH interacts directly with p53. In particular, it is interesting to note that this interaction is not mediated by TBP which is not detectable in this fraction (Figure 3, lane 4).

TFIIH binds to both the N- and C-terminal domains of p53

GST–p53 fusion proteins were used to localize the domains of p53 that interact with TFIIH. The C-terminal amino acids (320–393) of p53 bound specifically to both the TFIIH and TBP complexes in the crude HeLa cell extract (Figure 2A). TFIIH was detected with p62 antibody as in Figure 1. Neither the oligomerization domain (320–362) nor the basic region (363–393) alone interacted with TFIIH or TBP (Figure 2B). p53 (1–320), deleted of the C-terminus, also interacted with TFIIH (Figure 2C, lanes 5–7), but apparently less efficiently than the whole protein, as shown by both the amount retained (compare lanes 4 and 7) and flowing through (lanes 2 and 5). These results show that TFIIH binds independently to two distinct regions of p53.

p53 and its C-terminal domain bind directly to 80 and 52 kDa polypeptides in a purified TFIIH fraction

Far Western blotting was used to identify the subunits of TFIIH that interact directly with p53. Crude and purified fractions of TFIIH (heparin 0.6 M and heparin HPLC) were probed with p53 labelled with ^{32}P by casein kinase II (Meek *et al.*, 1990; Hupp *et al.*, 1992). Two major 80 and 52 kDa bands were detected in the purified fraction (Figure 3, lane 2). They were also clearly discernable on the original autoradiogram in the crude fraction, amongst other bands (lane 1). The 80 kDa band co-migrated precisely with ERCC2 revealed with a specific antibody (lanes 3 and 4). The 52 kDa band does not correspond to a known component of TFIIH and is unlikely to be p53, which was undetectable in this fraction with the PAb 240 antibody (result not shown). There was a weak 62 kDa band with similar mobility to the p62 subunit of TFIIH (lane 2). Several other bands were detected with labelled p53 in the purified fraction, most notably above 100 kDa. Using the casein kinase II-labelled C-terminus of p53 as a probe, we uniquely detected the 80 and 52 kDa bands in the TFIIH purified fraction, even after longer autoradio-

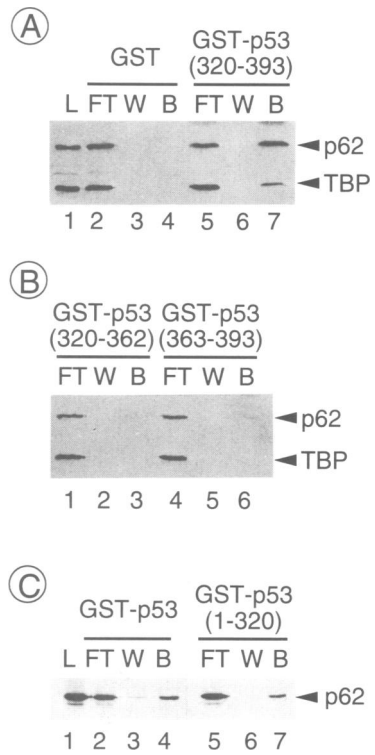

Fig. 2. TFIIH interacts with both the 320–393 (**A**) and 1–320 (**C**) regions of p53, and TBP with the 320–393 region. The 0.6 M heparin–Sepharose fraction was loaded on mini-columns containing the indicated GST proteins. One-tenth of the load (L), flow through (FT), wash (W) and 1/3 of the bound (B) fractions were analysed by SDS–PAGE. Western blots were probed with mAbs against the p62 subunit of TFIIH (A–C) and against TBP (A and **B**). The arrows point to the specific bands.

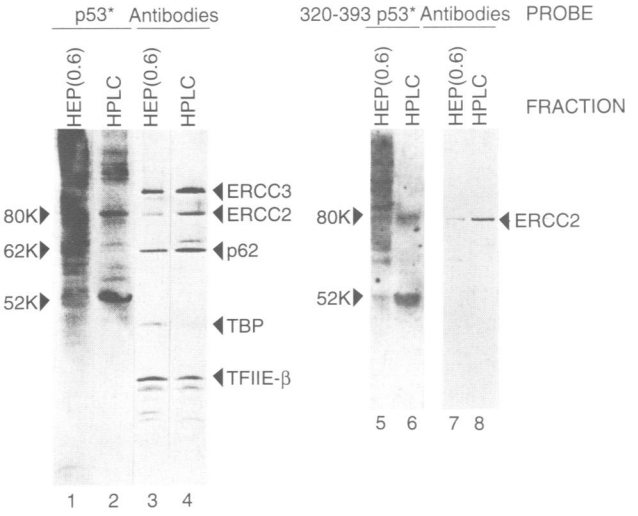

Fig. 3. p53 interacts with proteins of 80, 62 and 52 kDa molecular weight in far Western blotting. Proteins from crude [heparin–Sepharose 0.6 M; HEP (0.6), lanes 1, 3, 5 and 7] and more extensively purified (heparin–5PW column; HPLC, lanes 2, 4, 6 and 8) fractions were resolved on SDS–PAGE, transferred to nitrocellulose membranes and probed with ^{32}P-labelled p53 (p53*, lanes 1 and 2) or its C-terminus (320–393 p53*, lanes 5 and 6), or with antibodies against ERCC3, ERCC2, p62, TBP and TFIIE-β (lanes 3 and 4) or ERCC2 (lanes 7 and 8). Arrowheads indicate the corresponding bands.

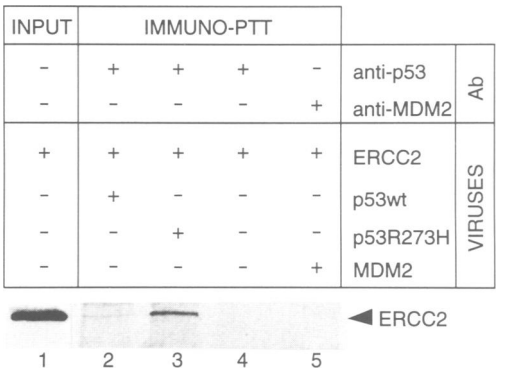

Fig. 4. ERCC2 co-immunoprecipitates with p53 when they are co-expressed *in vivo*. Sf9 cells were infected with baculoviruses expressing ERCC2 alone (lanes 1 and 4), or with either p53 (wild-type, lane 2; mutant R273H, lane 3) or MDM2 (lane 5). Whole cell extracts were immunoprecipitated with the p53 mAb DO-1 (lanes 2–4) or the MDM2 polyclonal antibodies 365 (lane 5). One-tenth of the ERCC2 input is shown in lane 1.

graphic exposure (lane 6). Again the 80 kDa band co-migrated precisely with ERCC2 (lanes 7 and 8). These bands were also detected in the crude fraction, both visually (lane 5) and by scanning (not shown). The 80 kDa band increased in intensity during TFIIH purification (lanes 5 and 6) to the same extent as ERCC2 (lanes 7 and 8). Interestingly, the 52 kDa band was also enriched to a similar extent, suggesting that it might be associated with TFIIH. The results were similar with p53 C-terminal probes labelled in three different ways (with casein kinase II, protein kinase C or protein kinase A on an external tag; not shown), indicating that the interactions were not affected by phosphorylation on a particular site. The data suggest that p53 interacts directly with ERCC2.

ERCC2 interacts with p53 in vivo and in vitro

We investigated interactions with isolated ERCC2 using two additional approaches, co-immunoprecipitation from infected insect cells (Figure 4) and GST columns (Figure 5). Sf9 insect cells were co-infected with baculoviruses expressing ERCC2 and p53. Crude extracts were immunoprecipitated with a p53 mAb (DO-1). Immunoprecipitates were analysed on Western blots with ERCC2 mAbs. ERCC2 was found to co-immunoprecipitate with wild-type p53 (Figure 4, lane 2). Several control infections were used to confirm that the interactions were specific. The p53 antibody did not co-immunoprecipitate ERCC2 when only the ERCC2 virus was used for infection (lane 4), showing that exogenous p53 was required and that the p53 antibody did not cross-react with ERCC2. MDM2 antibodies did not bring down ERCC2 with co-expressed MDM2 (lane 5), showing that ERCC2 was not trapped non-specifically in immunoprecipitates. With affinity columns, *in vitro* translated ERCC2 was retained on GST–p53 (Figure 5, lane 3) but not on GST (lane 2). In conclusion, we have shown by three different techniques that p53 interacts with ERCC2.

ERCC2–p53 interactions require the C-terminus of p53 as well as the N-terminus of ERCC2

p53 and ERCC2 interactions were investigated using GST fusion proteins. p53 (320–393) retained *in vitro* translated ERCC2 (Figure 5A, lane 5), whereas the other part of

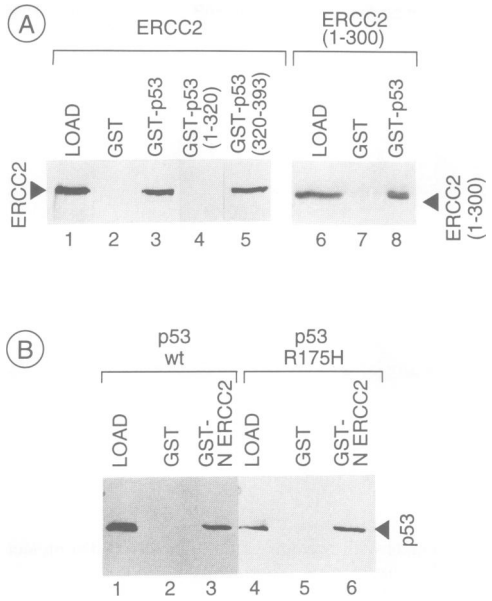

Fig. 5. Interactions between p53 and ERCC2. (**A**) The C-terminus of p53 binds the N-terminal region of ERCC2. *In vitro* translated ERCC2 (lanes 1–5) was loaded on mini-columns containing GST (lanes 2), GST–p53 (lane 3), GST–p53 (1–320) (lane 4), GST–p53 (320–393) (lane 5). *In vitro* translated N-terminal domain (1–300) of ERCC2 (lanes 6–8) was loaded on columns containing GST (lane 7) and GST–p53 (lane 8). One-tenth of the load (LOAD, lanes 1 and 6) and 1/10 (lanes 2–4, 7 and 8) or 1/3 (lane 5) of the bound proteins were analysed on SDS–polyacrylamide gels. (**B**) Wild-type p53 and the R175H mutant interact with the N-terminal domain of ERCC2 (1–300, N ERCC2). Purified wild-type (p53 wt, lanes 1–3) and p53 R175H (lanes 4–6) were loaded on GST (lanes 2 and 5) or GST–N ERCC2 (lanes 3 and 6) columns, and 1/10 of the input (LOAD, lanes 1 and 4) and 1/3 of the bound fractions (lanes 2, 3, 5 and 6) were analysed by SDS–PAGE and Western blotting with a p53-specific antibody (DO-1). The arrowheads indicate the specific bands.

p53 (1–320) did not (lane 4). These and the Far Western results indicate that the p53 C-terminus is necessary and sufficient for the interaction with ERCC2. Full-length p53 specifically retained N ERCC2, the N-terminal part of ERCC2 (1–300, lanes 6–8). Conversely, N ERCC2 retained p53 (Figure 5B, lanes 1–3). Both proteins had been purified extensively, p53 from Sf9 cells, GST–N ERCC2 from bacteria, indicating that they contact each other directly. The N-terminal 300 amino acids of ERCC2 contain the ATP binding site (Hoeijmakers, 1993). They are probably essential for p53 binding since an ERCC2 mutant lacking most of this sequence (247–760) did not bind p53 (results not shown).

p62–p53 interactions require the N-terminus of p53

In vitro translated p62 bound specifically to GST–p53 (Figure 6, lanes 1–3) but less efficiently than ERCC2 (compare lanes 1–3 with Figure 5A lanes 1–3 done at the same time). p53 (1–320) bound p62 (lane 4) whereas p53 (320–393, lane 5) did not. These results show that different regions of p53 interact with p62 and ERCC2, the N-terminal 320 amino acids with p62, and the C-terminal 73 amino acids with ERCC2. The affinity is apparently weaker for p62, as indicated by both affinity chromatography and Far Western blotting.

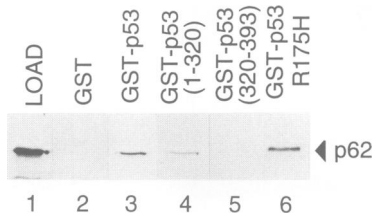

Fig. 6. p62 interacts with the N-terminal region of p53 (1–320) and with the R175H mutant. *In vitro* translated p62 was loaded on mini-columns containing GST (lane 2), GST–p53 (lane 3), GST–p53 (1–320) (lane 4), GST–p53 (320–393) (lane 5), GST–p53 (R175H) (lane 6). One-tenth of both the bound and input (LOAD) proteins were analysed.

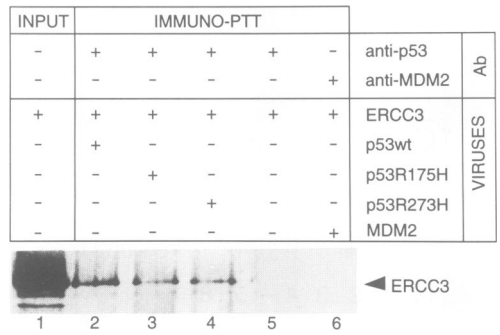

Fig. 7. p53 co-immunoprecipitates with ERCC3 when they are co-expressed *in vivo*. Sf9 cells were infected with baculoviruses expressing ERCC3 alone (lanes 1 and 5), or with either p53 (wild-type, lanes 2; p53 R175H, lane 3; R273H, lane 4) or MDM2 (lane 6). Whole cell extracts were immunoprecipitated with a p53 mAb DO-1 (lanes 2–4) or MDM2 polyclonal antibodies 365 (lane 6). One-tenth of the ERCC3 input is shown in lane 1.

ERCC3 interacts with p53

Interactions between ERCC3 and p53 were studied in co-infection experiments in Sf9 cells, as for ERCC2 above. ERCC3 was brought down by p53 antibodies when it was co-expressed with p53 (Figure 7, lane 2) but not when it was expressed alone (lane 5). It was not precipitated with MDM2 antibodies when it was co-expressed with MDM2 (lane 6). These results show that ERCC3 interacts specifically with p53 *in vivo*. The interactions were not detected by Far Western blotting, possibly because ERCC3 does not renature appropriately.

p53 mutants interact with TFIIH subunits

We investigated interactions between TFIIH subunits and two p53 mutants: R175H, that alters the conformation of the DNA binding domain, and R273H, that substitutes an amino acid that contacts DNA (Cho *et al.*, 1994). In column assays, GST–N ERCC2 specifically retained p53 R175H (Figure 5B, lanes 4–6) and GST–p53 R175H retained p62 (Figure 6, lanes 1, 2 and 6). In co-infections, ERCC3 co-immunoprecipitated with R175H (Figure 7, lane 3). These data show that p53 R175H interacts with ERCC2, ERCC3 and p62. In co-infection assays, p53 R273H interacted with both ERCC2 (Figure 4, lane 3) and ERCC3 (Figure 7, lane 4). These results, together with those of Wang *et al.* (1995), show that the R175H and R273H mutations do not affect p53 binding to ERCC2 and ERCC3.

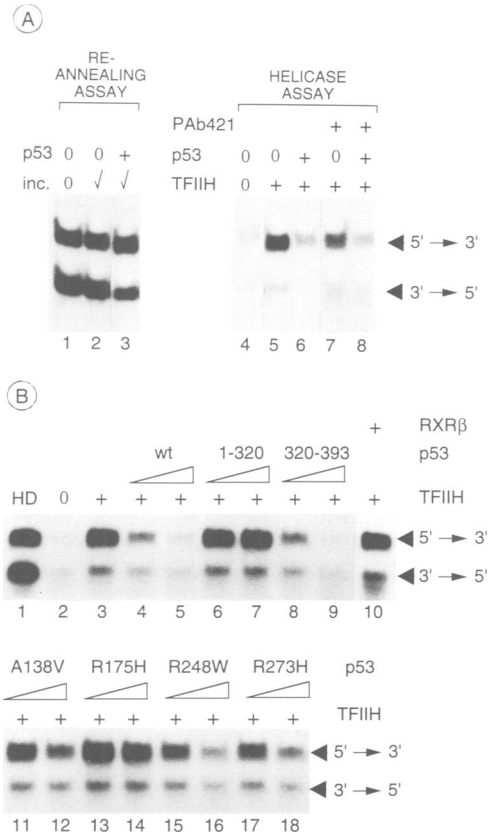

Fig. 8. Effects of wild-type and mutant p53 proteins on TFIIH helicase activities. The helicase assays (Schaeffer *et al.*, 1994) contained purified p53 and TFIIH (2 μl hydroxyapatite fraction, Gerard *et al.*, 1991). The arrowheads point to oligonucleotides dissociated from the probe. 5′→3′ and 3′→5′ indicate the direction of strand displacement due to the ERCC2 and ERCC3 helicases, respectively. (**A**) Re-annealing assay (lanes 1–3). DNA strand re-annealing by wild-type p53 is undetectable in the conditions of the helicase assay. Heat-denatured probe (lanes 1–3, see undenatured probe in lane 4) before (lane 1) and after (lanes 2 and 3) incubation in the presence (lane 3) or absence (lane 2) of p53. Helicase assay (lanes 4–8). Wild-type p53 inhibits the TFIIH helicases (lanes 4–6) and PAb421 does not affect inhibition (lanes 7 and 8). The helicase probe was incubated in the absence (lane 4) or presence (lane 5–8) of TFIIH with wild-type p53 (0.1 μM, lanes 6 and 8) and PAb421 (2 μg, lanes 7 and 8). (**B**) Helicase assay. p53 mutants inhibit TFIIH helicases to different extents. The native probe (lanes 2–18) was incubated without (0, lane 2) or with (+, lanes 3–18) TFIIH and with wild-type p53 (0.05 μM, lane 4; 0.2 μM, lane 5), p53(1–320) (0.05 μM, lane 6; 0.2 μM, lane 7), purified RXRβ (0.2 μM, lane 10), p53 (320–393) (0.2 μM, lane 8; 0.9 μM, lane 9), point mutated p53s (as indicated in lanes 11–18; 0.05 μM, odd lanes; 0.2 μM, even lanes) and HD (lane 1) = heat-denatured probe.

Wild-type and mutant p53s have different effects on the helicase activities of TFIIH

TFIIH has both 5′→3′ and 3′→5′ helicase activities, due to ERCC2 and ERCC3, respectively (Schaeffer *et al.*, 1994). Helicase activities were measured with a probe consisting of different sized labelled oligonucleotides (23mer and 27mer for ERCC3 and ERCC2, respectively) hybridized to the extremities of linear single-stranded M13 phage DNA. The helicases track in either direction from the central single-stranded part of the probe and displace the corresponding oligonucleotides, which are detected by non-denaturing gel electrophoresis (Figure 8A, see bands labelled 5′→3′ and 3′→5′; compare lane 4, the 'starting native' probe, with both lane 5, in which TFIIH has displaced the oligonucleotides, and lane 1, the heat-denatured probe). Wild-type p53 reproducibly inhibited both helicases, with a greater effect on ERCC2 (Figure 8A, lane 6, and B, lanes 4 and 5). Another transcription factor, RXRβ, did not significantly affect the helicase activities (Figure 8B, lane 10). p53 has been shown to catalyse re-annealing of complementary single-stranded oligonucleotides (Oberosler *et al.*, 1993; Bakalkin *et al.*, 1994; Wu *et al.*, 1995), a separate reaction that would give the same result as direct inhibition of the helicases. Under our assay conditions, p53 did not significantly stimulate re-annealing of dissociated oligonucleotides (Figure 8A, lanes 1–3 and results not shown). Moreover, p53 inhibition was not counteracted by PAb 421 (Figure 8A, compare lanes 7 and 8 with 5 and 6), that binds to the C-terminus of p53 and inhibits its strand re-annealing activity (Wu *et al.*, 1995). PAb421 did not inhibit binding of p53 to TFIIH (not shown). Our results indicate that wild-type p53 directly inhibits both TFIIH helicases.

Mutated p53 proteins were less effective inhibitors of TFIIH helicases. The C-terminal amino acids (320–393) inhibited (Figure 8B, lanes 8 and 9), but less efficiently than wild-type (compare lanes 8 and 5 with equivalent molar amounts of protein), whereas the N-terminal part had no effect (Figure 8B, lanes 6 and 7). Several mutant proteins inhibited less efficiently than wild-type (A138V, R248W, R273H, lanes 11–12 and 15–18), similar to the isolated C-terminus. Surprisingly, the R175H mutant had virtually no effect, even though it binds efficiently to TFIIH subunits (see above and the Discussion).

TFIIH relieves wild-type p53-mediated inhibition of transcription in vitro

The effects of p53–TFIIH interactions on *in vitro* transcription were studied with a reconstituted system (Gerard *et al.*, 1991), in which the effects of p53 in the absence and the presence of TFIIH can be compared. As expected from previous studies (Parvin and Sharp, 1993), specific transcription from the IgH promoter does not absolutely require TFIIH when the template is supercoiled (Figure 9, lane 1), whereas TFIIH is essential when the template is linear (not shown). Wild-type p53 and, to a lesser extent, the C-terminus (320–393), reproducibly inhibited TFIIH-independent transcription (Figure 9, lanes 2, 3 and 9), whereas mutants R175H, R248H and p53 (1–320) were less efficient (lanes 4–8). These results show that inhibition of transcription by p53, in the absence of a specific DNA binding site, does not require TFIIH. TFIIH stimulated transcription to levels that were similar for supercoiled and linear templates (lanes 10 and 21), presumably because it overcomes topological requirement, although other effects cannot be excluded. TFIIH overcame inhibition by wild-type p53, leading to an ~2-fold activation (lanes 11, 12 and 22). In contrast, TFIIH did not completely overcome inhibition by the C-terminus (lanes 19, 20 and 26). TFIIH also overcame the weaker inhibition by the p53 mutants, and gave a smaller stimulation than with wild-type p53 [see p53 R175H, p53 R248W and p53 (1–320) in lanes 13–18 and 23–25]. Similar results were obtained with the adenovirus major late promoter (not shown). These data show that TFIIH can relieve inhibition of transcription by p53.

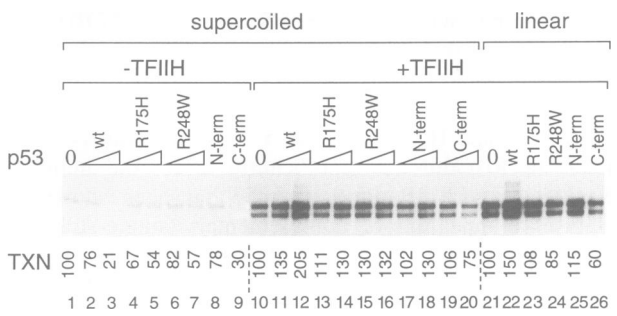

Fig. 9. Effects of p53 on TFIIH-independent and -dependent transcription *in vitro*. Supercoiled or linear pμ(-47)-(G-) were incubated in a reconstituted transcription system in the absence (lanes 1–9) or presence (lanes 10–26) of purified TFIIH (heparin–5PW). The reactions also contained either no additional proteins (lanes 1, 10 and 21), wild-type p53 [0.2 μM (lanes 2 and 11); 0.6 μM (lanes 3, 12 and 22)], p53 R175H [0.2 μM (lanes 4 and 13); 0.6 μM (lanes 5, 14 and 23)], p53 R248W [0.2 μM (lanes 6 and 15); 0.6 μM (lanes 7, 16 and 24)], p53 (1–320) [N-term; 0.2 μM (lane 17); 0.6 μM (lanes 8, 18 and 25)] and p53 (320–393) [C-term; 0.4 μM (lane 9), 0.2 μM (lane 19); 0.6 μM (lanes 20 and 26)]. Reactions were incubated for 60 min at 30°C and the products were analysed on denaturing polyacrylamide gels. The specific transcripts are shown. TXN = the percentage of transcription relative to the controls without p53 (lanes 1, 10 and 21), as estimated by phosphorimaging.

p53 does not affect NER in vitro

NER was studied *in vitro* using HeLa whole cell extracts and UV-irradiated DNA, that was treated with *Nth* endonuclease to remove 'non-specific' damage (Wood *et al.*, 1995), and extensively purified. A mixture of UV-modified and untreated plasmids (+ and – in the upper part of Figure 10B) was incubated under standard reaction conditions (see Wood *et al.*, 1995). As expected for NER, only the damaged DNA was labelled (compare + and –, in Figure 10B, lane 1, lower panel). We used 40 min incubations, well within the linear range (Figure 10A), to study the effect of p53. Added wild-type p53 did not affect NER (Figure 10B, compare lanes 2–5 with lane 1), even when a nearly 10-fold higher concentration was used than is necessary to inhibit TFIIH helicases (see Figure 8A, lanes 5 and 6). The HeLa extracts contained very little endogenous p53 (data not shown), in agreement with other studies (Haupt *et al.*, 1995). Furthermore, exogenous p53 was not degraded during the incubation (not shown). Similarly, p53 did not inhibit NER with extracts from primary lymphoblasts (GM01953) and fibroblasts (GM03348C) (not shown). These results agree with studies in other laboratories (Sancar, 1995 and personal communications; R.Wood, T.Hupp and D.Lane, personal communications). We also found that NER was unaffected by several mutants [p53 R175H (lanes 6–9); p53 (1–320) and p53 (320–393), not shown].

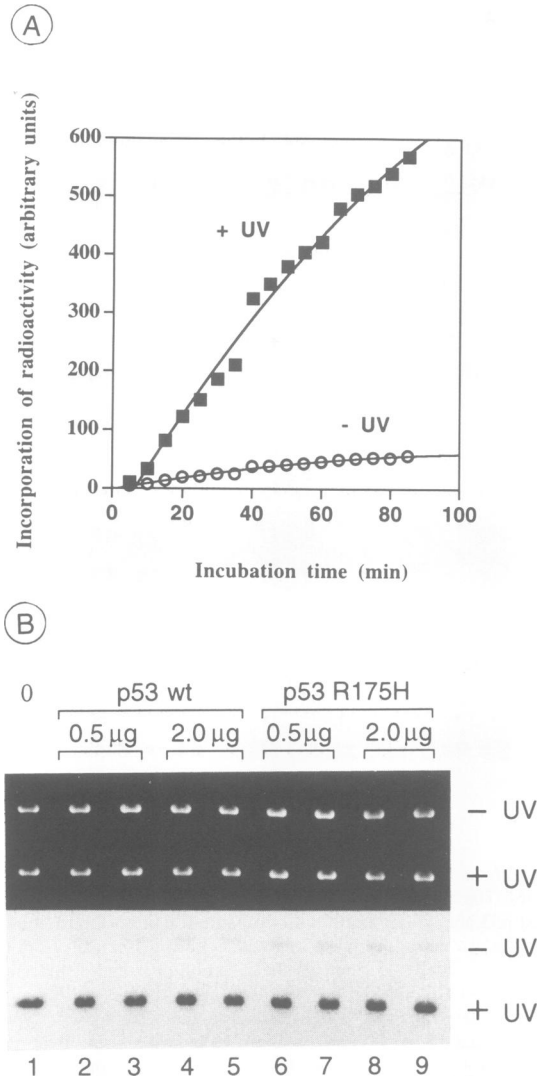

Fig. 10. Effect of p53 on NER *in vitro*. DNA repair reactions contained HeLa whole cell extracts (100 μg) and a mixture of UV-damaged (+UV) and control (–UV) plasmids (200 ng of each). (**A**) Kinetics of DNA repair synthesis in the absence of added p53. HeLa cell extracts were incubated for various times, incorporated radioactivity was quantified by phosphorimaging and plotted in arbitrary units. (**B**) Effect of p53 on DNA repair synthesis. HeLa extracts were pre-incubated without p53 (lane 1) or with the indicated amounts (0.5 μg = 0.25 μM; 2.0 μg = 0.9 μM) of wild-type (lanes 2–5) or mutant (R175H, lanes 6–9) p53, and then incubated for 40 min at 30°C. The products were separated in 1% agarose gels. The ethidium bromide-stained gel (upper panel) was dried and exposed for autoradiography (lower panel).

Discussion

Multiple interactions between the complex factors p53 and TFIIH

We have shown by different approaches that p53 interacts with the TFIIH complex that is involved in multiple steps in transcriptional initiation, DNA repair and possibly cell cycle control (Seroz *et al.*, 1995). p53 interacts with highly purified TFIIH, suggesting that it does not require additional factors, notably TFIID that also interacts with p53. The interactions are complex, since two distinct regions of p53 contact three different subunits of TFIIH, ERCC2, ERCC3 and p62. Interestingly, the viral transcription factor, EBNA2, also interacts with ERCC2 and p62 (Tong *et al.*, 1995). The N-terminal transactivation domain of p53 contacts the p62 subunit [see results and Xiao *et al.* (1994)]. The C-terminus binds directly to the N-terminal half of the ERCC2 helicase (our results), and the related domain of ERCC3 (Wang *et al.*, 1995). The C-terminus of p53 is involved in many processes, including oligomerization, regulation of specific DNA binding, single strand re-annealing, nuclear localization and binding to a variety of factors. The N-terminus of ERCC2 contains

the ATP binding site (Hoeijmakers, 1993). Related sequences are found in the ERCC6 and RAD3 helicases that also interact with p53 (Wang *et al.*, 1995 and our unpublished results). Interestingly, p53 was first isolated through its ability to complex with a viral helicase, SV40 large T (reviewed in Ludlow, 1993). Sakurai *et al.* (1994) reported that p53 binds to an unidentified helicase from HeLa cells whose activity has the same polarity as ERCC2. These observations suggest that p53 has an intrinsic affinity for helicases.

Direct inhibition of helicase activity by p53

Wild-type p53 inhibits both helicase activities of TFIIH. A number of observations point to a direct effect, rather than an indirect result of p53 re-annealing dissociated DNA strands. p53 alone does not detectably re-anneal the probe during the incubation. The mAb PAb421 does not influence p53's effects on the helicases, even though it blocks the ability of p53 to re-associate single-stranded DNA (Wu *et al.*, 1995). There are various ways by which p53 could inhibit the helicases. p53 could prevent ATP binding by interacting with ATP binding domains of helicases. However, p53 does not block the ATPase activity of TFIIH (Wang *et al.*, 1995). An interesting possibility is that p53 could displace ERCC2 from TFIIH. This would explain greater inhibition of ERCC2 than ERCC3, even though isolated ERCC3 interacts efficiently with p53 (our results and Wang *et al.*, 1994, 1995). Dissociated ERCC2 has no helicase activity, but stimulates both helicase activities when added back to ERCC2-depleted TFIIH (Schaeffer *et al.*, 1994).

Cancer-associated mutations in the p53 core domain affect activities mediated by the C-terminus

Mutant p53s that are found in tumours inhibit TFIIH helicases less efficiently than wild-type. Based on the crystal structure of the core domain (Cho *et al.*, 1994), p53 mutants have been classified into two groups: those with changes in amino acids that interact with DNA and retain the wild-type conformation (R273H and R248W) and those with a disrupted structure (R175H). The 'contact' mutants inhibit less efficiently than wild-type, but as efficiently as the isolated C-terminal domain, whereas p53 lacking the C-terminus has no effect on the helicases. Apparently, inhibition is mediated by the C-terminal domain and the linked DNA binding core domain affects its activity. 'Contact' mutations may abrogate the activity of the core domain, leaving an activity due to the C-terminal domain. Interestingly, R175H is virtually inactive, even though it binds to the three subunits of TFIIH as efficiently as wild-type p53. The conformation of R175H is thought to be severely affected, to such an extent that it interacts with HSC70 (Hinds *et al.*, 1990). This unusual conformation of the core domain could also block the activity of the C-terminus. p53 R175H is a 'gain of function' mutant that appears to have acquired transforming activity (Dittmer *et al.*, 1993). An interesting possibility is that there is a link with degree of inhibition of TFIIH helicases.

p53 and the C-terminus inhibit *in vitro* transcription in the absence of TFIIH, as expected if inhibition results from interactions between the N- and C-terminal domains of p53 and TFIID (Liu *et al.*, 1993; Mack *et al.*, 1993; Seto *et al.*, 1993; Truant *et al.*, 1993; Horikoshi *et al.*, 1995). Surprisingly, we found that both mutations in the core domain and deleting the C-terminal domain abrogate inhibition. Apparently, as with the helicase assays, the linked core domain affects the activity of the C-domain. A number of studies have shown the converse, that the C-terminus affects DNA binding by the core domain (Hupp *et al.*, 1992, 1995; Jayaraman and Prives, 1995). These effects could be due to intramolecular interactions between domains of p53, or other mechanisms. The core domain of wild-type p53 binds to long stretches of single-stranded DNA (Bakalkin *et al.*, 1995). It may interact with single-stranded DNA in the assays and thereby affect the activity of the linked C-terminal domain. Mutations could modify these p53–DNA interactions and abrogate these effects.

TFIIH overcomes inhibition of transcription by p53

TFIIH reproducibly converts inhibition of transcription by wild-type p53 to a weak activation and also partially suppresses inhibition by the C-terminus. There are a number of potential mechanisms. TFIIH helicases may counteract promoter closing by p53 or the C-terminus, either by sterically hindering their access to the DNA or by independently opening the DNA. Alternatively, TFIIH may affect p53–TBP or p53–TFIID interactions (Figure 2A and Liu *et al.*, 1993; Mack *et al.*, 1993; Seto *et al.*, 1993; Truant *et al.*, 1993; Horikoshi *et al.*, 1995; Lu and Levine, 1995). Nevertheless, these mechanisms do not explain weak but reproducible TFIIH-dependent activation of transcription by wild-type p53, even in the absence of a specific p53 DNA binding site. TFIIH may help recruit p53 to the promoter by providing stabilizing contacts for p53. Alternatively, TFIIH may change the activity of p53, by altering its conformation or state of phosphorylation. An interesting possibility suggested by our data is that changes in TFIIH in the cell could affect transcriptional regulation by p53.

Involvement of p53 in NER

NER deficiencies favour cancerogenesis. p53 could intervene in these processes through its interactions with several components of the NER machinery, TFIIH and RPA (Dutta *et al.*, 1993). From experiments in several laboratories, including ours, p53 does not affect NER *in vitro* (Sancar, 1995 and personal communications; R.Wood, D.Meek and D.Lane, personal communications). There are contradictory reports on the effects of p53 on NER. Several studies describe slow and less efficient NER in cells with down-regulated or mutated p53 (Ford and Hanawalt, 1995; Havre *et al.*, 1995; Smith *et al.*, 1995; Wang *et al.*, 1995). On the other hand, cells from p53 –/– mice are as efficient in DNA repair as their wild-type counterparts (Ishizaki *et al.*, 1994; Sands *et al.*, 1995). p53 might affect NER indirectly through its downstream target, Gadd45. However, there are conflicting reports on the effects of Gadd45 on NER (Smith *et al.*, 1994; Kazantsev *et al.*, 1995). p53–TFIIH interactions do not affect *in vitro* repair directly, but there could be other consequences during the repair process. One intriguing possibility is that, during repair, the cyclin H–CDK7 component of TFIIH could phosphorylate p53, by analogy

with phoshorylation by other cyclin–CDKs (Wang and Prives, 1995). Phosphorylated p53, bound to excised and damaged DNA, may have altered transcriptional and apoptotic properties.

Perspectives

Our results provide an enticing conceptual framework for integrating the properties of two complex factors. Our working hypothesis is that the interaction of p53 with TFIIH is a direct manner by which p53 expresses its functions as the guardian of the genome. In response to genotoxic stress, p53 could, through the interaction with one factor, TFIIH, intervene at three critical levels, transcription initiation, NER and the cell cycle.

Materials and methods

Protein expression and purification

GST fusion proteins were expressed in *Escherichia coli* BL21 grown at 28°C and purified on gluthatione–Sepharose in the presence of 10 mM dithiothreitol (DTT) as described previously (Léveillard and Verma, 1993). p53 C-terminal polypeptide (320–393) was digested from the fusion protein by thrombin and further purified on benzamidine–Sepharose. All other p53 proteins were purified from *Spodoptera frugiperda* (Sf9) insect cells infected with appropriate recombinant baculoviruses by affinity chromatography on agarose-coupled p53 mAbs PAb421 or DO-1. Proteins were eluted with antibody-specific peptides (10 mM, final concentration), purified on Superose 12 or on MonoQ/MonoS columns (SMART, Pharmacia), verified to be nuclease free and kept in storage buffer (20 mM HEPES, pH 7.9, 50 mM KCl, 20% glycerol, 5 mM DTT, 0.1 mM EDTA, 0.02% NP-40) at –80°C.

In vitro transcription/translation

Plasmids for in vitro binding studies. Plasmids [pGOp53, pGOp53(1–320), pGOp53(320–393), pGOp53(320–362) and pGOp53(363–393] contain wild-type p53 coding sequences in pGOMTT (a modified pGEX plasmid containing two thrombin cleavage sites) and were a gift from B.Tocqué. pBSKERCC2 contains the insert from p2E-ER2 (a modified version of p2E-ER2-14; Weber *et al.*, 1990) in pBluescript (Stratagene). pETp62 is described by Fisher *et al.* (1992). pBSKΔNERCC2 lacks the 5′ 670 bp fragment of the ERCC2 cDNA, allowing translation from Met247.

Transcription/translation. Proteins translated in rabbit reticulocyte lysates (Promega, manufacturer's procedure) were treated immediately for 1 h at 30°C with a cocktail of nucleases (RNase A, T1 and DNase I), and purified on heparin–agarose.

Protein–protein interaction assays and immunodetection

Mini-column assays. Glutathione–Sepharose (40 µl) with bound GST fusion proteins packed in mini-columns (yellow pipetman tips) were equilibrated with binding buffer [20 mM Tris–HCl, pH 7.5, 1 mM DTT, 5% glycerol, 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 2.5 µg/ml proteinase inhibitors] + 50 or 150 mM KCl. Columns were loaded with either the *in vitro* translation products or partially purified HeLa cell fraction (0.6 M eluate from a heparin–ultrogel column) or more extensively purified TFIIH fraction (0.3 M KCl fraction from a heparin–5PW column; Gerard *et al.*, 1991) and washed with 10 volumes of binding buffer containing different KCl concentrations, as specified in the figure legends. The fractions were analysed by SDS–PAGE and either processed for fluorography or transferred to nitrocellulose membranes and revealed by Western blotting using standard protocols and the mAbs: 3G3 (anti-TBP), 3c9 (anti-p62), 2F6 (anti-ERCC2), 2G12 (anti-ERCC3), 7G5 (anti-CTD pol II), 1C2 (anti-TFIIE-β) and PAb 240 (anti-p53).

Far Western blotting. This method was modified from Lee *et al.* (1991) as follows. Eluted GST–p53 (320–393) and bacterially expressed and purified full-length p53 were labelled with [γ-^{32}P]ATP and casein kinase II. GST–p53 (320–393) was re-purified on glutathione–Sepharose and the p53 sequence was isolated after thrombin cleavage. Labelled full-length p53 was re-purified on a heparin–Sepharose column.

Co-immunoprecipitation. This method was adapted from Hannon *et al.* (1993). Briefly, 10^7 Sf9 cells were infected with either p53 (wild-type or R175H and R273H) or MDM2 baculoviruses at 2 p.f.u. per cell and either with ERCC2 or ERCC3 baculoviruses at 5 p.f.u. per cell and incubated for 50 h at 27°C in Grace's medium. Cells were lysed (20 mM HEPES, pH 7.9, 150 mM NaCl, 0.2% NP-40, 3 mM DTT, 20% glycerol, 0.1 mM EDTA, 0.5 mM PMSF), pre-cleared with protein A–Sepharose and immunoprecipitated for 2 h at 4°C with either p53 mAb DO-1 or with polyclonal antibodies against MDM2 (365Ab) bound to protein A–Sepharose. After washing in the lysis buffer, bound fractions were resolved on SDS–PAGE, transferred to nitrocellulose membranes and probed with mAbs 2F6 (anti-ERCC2) or 2G12 (anti-ERCC3).

Functional assays

Helicases. The conditions are described in Schaeffer *et al.* (1994). For the re-annealing experiment, the DNA substrate was denatured by boiling the reaction mixture for 2 min prior to the addition of ATP and proteins.

In vitro transcription. The reactions contained recombinant human TBP, TFIIB, TFIIE and purified TFIIF, TFIIH and RNA pol II, as described previously (Fisher *et al.*, 1992). The templates were linear (60 ng per 25 µl reaction) or supercoiled (30 ng per 25 µl) pµ-47-(G-) plasmids containing the IgH promoter and 180 bp G-less cassette (Parvin and Sharp, 1993). The reactions were incubated at 30°C for 60 min and terminated by the addition of 150 µl of stop mix containing 1.2 M LiCl, 25 mM EDTA, 0.005% SDS, and 60 µl/ml tRNA. The reaction products were extracted with phenol, followed by chloroform extraction, ethanol precipitated, separated on 8% polyacrylamide gels containing 8.3 M urea and dried gels were exposed for autoradiography and quantified by phosphorimaging.

In vitro DNA repair. The protocol is described in Wood *et al.* (1995). The substrates were purified UV-irradiated pUC19 or *N*-acetylamino-fluorene (N-AAF)-modified pBluescript (kindly provided by R.Fuchs) and mock-treated pUC19 with a 1.2 kb insert (200 ng of each plasmid per reaction), and HeLa whole cell extracts were the source of NER proteins. p53 proteins were pre-incubated with the cell extracts (100 µg of total protein) for 5 min at 25°C, then DNA repair reactions were carried out at the same temperature for another 40 min. Reaction products were extracted with phenol–chloroform, digested with *Eco*RI and separated on 1% agarose gels. Gels were stained with ethidium bromide, photographed, dried, exposed for autoradiography and quantified by phosphorimaging.

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References

Akoulitchev,S., Mäkelä,T.P., Weinberg,R.A. and Reinberg,D. (1995) Requirement for TFIIH kinase activity in transciption by RNA polymerase II. *Nature*, **377**, 557–560.

Bakalkin,G., Yakovleva,T., Selivanova,G., Magnusson,K.P., Szekely,E., Klein,G., Terenius,L. and Wiman,K.G. (1994) p53 binds single-stranded DNA ends and catalyses DNA renaturation and strand transfer. *Proc. Natl Acad. Sci. USA*, **91**, 413–417.

Bakalkin,G. *et al.* (1995) p53 binds single-stranded DNA ends through the C-terminal domain and internal DNA segments via the middle domain. *Nucleic Acids Res.*, **23**, 362–369.

Baker,S.J., Markowitz,S., Fearon,E.R., Willson,J.K.U. and Vogelstein,B.

(1990) Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science*, **249**, 912–915.

Bargonetti,J., Manfredi,J.J., Chen,X., Marshak,D.R. and Prives,C. (1993) A proteolytic fragment from the central region of p53 has marked sequence-specific DNA-binding activity when generated from wild-type but not from oncogenic mutant p53 protein. *Genes Dev.*, **7**, 2565–2574.

Bodmer,W., Bishop,T. and Karran,P. (1994) Genetic steps in colorectal cancer. *Nature Genet.*, **6**, 217–219.

Caelles,C., Helmberg,A. and Karin,M. (1994) p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature*, **370**, 220–223.

Caron de Fromentel,C. and Soussi,T. (1992) TP53 tumor suppressor gene: a model for investigating human mutagenesis. *Gene Chromosomes Cancer*, **4**, 1–15.

Cho,Y., Gorina,S., Jeffrey,P.D. and Pavletich,N.P. (1994) Crystal structure of a p53 tumor suppressor–DNA complex: understanding tumorigenic mutations. *Science*, **265**, 346–355.

Clarke,A.R., Purdie,C.A., Harrison,D.J., Morris,R.G., Bird,C.C., Hooper,M.L. and Wyllie,A.H.H. (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature*, **362**, 849–852.

Clore,G.M., Ernst,J., Cubb,R., Omichinski,J.G., Poindexter Kennedy, W.M., Sakagushi,K., Appella,E. and Gronenborn,A.M. (1995) Refined solution structure of the oligomerisation domain of the tumour suppressor p53. *Nature Struct. Biol.*, **2**, 321–333.

Cox,L.S., Hupp,T., Midgley,C.A. and Lane,D.P. (1995) A direct effect of activated human p53 on nuclear DNA replication. *EMBO J.*, **14**, 2099–2105.

Dittmer,D., Pati,S., Zambetti,G., Chu,S., Teresky,A.K., Moore,M., Finlay,C. and Levine,A.J. (1993) Gain of function mutations in p53. *Nature Genet.*, **4**, 42–45.

Donehower,L.A., Harvey,M., Slagle,B.L., McArthur,M.J., Montgomery, C.A.,Jr, Butel,J.S. and Bradley,A. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*, **356**, 215–221.

Drapkin,R., Reardon,J.T., Ansari,A., Huang,J.C., Zawel,L., Ahn,K., Sancar,A. and Reinberg,D. (1994) Dual role of TFIIH in DNA excision repair and in transcription by RNA polymerase II. *Nature*, **368**, 769–772.

Dubs-Poterszman,M.C., Tocque,B. and Wasylyk,B. (1995) MDM2 transformation in the absence of p53 and abrogation of the p107 G1 cell-cycle arrest. *Oncogene*, **11**, 2445–2449.

Dutta,A., Ruppert,J.M., Aster,J.C. and Winchester,E. (1993) Inhibition of DNA replication factor RPA by p53. *Nature*, **365**, 79–82.

El-Deiry,W.S. *et al.* (1993) WAF-1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825.

Feaver,W.J., Svejstrup,J.Q., Bardwell,L., Bardwell,A.J., Buratowski,S., Gulyas,K.D., Donahue,T.F., Friedberg,E.C. and Kornberg,R.D. (1993) Dual roles of a multiprotein complex from *S.cerevisiae* in transcription and DNA repair. *Cell*, **75**, 1379–1387.

Feaver,W.J., Svejstrup,J.Q., Henry,N.L. and Kornberg,R.D. (1994) Relationship of CDK-activating kinase and RNA polymerase II CTD kinase TFIIH/TFIIK. *Cell*, **79**, 1103–1109.

Fields,S. and Jang,S.K. (1990) Presence of a potent transcription activating sequence in the p53 protein. *Science*, **249**, 1046–1048.

Fisher,F., Gerard,M., Chalut,C., Lutz,Y., Humbert,S., Kanno,M., Chambon,P. and Egly,J.M. (1992) Cloning of the 62-Kilodalton component of basic transcription factor BTF2. *Science*, **257**, 1392–1395.

Fisher,R.P. and Morgan,D.O. (1994) A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase. *Cell*, **78**, 713–724.

Flejter,W.L., McDaniel,L.D., Johns,D., Friedberg,E.C. and Schutz,R.A. (1992) Correction of xeroderma pigmentosum complementation group D mutant cell phenotype by chromosome and gene transfer: involvement of the human ERCC2 DNA repair gene. *Proc. Natl Acad. Sci. USA*, **89**, 261–265.

Flores,O., Lu,H. and Reinberg,D. (1992) Factors involved in specific transcription by mammalian RNA polymerase II. *J. Biol. Chem.*, **267**, 2786–2793.

Ford,J.M. and Hanawalt,P.C. (1995) Li–Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. *Proc. Natl Acad. Sci. USA*, **92**, 8876–8880.

Gerard,M., Fisher,L., Moncollin,V., Chipoulet,J.M., Chambon,P. and Egly,J.M. (1991) Purification and interaction properties of the human RNA polymerase B (II) general transcription factor BTF2. *J. Biol. Chem.*, **266**, 20940–20945.

Goodrich,J.A. and Tjian,R. (1994) Transcription factor IIE and IIH and ATP hydrolysis direct promoter clearance by RNA polymerase II. *Cell*, **77**, 145–156.

Hannon,G.J., Demetrick,D. and Beach,D. (1993) Isolation of the Rb-related p130 through its interaction with CDK2 and cyclins. *Genes Dev.*, **7**, 2378–2391.

Harris,C.C. (1993) p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science*, **262**, 1980–1981.

Haupt,Y., Rowan,S., Shaulian,E., Vousden,K.H. and Oren,M. (1995) Induction of apoptosis in HeLa cells by trans-activation-deficient p53. *Genes Dev.*, **9**, 2170–2183.

Havre,P.A., Yuan,J., Hedrick,L., Cho,K.R. and Glazer,P.M. (1995) p53 inactivation by HPV16 results in increased mutagenesis in human cells. *Cancer Res.*, **55**, 4420–4424.

Hinds,P.W., Finlay,C.A., Quartin,R.S., Baker,S., Fearon,E.R., Vogelstein,B. and Levine,A.J. (1990) Mutant p53 cDNAs from human colorectal carcinomas can cooperate with ras in transformation of primary rat cells: a comparison of the 'hot spot' phenotypes. *Cell Growth Differ.*, **1**, 571–580.

Hoeijmakers,J.H.J. (1993) Nucleotide excision repair II: from yeast to mammals. *Trends Genet.*, **9**, 211–217.

Hollstein,M., Sidransky,D., Vogelstein,B. and Harris,C.C. (1991) p53 mutations in human cancers. *Science*, **253**, 49–53.

Horikoshi,N., Usheva,A., Chen,J., Levine,A.J., Weinmann,R. and Shenk,T. (1995) Two domains of p53 interact with the TATA-binding protein, and the adenovirus 13S E1A protein disrupts the association, relieving p53-mediated transcriptional repression. *Mol. Cell. Biol.*, **15**, 227–234.

Hupp,T.R., Meek,D.W., Midgley,C.A. and Lane,D.P. (1992) Regulation of the specific DNA binding function of p53. *Cell*, **71**, 875–886.

Hupp,T.R., Sparks,A. and Lane,D.P. (1995) Small peptides activate the latent sequence-specific DNA binding function of p53. *Cell*, **83**, 237–245.

Ishizaki,K., Ejima,Y., Matsunaga,T., Hara,R., Sakamoto,A., Ikenaga,M., Ikawa,Y. and Aizawa,S.I. (1994) Increased UV-induced SCEs but normal repair of DNA damage in p53-deficient mouse cells. *Int. J. Cancer*, **57**, 254–257.

Jayaraman,L. and Prives,C. (1995) Activation of sequence-specific DNA binding by short single strands of DNA requires the p53 C-terminus. *Cell*, **81**, 1021–1029.

Jeffrey,P.D., Gorina,S. and Pavletich,N.P. (1995) Crystal structure of the tetramerization domain of the p53 tumour suppressor at 1.7 angströms. *Science*, **267**, 1498–1502.

Kastan,M.B., Onyiye,O., Sidransky,D., Vogelstein,B. and Craig,R.W. (1991) Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.*, **51**, 6304–6311.

Kazantsev,A., Sancar,A., Kearsey,J.M., Shivji,M.K.K., Hall,P.A. and Wood,R.D. (1995) Does the p53 up-regulated Gadd45 protein have a role in excision repair? *Science*, **270**, 1003–1006.

Lane,D.P. (1992) p53, guardian of the genome. *Nature*, **358**, 15–16.

Lane,D.P. (1993) A death in the life of p53. *Nature*, **362**, 786–787.

Lee,S., Elenbaas,B., Levine,A.J. and Griffith,J. (1995) p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. *Cell*, **81**, 1013–1020.

Lee,W.S., Kao,C.C., Bryant,G.O., Liu,X. and Berk,A.J. (1991) Adenovirus E1A activation domain binds the basic repeat in the TATA box transcription factor. *Cell*, **67**, 365–376.

Léveillard,T. and Verma,I. (1993) Diverse molecular mechanisms of inhibition of NF-κB/DNA binding complexes by IκB proteins. *Gene Exp.*, **3**, 135–150.

Liu,X., Miller,C.W., Koeffler,P.H. and Berk,A.J. (1993) The p53 activation domain binds the TATA box-binding polypeptide in Holo-TFIID, and neighboring p53 domain inhibits transcription. *Mol. Cell. Biol.*, **13**, 3291–3300.

Livinstone,L.R., White,A., Sprouse,J., Livanos,E., Jacks,T. and Tlsty,T.D. (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell*, **70**, 923–935.

Lowe,S.W., Schmitt,E.M., Smith,S.W., Osborne,B.A. and Jacks,T. (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature*, **362**, 847–849.

Lu,H. and Levine,A.J. (1995) Human $TAF_{II}31$ protein is transcriptional coactivator of the p53 protein. *Proc. Natl Acad. Sci. USA*, **92**, 5154–5158.

Lu,H., Zawel,L., Fisher,L., Egly,J.M. and Reinberg,D. (1992) Human general transcription factor IIH phosphorylates the C-terminal domain of RNA polymerase II. *Nature*, **358**, 641–645.

Ludlow,J.W. (1993) Interactions between SV40 large-tumor antigen and the growth suppressor proteins pRB and p53. *FASEB J.*, **7**, 866–871.
Mack,D.H., Vartikar,J., Pipas,J.M. and Laimins,L.A. (1993) Specific repression of TATA-mediated but not initiator-mediated transcription by wild-type p53. *Nature*, **363**, 281–283.
Maxon,M.E., Goodrich,J.A. and Tjian,R. (1994) Transcription factor IIE binds preferentially to RNA polymerase IIa and recruits TFIIH: a model for promoter clearance. *Genes Dev.*, **8**, 515–524.
Meek,D.W., Simon,S., Kikkawa,U. and Eckhart,W. (1990) The p53 tumour suppressor protein is phosphorylated at serine 389 by casein kinase II. *EMBO J.*, **9**, 3253–3260.
Miyashita,T. and Reed,J.C. (1995) Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell*, **80**, 293–299.
Momand,J., Zambetti,G.P., Olson,D.C., George,D. and Levine,A.J. (1992) The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell*, **69**, 1237–1245.
Oberosler,P., Hloch,P., Ramsperger,U. and Stahl,H. (1993) p53-catalyzed annealing of complementary single-stranded nucleic acids. *EMBO J.*, **12**, 2389–2396.
Ohkuma,Y. and Roeder,R.G. (1994) Regulation of TFIIH ATPase and kinase activities by TFIIE during active initiation of complex formation. *Nature*, **368**, 160–163.
Ossipow,V., Tassan,J.P., Nigg,E.A. and Schibler,U. (1995) A mammalian RNA polymerase II holoenzyme containing all components required for promoter-specific transcription initiation. *Cell*, **83**, 137–146.
Parvin,J.D. and Sharp,P.A. (1993) DNA topology and a minimal set of basal factors for transcription by RNA polymerase II. *Cell*, **73**, 533–540.
Pavletich,N.P., Chambers,K.A. and Pabo,C.O. (1993) The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots. *Genes Dev.*, **7**, 2556–2564.
Reed,M., Wang,Y., Mayr,G., Anderson,M.E., Schwedes,J.F. and Tegtmeyer,P. (1993) p53 domains: suppression, transformation, and transactivation. *Gene Exp.*, **3**, 95–106.
Reed,M., Woelker,B., Wang,P., Wang,Y., Anderson,M.E. and Tegtmeyer,P. (1995) The C-terminal domain of p53 recognizes DNA damaged by ionizing radiation. *Proc. Natl Acad. Sci. USA*, **92**, 9455–9459.
Roy,R., Adamczewski,J.P., Seroz,T., Vermeulen,W., Tassan,J.P., Schaeffer,L., Nigg,E.A., Hoeijmakers,J.H.J. and Egly,J.M. (1994) The MO15 cell cycle kinase is associated with the TFIIH transcription–DNA repair factor. *Cell*, **79**, 1093–1101.
Sakurai,T., Suzuki,M., Sawazaki,T., Ishii,S. and Yoshida,S. (1994) Anti-oncogene product p53 binds DNA helicase. *Exp. Cell Res.*, **215**, 57–62.
Sancar,A. (1995) Excision repair in mammalian cells. *J. Biol. Chem.*, **270**, 15915–15918.
Sands,A.T., Sukaokar,M.B., Sanchez,A., Marth,J.E., Donehower,L.A. and Bradley,A. (1995) p53 deficiency does not affect the accumulation of point mutations in a transgene target. *Proc. Natl Acad. Sci. USA*, **92**, 8517–8521.
Schaeffer,L., Roy,R., Humbert,S., Moncollin,V., Vermeulen,W., Hoeijmakers,J.H.J., Chambon,P. and Egly,J.M. (1993) DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science*, **260**, 58–63.
Schaeffer,L., Moncollin,V., Roy,R., Staub,A., Mezzina,M., Sarasin,A., Weeda,G., Hoeijmakers,J.H.J. and Egly,J.M. (1994) The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. *EMBO J.*, **13**, 2388–2392.
Seroz,T., Hwang,J.R., Moncollin,V. and Egly,J.M. (1995) TFIIH: a link between transcription, DNA repair and cell cycle regulation. *Curr. Opin. Genet. Dev.*, **5**, 217–221.
Seto,E., Usheva,A., Zambetti,G.P., Momand,J., Horikoshi,N., Weinmann,R., Levine,A.J. and Shenk,T. (1992) Wild-type p53 binds to the TATA-binding protein and represses transcription. *Proc. Natl Acad. Sci. USA*, **89**, 12028–12032.
Shaulian,E., Zauberman,A., Ginsberg,D. and Oren,M. (1992) Identification of a minimal transforming domain of p53: negative dominance through abrogation of sequence-specific DNA binding. *Mol. Cell. Biol.*, **12**, 5581–5592.
Shaulian,E., Haviv,I., Shaul,Y. and Oren,M. (1995) Transcriptional repression by the C-terminal domain of p53. *Oncogene*, **10**, 671–680.
Shaw,P., Bovey,R., Tardy,S., Sahli,R., Sordat,B. and Costa,J. (1992) Induction of apoptosis by wild-type p53. *Proc. Natl Acad. Sci. USA*, **89**, 4495–4499.
Shibata,D., Peinado,M.A., Ionov,Y., Malkhosyan,S. and Perucho,M. (1994) Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. *Nature Genet.*, **6**, 273–281.
Shiekhattar,R., Mermelstein,F., Fisher,R.P., Drapkin,R., Dynlatch,B., Wessling,H.C., Morgan,D.O. and Reinberg,D. (1995) Cdk-activating kinase complex is a component of human transcription factor TFIIH. *Nature*, **374**, 283–287.
Smith,M.L., Chen,I.T., Zhan,Q., Bae,I., Chen,C.Y., Gilmer,T.M., Kastan,M.B., O'Connor,P.M. and Fornace,A.J.,Jr (1994) Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science*, **266**, 1376–1380.
Smith,M.L., Chen,I.T., Zhan,Q., O'Connor,P.M. and Fornace,A.J.,Jr (1995) Involvement of the p53 tumor suppressor in repair of u.v.-type DNA damage. *Oncogene*, **10**, 1053–1059.
Stürzbecher,H.-W., Brain,R., Addison,C., Rudge,K., Remm,M., Grimaldi,M., Keenan,E. and Jenkins,J.R. (1992) A C-terminal α-helix plus basic region motif is the major structural determinant of p53 tetramerization. *Oncogene*, **7**, 1513–1523.
Tong,X., Drapkin,R., Reinberg,D. and Kieff,E. (1995) The 62- and 80-kDa subunits of transcription factor IIH mediate the interaction with Epstein–Barr virus nuclear protein 2. *Proc. Natl Acad. Sci. USA*, **92**, 3259–3263.
Truant,R., Xiao,H., Ingles,C.J. and Greenblatt,J. (1993) Direct interaction between the transcription activation domain of human p53 and the TATA box-binding protein. *J. Biol. Chem.*, **268**, 2284–2287.
van Vuuren,A.J. *et al.* (1994) Correction of xeroderma pigmentosum repair defect by basal transcription factor BTF2 (TFIIH). *EMBO J.*, **13**, 1645–1653.
Wang,E.H., Friedman,P.N. and 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.
Wang,X.W., Forrester,K., Yeh,H., Feitelson,M.A., Gu,J.R. and Harris,C.C. (1994) Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc. Natl Acad. Sci. USA*, **91**, 2230–2234.
Wang,X.W. *et al.* (1995) p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nature Genet.*, **10**, 188–195.
Wang,Y. and Prives,C. (1995) Increased and altered DNA binding of human p53 by S and G2/M but not G1 cyclin-dependent kinases. *Nature*, **376**, 88–91.
Wang,Y., Reed,M., Wang,P., Stenger,J.E., Mayr,G., Anderson,M.E., Schwedes,J.F. and Tegtmeyer,P. (1993) p53 domains: identification and characterization of two autonomous DNA-binding regions. *Genes Dev.*, **7**, 2575–2586.
Weber,C.A., Salazar,E.P., Stewart,S.A. and Thompson,L.H. (1990) ERCC2: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. *EMBO J.*, **9**, 1437–1447.
Weeda,G., van Ham,R.C.A., Vermeulen,W., Bootsma,D., van der Eb,A.J. and Hoeijmakers,J.H.J. (1990) A presumed DNA helicase encoded by ERCC-3 is involved in the human DNA repair disorders xeroderma pigmentosum and Cockayne's syndrome. *Cell*, **62**, 777–791.
Wood,R.D., Biggerstaff,M. and Shivji,M.K.K. (1995) Detection and measurement of nucleotide excision repair synthesis by mammalian cell extracts *in vitro*. *Methods: A companion to Methods in Enzymology*, **7**, 163–175.
Wu,W., Bayle,H., Elenbaas,B., Pavletich,N.P. and Levine,A.J. (1995) Alternatively spliced forms in the carboxy-terminal domain of the p53 protein regulate its ability to promote annealing of complementary single strands of nucleic acids. *Mol. Cell. Biol.*, **15**, 497–504.
Xiao,H. *et al.* (1994) Binding of basal transcription factor TFIIH to the acidic activation domains of VP16 and p53. *Mol. Cell. Biol.*, **14**, 7013–7024.
Yew,P.R. and Berk,A.J. (1992) Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. *Nature*, **357**, 82–85.
Yin,Y., Tainsky,M.A., Bishoff,F.Z., Strong,L.C. and Wahl,G.M. (1992) Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. *Cell*, **70**, 937–948.
Yonish-Rouach,E., Resnitzky,D., Lotem,J., Sachs,L., Kimchi,A. and Oren,M. (1991) Wild-type p53 induces apoptosis of myeloid leukeamic cells that is inhibited by interleukin-6. *Nature*, **352**, 345–347.
Zawel,L., Kumar,K.P. and Reinberg,D. (1995) Recycling of the general transcription factors during RNA polymerase II transcription. *Genes Dev.*, **9**, 1479–1490.

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