Direct involvement of p53 in the base excision repair pathway of the DNA repair machinery

Hagai Offer[a], Roland Wolkowicz[a], Devorah Matas[a], Sara Blumenstein[b], Zvi Livneh[b], Varda Rotter[a,*]

[a] *Department of Molecular Cell Biology, The Weizmann Institute of Science, 76100 Rehovot, Israel*
[b] *Department of Biological Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel*

Received 15 March 1999

Abstract The p53 tumor suppressor that plays a central role in the cellular response to genotoxic stress was suggested to be associated with the DNA repair machinery which mostly involves nucleotide excision repair (NER). In the present study we show for the first time that p53 is also directly involved in base excision repair (BER). These experiments were performed with p53 temperature-sensitive (ts) mutants that were previously studied in in vivo experimental models. We report here that p53 ts mutants can also acquire wild-type activity under in vitro conditions. Using ts mutants of murine and human origin, it was observed that cell extracts overexpressing p53 exhibited an augmented BER activity measured in an in vitro assay. Depletion of p53 from the nuclear extracts abolished this enhanced activity. Together, this suggests that p53 is involved in more than one DNA repair pathway.

Key words: p53; Temperature-sensitive mutant; DNA repair; Base excision repair

1. Introduction

The p53 tumor suppressor gene plays a central role in the cellular response to genotoxic stress [1–5]. Activated wild-type p53 may lead to the onset of apoptosis [4,6–11] or alternatively turn on the DNA repair machinery [2–5,12] leading to the completion of the cell cycle.

It is well accepted that p53 is essential for the maintenance of the normal genome integrity and stability [1–3,13–15]. Data accumulated from several laboratories have suggested that p53 plays a role in mediating the response to DNA damage, by orchestrating both G_1 growth arrest and induction of apoptosis. Levels of p53 rapidly increase upon DNA damage, mainly through the stabilization of the p53 protein [1–4,15]. In general, it is believed that induction of p53-dependent cell growth arrest [16,17] is expected to permit DNA repair. However, when damage is irreparable and the DNA repair machinery fails, the multi-step apoptotic process is induced [18–20].

The involvement of wild-type p53 in DNA repair has previously been suggested. Indeed, p53 was shown to specifically transactivate the GADD-45 gene [21]. Furthermore, p53 was found to form complexes with proliferating cell nuclear antigen (PCNA) or with replication protein A (RPA) which are directly involved in DNA replication and repair [21–23]. p53 was also shown to form a complex with XPB (ERCC3) and RPA [24,25], both part of the multifactor complex involved in nucleotide excision repair. p53 was suggested to modulate the nucleotide excision repair (NER) pathway [12,26–30], where it was shown to be associated with DNA excision repair mediated by polymerase δ or ε [31]. Recently, it was found that the C-terminus of wild-type p53 may directly bind single-stranded DNA that catalyzes renaturation and annealing of complementary DNA strands [32]. Wild-type p53 protein, but not mutant p53, exhibits 3′-to-5′ exonuclease activity [33–35].

The DNA repair-related activity of p53 was shown to be controlled by the C-terminus. This part of the molecule was shown to bind XPB, an analog of the yeast RAD3, and XPD, constituents of transcription factor IIH (TFIIH) and CSB, the strand-specific DNA repair enzyme [30], to interact with the human Rad51 [36,37], RPA [21] and ERCC3 [30,38]. The C-terminal domain of the human p53 molecule binds non-specific single-stranded [27,39,40] and mismatched DNA [28]. This domain of the murine regularly spliced p53 form (p53RS) also contains a DNA helicase activity [28,29,39,41], characteristic of many DNA repair proteins.

DNA repair is a complex process involving several pathways designed to address the various types of damaged DNA encountered by cells following both external and internal signals [42]. Base excision repair (BER) and NER are two major DNA repair mechanisms [42–45]. NER is involved mainly in the removal of the major UV-induced photoproducts caused by sunlight and other bulky DNA lesions [42–44]. BER acts continuously on both spontaneous and induced DNA damage caused by hydrolysis, oxygen free radicals, and simple alkylating agents [45,46]. BER is initiated by the hydrolysis of the *N*-glycosylic bond linking a modified base to the deoxyribose, which excises the base residue in the free form. As a result of glycosylase activity, apurinic or apyrimidinic (AP) sites are generated. The removal of AP sites is initiated by a second class of BER enzymes, the apurinic/apyrimidinic endonucleases. AP endonucleases produce incisions at the AP sites which are further excised by DNA deoxyribophosphodiesterase. The resulting single nucleotide gap is filled by repair synthesis and DNA repair is completed by DNA ligase [42,45].

The association of p53 with the DNA repair machinery was mostly observed in model systems in which the NER pathways were measured [12,21,22,23,26–30]. To further elucidate the molecular mechanism which underlies the involvement of p53 in DNA repair, we focused our research on evaluating the activity of the p53 protein in the BER pathway. The observation that this is a predominant pathway essential for the maintenance of DNA integrity makes BER an attractive model for getting further insight into the possible role of p53 in DNA repair. In our study, we took advantage of our novel obser-

*Corresponding author. Fax: (972) (8) 9465265.
E-mail: lcrotter@wiccmail.weizmann.ac.il

PII: S0014-5793(99)00505-0

vation that temperature-sensitive (ts) mutant p53 proteins of murine and human origin from cells grown at the non-permissive temperature gained wild-type activity when shifted to the permissive temperature in vitro. We found that nuclear extracts containing active p53 exhibited higher DNA repair synthesis compared to those without p53. Furthermore, exhaustion of p53 by anti-p53 monoclonal antibodies abolished the p53-dependent DNA repair synthesis. Interestingly, at the non-permissive temperature, extracts containing mutant p53 consistently exhibited lower DNA repair synthesis than p53 null nuclear cell extracts.

2. Materials and methods

2.1. Construction of DNA molecules containing AP sites

The plasmids pSP65 (3 kb) and pSP65-8-3 (4.5 kb) were purified using the Qiagen kit and further purified on a CsCl gradient. Depurination of pSP65 was carried out by heating 11 μg DNA in 40 μl buffer containing 0.01 M sodium citrate and 0.1 M KCl (pH 5.0) at 70°C for 45'. The depurinated DNA was neutralized by the addition of 10 μl of 0.2 M Tris-HCl (pH 7.8). pSP65 served as the AP+ plasmid which contains the AP sites while pSP65-8-3, AP−, served as control.

2.2. DNA repair assay

Cell line M1/2, a p53 non-producer myeloid cell line and derived clones expressing the murine p53 135val ts mutant [47], established previously were used in our experiments [48] as well as novel clones expressing the human 143 Val→Ala, also known as temperature-sensitive mutations [49], were used. The M1/2 clones expressing the human ts143 p53 mutant were generated by infecting the M1/2 parental cells with p53 retroviral stocks obtained from 293GP packaging cell line [50], transfected with the corresponding cDNA construct. Nuclear extracts were prepared by a modified protocol according to that described before [51]. Briefly, 10^6–10^7 cells were washed twice with cold PBS, cell pellets were resuspended in 400 μl buffer A (10 mM HEPES-KOH pH 7.9; 1.5 mM $MgCl_2$; 10 mM KCl; 0.5 mM DTT; 0.2 mM PMSF; 10 μ/ml leupeptin; 1 μg/ml pepstatin and 10 μg/ml aprotinin) by gentle pipetting. After 15' incubation on ice, 25 μl of 10% NP-40 was added and vortexing was performed vigorously for 10'. After centrifugation, cell pellets were resuspended in 150–300 μl of buffer C (20 mM HEPES-KOH pH 7.9; 25% glycerol; 420 mM NaCl; 1.5 mM $MgCl_2$; 0.2 mM EDTA; 0.5 mM DTT; 0.2 mM PMSF; 10 μg/ml leupeptin; 1 μg/ml pepstatin and 10 μg/ml aprotinin). Tubes were transferred to a rotating platform for 15' at 4°C. After centrifugation the supernatant was kept at −70°C.

Extracts were analyzed for protein concentration (Bradford assay). The DNA repair assay was carried out in 25 μl containing 40 mM Tris pH 7.6, 12 mM $MgCl_2$, 1 mM DTT, 0.1 mM dTTP, dATP, dCTP, 0.01 mM dGTP, 3% PEG, 0.3 μg depurinated pSP65 plasmid (treated for 45', as described above), 0.3 μg untreated plasmid, 0.25 μl [α-^{32}P]dGTP, 30 mM KCl, and 0.5–1.5 μg of nuclear extracts.

Samples were incubated at 37°C or 32°C for 15'. 5 μl of stop buffer (120 mM EDTA, 1.2% SDS) was added to each sample and incubated for 10' at 60°C. 20 μg of proteinase K was added and incubated at 37°C for 1 h. Then 170 μl TE was added, phenol/chloroform extracted and ethanol precipitated. DNA was linearized with *Bam*HI and fractionated through a 0.7% agarose gel in TBE. Gels were UV photographed, dried and analyzed by phosphorimaging. Specific DNA repair synthesis was calculated by dividing net counts in the phosphorimager (taken as PSL−background:AP− in each individual lane) by the DNA content assessed by UV incorporation. The final numbers of DNA repair synthesis (%) are the ratio of an average of duplicates in each sample as a percentage of that in M1/2 cell extract used in each experiment. Standard deviation was calculated in all experiments.

2.3. DNA mobility shift assay

The DNA mobility shift mobility assay was performed as previously described [52]. Briefly, 10–20 fmol of radiolabeled DNA was mixed with 5 μg of nuclear extract. 1 μl of PAb-421 ascitic fluid, 2 μg (2 μl) of poly dI-dC and 10 μl of buffer (25 mM Tris-HCl, 100 mM KCl, 6.25 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT and 10% glycerol) were added. The reactions were incubated for 30' at 32°C or 37°C, loaded on a 4% polyacrylamide gel and electrophoresed.

2.4. Monoclonal antibodies

Anti-p53 monoclonal antibodies PAb-421 [53] and PAb-419 anti-LTAg of SV40, used as a negative control, were obtained either from supernatants of growing hybridoma cell lines or from ascitic fluids of hybridoma-bearing syngeneic mice.

2.5. p53 protein analysis by FACS

Cells were washed in PBS and fixed for 1.5 h at 4°C in 70% methanol at a concentration of $0.5–2\times10^6$ cells/ml. Fixed cells were incubated for 30' on ice with 1 μg of anti-p53 monoclonal antibody PAb-421. Cells were washed twice with PBS containing 0.5% w/v BSA (fraction V) and 0.02% w/v sodium azide (PBA solution), and incubated for 30' on ice with a second donkey anti-mouse or rabbit anti-mouse IgG (H+L chains) FITC-conjugated antibody (Jackson Immunoresearch) diluted 1:50 in 50 μl PBA solution per sample.

2.6. Depletion of p53 protein

Two μg of nuclear cell extracts was mixed with 5 μl of 50% Sepharose-protein A conjugated with anti-p53 monoclonal antibodies (PAb-421) or non-conjugated beads or no beads at all, in total volume of 20 μl in buffer C (see Section 2.2). Mixtures were incubated at 4°C for 1 h on a rocking tray. Finally, mixtures were centrifuged and DNA repair synthesis was determined in supernatants.

3. Results

3.1. Augmentation of BER activity by p53 at the permissive temperature

The in vitro DNA repair assay that we adopted [54,55] consisted of two different sized plasmids: AP+ (3 kb), containing AP sites, and AP− (4.5 kb), lacking AP sites, incubated together with a nuclear cell extract and radioactively labeled nucleotide ([α-^{32}P]dGTP). Upon completion of the reaction, the two plasmids were linearized, separated through an agarose gel and the levels of radioactivity, reflecting DNA repair synthesis, were measured by phosphorimaging.

In order to demonstrate the involvement of p53 in DNA repair, it was critical to obtain p53 protein in the active wild-type conformation and at sufficient levels to demonstrate specific activity in an in vitro DNA repair assay. The fact that wild-type p53 is a cell cycle control protein, expressed at very low levels in normal cells, makes it difficult to use nuclear extracts obtained from normal growing cells as a suitable source for that purpose. On the other hand, overexpression of wild-type p53 leads to massive cell death via apoptosis. For that reason, we studied DNA repair activity in cell lines expressing the well characterized p53*val*135 ts mutant [47]. At the permissive temperature (32°C) these cells express the active p53 form, whereas cells grown at the non-permissive temperature (37°C) express the mutant p53 form.

It was previously shown that various ts proteins may gain an active conformation following shift to permissive temperature under in vitro conditions [56–60]. In order to examine whether this is the case for p53, we measured the DNA repair synthesis of nuclear extracts obtained from cells grown at 37°C, but maintained in vitro for the DNA repair reaction at 32°C, to permit the acquisition of the active form. Fig. 1 demonstrates that the DNA repair synthesis mediated by p53ts61, a p53 producer cell line generated by infection of the parental M1/2 cell line with the retroviral vector containing the p53*val*135 ts inducible mutant [48], was significantly enhanced. Fig. 1A depicts the actual amount of DNA used in

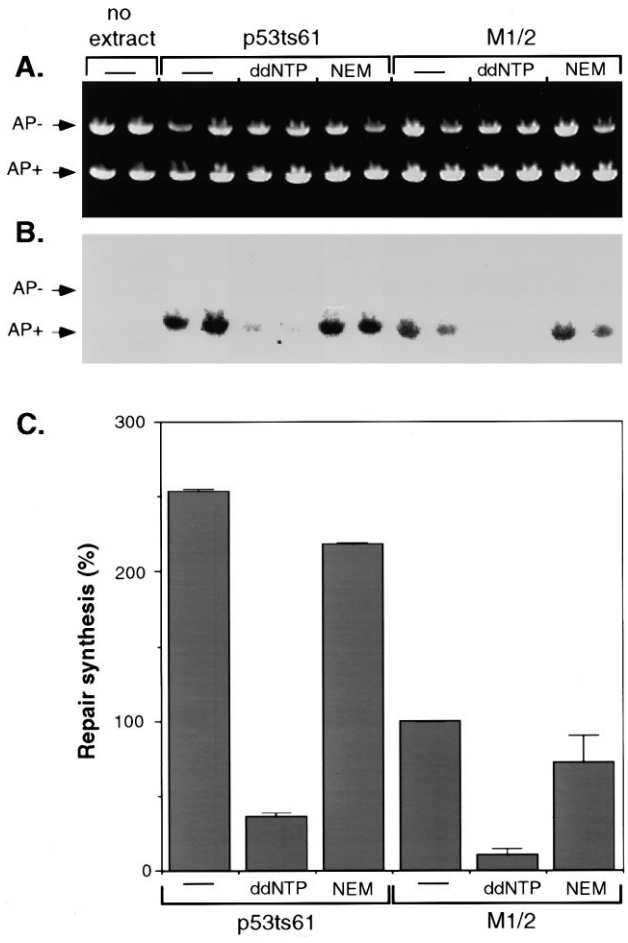

Fig. 1. p53 stimulates DNA base excision repair. Duplicate samples of linearized AP+ and AP− plasmids of the various reactions were separated by gel electrophoresis and exposed to UV (A) or analyzed for radioactivity by phosphorimaging (B). Repair synthesis (%) is calculated as described in Section 2 (C). Non-treated extracts (−), dideoxy-NTPs (ddNTP) and *N*-ethylmaleimide (NEM).

each reaction, while Fig. 1B represents the radioactive signal incorporated into the individual plasmids. Fig. 1C illustrates the relative DNA repair synthesis estimated as the ratio of radioactivity detected in the individual samples and that of M1/2 nuclear cell extract. The basal activity observed in the M1/2 cell extract was significantly enhanced by p53 (compare M1/2 with p53ts61 in Fig. 1C). A twofold increase in the DNA repair synthesis was consistently observed, thus suggesting that this increment is a result of p53 expression.

The model system that we were utilizing consists of highly proliferating cells. It was therefore essential to confirm that uptake of radioactive nucleotides is attributed to DNA repair synthesis rather than to DNA replication. The observation that activity was detected mainly with the AP+ plasmid and not with the AP− plasmid strongly suggests that the measured increment is attributed solely to DNA repair. It should be added that our experiments were performed at the range where repair synthesis correlated linearly with the number of AP sites (data not shown). This was further substantiated by the fact that the reactions were performed in the absence of ATP or any other energy source essential for DNA replication. Furthermore, we found that addition of ddNTPs, which blocks BER via inhibition of DNA polymerase β, reduced the specific radioactive incorporation (Fig. 1), while *N*-ethylmaleimide (NEM) (an inhibitor of all polymerases except β) [42,61] barely affected incorporation (see Fig. 1). In addition, incorporation was observed with radiolabeled dGTP but not with dCTP, typical for very short patch repair AP sites, generated under the experimental conditions, via BER (data not shown). This suggests that we are indeed measuring the BER pathway in this assay.

3.2. Kinetics of p53-dependent and -independent BER activity

To further establish the conclusion that enhanced DNA repair synthesis is a direct result of wild-type p53 activity gained under in vitro conditions, we compared the kinetics of DNA repair synthesis with nuclear cell extracts of M1/2 and p53ts61 cells grown at 37°C but maintained in vitro at either the permissive temperature (32°C) or the non-permissive temperature (37°C). As seen in Fig. 2A, a significant increase in DNA repair synthesis was observed only at 32°C. At 37°C, the kinetics of the DNA repair synthesis mediated by nuclear cell extracts of p53ts61 expressing mutant p53 was significantly lower than that induced by M1/2 (Fig. 2B). The observation that mutant p53 did not induce any DNA repair synthesis, but rather consistently seemed to reduce it, suggests that mutant p53 may act by a negative transdominant mechanism. It should be noted that BER activity promoted by extracts from M1/2 was higher at 37°C than at 32°C. The above results support the conclusion that although the cells were grown at 37°C, where they express the mutant conformation, when nuclear cell extracts are maintained at 32°C in vitro, p53 acquires the wild-type activity.

3.3. Acquisition of wild-type p53 DNA binding activity

To confirm the observation that the p53*val*135 ts mutant acquires its wild-type activity under in vitro conditions, we measured its specific DNA binding activity, a hallmark of the wild-type p53 protein [52,62,63]. Nuclear cell extracts obtained from cells grown at 37°C were mixed with radioactively labeled DNA oligonucleotide consisting of p53 responsive element at either 37°C or 32°C, in the presence or absence of anti-p53 monoclonal antibody PAb-421, and subjected to electromobility shift assay. As can be seen in Fig. 2C, a specific band shift with p53ts61 was observed only at 32°C. No band was evident at the non-permissive temperature (37°C). As expected, nuclear extracts of PLXSN, a cell line generated by infection of the parental M1/2 cell line with the empty retroviral vector, did not exhibit any specific supershifted band at either temperature. Recombinant wild-type p53 exhibited similar band shift patterns at both temperatures. Judged by these observations, we conclude that induction of wild-type p53 activity mediated by the p53*val*135 ts mutant may occur at the permissive temperature (32°C) in an in vitro cell-free system.

3.4. BER activity mediated by various p53 producer clones

To establish the correlation between p53 expression and DNA repair synthesis, p53*val*135 ts clones expressing variable levels of the p53 protein were compared. M1/2 and PLXSN represent p53 null controls, whereas clones p53ts74, p53ts75, p53ts53, p53ts64, p53ts61, and p53ts63 express increasing levels of p53 [48]. p53 protein levels, estimated by FACS analysis, using the PAb-421 anti-p53 monoclonal antibody, are presented in Fig. 3B. DNA repair synthesis induced by nu-

clear extracts obtained from the various p53 producer clones was compared. As can be seen in Fig. 3A, cell lines exhibiting various levels of p53 showed corresponding DNA repair synthesis. Again, p53-dependent DNA repair synthesis was restricted to 32°C.

3.5. BER activity mediated by the human p53 ts mutant

In the above experiments we used the well characterized murine p53 ts mutant. In order to exclude the possibility that our findings are restricted to the murine cDNA clone, we examined the activity of a human p53 ts mutant in our assay. To that end, we generated M1/2 p53 producer clones which express various levels of human p53 ts protein encoded by the p53*val*143 ts cDNA. We selected for our experiments two representative clones which express different levels of the human p53 protein. As shown in Fig. 4A, clone p53ts103 expresses lower levels of p53 protein than clone p53ts106. PLXSN is a parental derived clone generated by infection with the empty retrovirus. Nuclear extracts obtained from these clones were subjected to the DNA repair assay in vitro at the permissive temperature. As shown in Fig. 4B, repair synthesis mediated by cell extracts obtained from the human

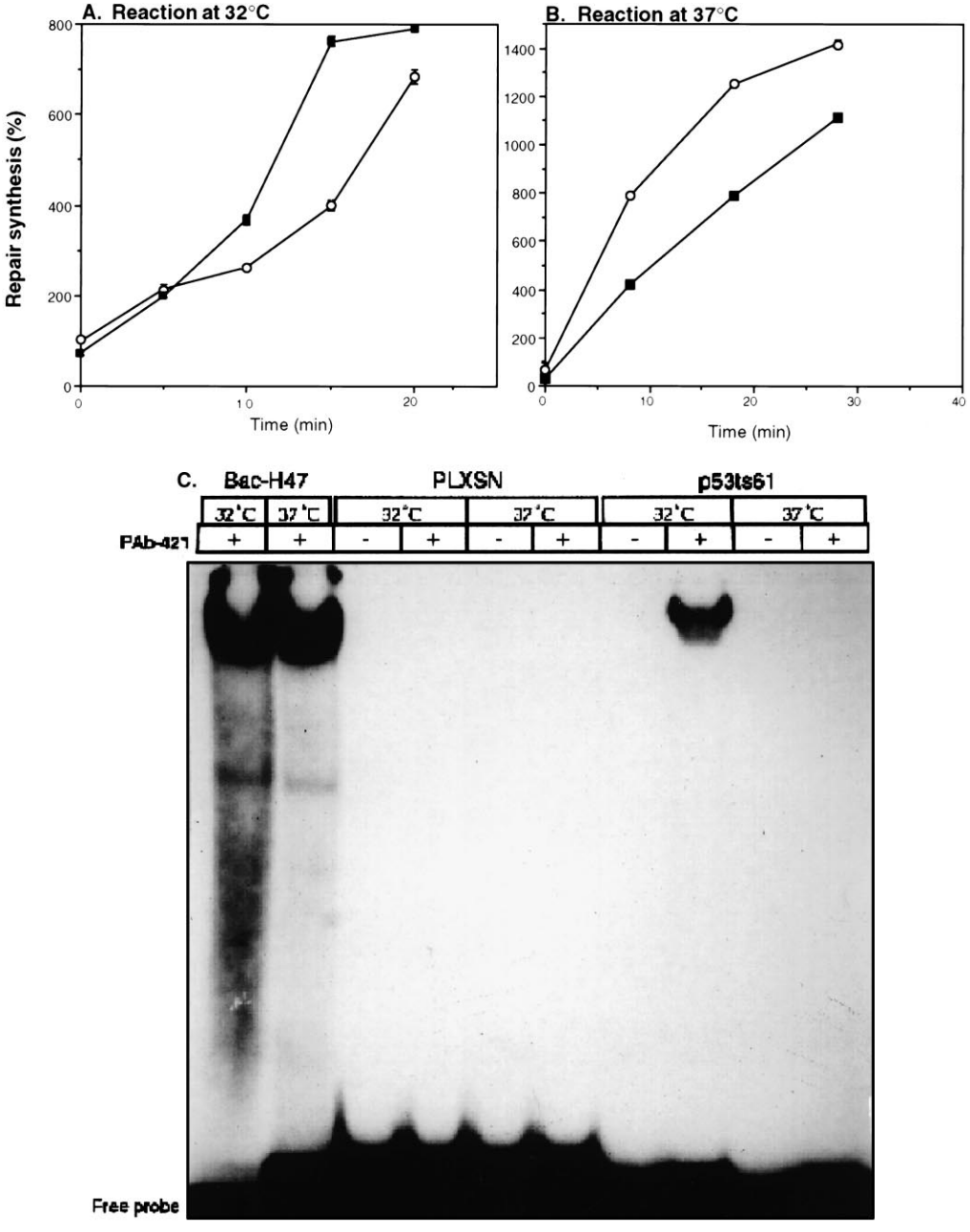

Fig. 2. Comparison between DNA repair synthesis mediated by p53 ts mutant at the permissive and the non-permissive temperatures. Time course of the relative DNA repair synthesis (%) at 32°C (A) and 37°C (B). Each point represents a duplicate; p53ts61 (■) and M1/2 clone (○) cell extracts. DNA binding activity of p53 at both temperatures (C). DNA mobility band shift analysis of recombinant wild-type p53 protein (Bac-H47), nuclear cell extracts of p53 non-producer PLXSN and p53ts61 cell extracts in the presence (+) or the absence (−) of PAb-421 at either 32°C or 37°C.

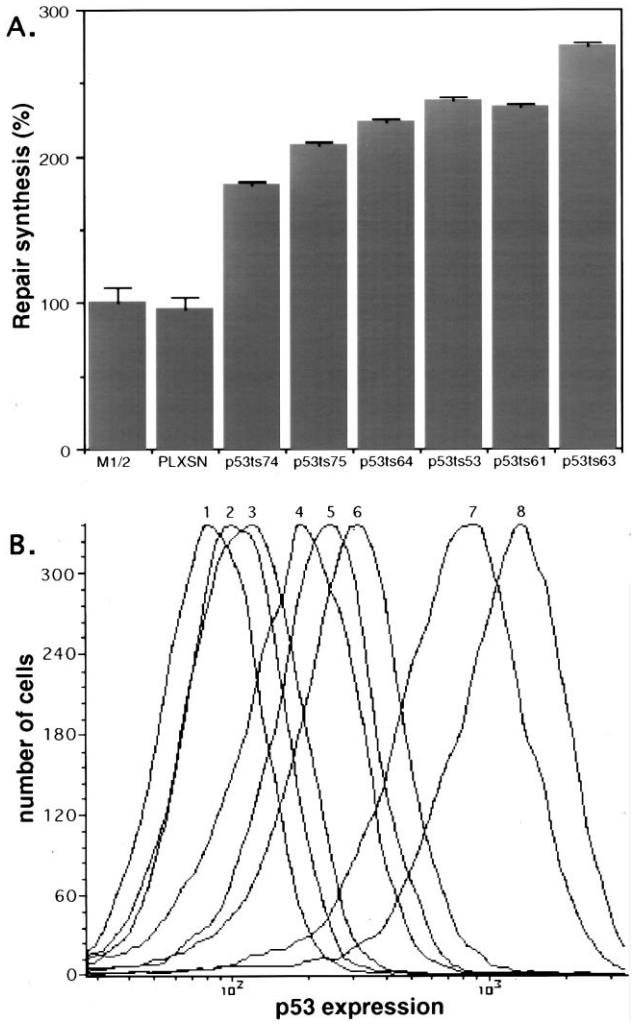

Fig. 3. DNA repair synthesis mediated by nuclear extracts obtained from cell lines expressing various levels of p53 protein. Graphic representation of the relative DNA repair synthesis (%) (A). Evaluation of p53 expression in the various p53 ts clones (M1/2:1; PLXSN:2; p53ts74:3; p53ts75:4; p53ts53:5; p53ts64:6; p53ts61:7; p53ts63:8) by FACS analysis; counts (number of cells) versus the level of fluorescent FITC (p53 expression)(B).

ts producer cells exhibited a significantly higher repair synthesis. Furthermore, clone p53ts106 seems to exhibit higher DNA repair synthesis than that induced by nuclear extracts derived from the p53ts103 clone. These results suggest that our methodology of using an in vitro system for the acquisition of wild-type conformation to p53 ts mutant is not unique to the murine-derived ts clones, but rather represents a more general trend. Furthermore, BER activity seems to correlate with p53 protein levels. It should be noted that fold induction in DNA repair was consistently higher with the human p53 ts mutant than that observed above with the mouse one.

3.6. Reduction in BER activity following p53 depletion

To further correlate p53 and DNA repair we depleted the nuclear extracts of their p53 content and measured whether this would obliterate the p53-dependent DNA repair. The various nuclear cell extracts were treated with anti-p53 monoclonal antibodies and the specific complexes were conjugated onto Sepharose-protein A beads. Fig. 5 shows that the enhanced DNA repair synthesis of p53ts61 was significantly reduced following depletion of the p53 protein (see Fig. 5C: p53ts61+PAb-421). This reduction was not observed when either Sepharose-protein A (p53ts61+protein A) or a control of anti-large T antigen antibody was added (p53ts61+PAb-419). As expected, no significant effect was noted when M1/2 cells were treated similarly. Our attempts to restore DNA repair activity to the depleted extracts by adding recombinant p53 were not successful. This may be due to the fact that the recombinant p53 protein may not be correctly processed to mediate such an activity.

4. Discussion

Genomic stability is central for the maintenance of the normal life of the cell. Wild-type p53 plays a key physiological role in these pathways [1–3,13–15]. External and internal stress signals were shown to activate the p53 that may induce DNA repair leading to the completion of the cell cycle, or alternatively, apoptosis and cell differentiation leading to exit from the cell cycle. Although several models have been proposed for the function of the activated p53, it is still unclear what determines the onset of the specific p53-dependent pathway in a given cell. Most of the reports are focused on the analysis of the role of p53 in apoptosis. However, less has been reported on the nature of its involvement in DNA repair. This might be due to the fact that in most in vivo inducible p53 experimental models, onset of p53 expression leads to an immediate apoptotic response which could have overridden the possible induction and/or detection of the DNA repair pathways. Such a scenario is supported by a recent study showing that cellular stress response to UV irradiation depends on UV dose. p53-dependent repair occurs following exposure to low UV doses while apoptosis follows exposure to high doses of UV [64]. It is possible that in all p53 inducible experimental systems p53 protein levels produced are comparable to amounts that already induce apoptosis and thus do not permit the detection of DNA repair, which requires moderate levels of the protein.

Several molecular mechanisms by which p53 functions in apoptosis have been described (for a review see [65]). Activated wild-type p53 was shown to directly transactivate apoptosis-related genes by interaction with the p53 responsive sequences found in promoter genes such the Bax gene, a positive apoptosis-related gene [66]. The CD95/Fas/Apo1, a member of the cell surface death-induced receptor family, is directly induced by wild-type p53 expression [67,68]. In this case, a p53 responsive element was found in the first intron of the CD95 gene [69]. Recently, it was suggested that the p53-CD95 association may be controlled by yet another mechanism, where p53 was suggested to stimulate the trafficking of the CD95/Fas death receptor from the Golgi system to the cell surface [70]. Another interesting mode of interaction of wild-type p53 with members of apoptosis-related genes consists of a direct protein-protein interaction. For example, the caspases, a family of cysteine aspartate proteases whose function is central to the apoptotic process, were found to complex with wild-type p53 protein [71].

Much less is known about the molecular mechanism that underlies the nature of wild-type p53 involvement in the DNA repair machinery. Several studies indicated that the involvement of p53 in NER is associated with protein complex for-

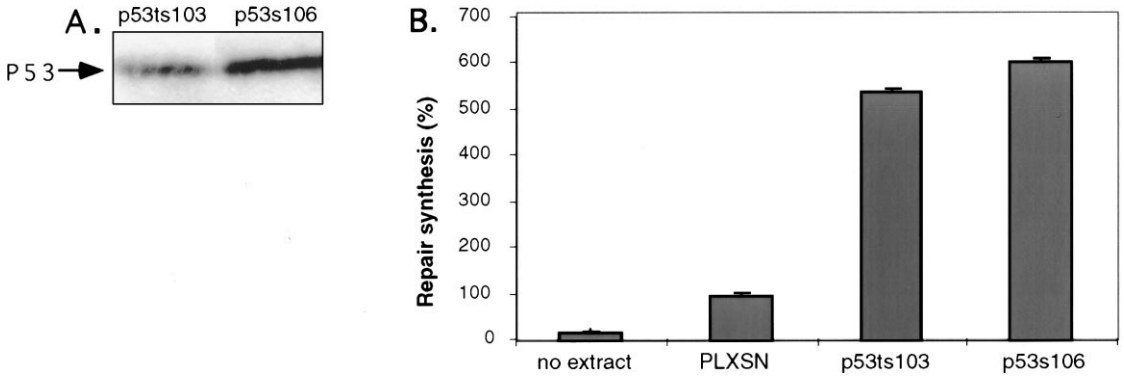

Fig. 4. DNA repair synthesis mediated by nuclear extracts obtained from cell lines expressing the human p53 ts mutant at the permissive temperature. Western blot analysis of PLXSN, p53 non-producer, and p53ts103; p53ts106, human-derived p53 producer clones (A). Graphic representation of the relative DNA repair synthesis (%) mediated by the different p53 ts human expressing clones (B).

mation through its C-terminus with XPD, XPB and CSB, NER-specific proteins [22,24,30,72]. Li-Fraumeni-derived fibroblasts homozygous for p53 were found to be deficient in the removal of UV-induced dimers but still proficient in the transcription-coupled repair pathway [24]. Recently, it was found that expression of p53 resulted in the recovery of normal levels of cyclobutane pyrimidine dimers and 6-4 photoproduct in genomic DNA alters the transcription-coupled repair of the cyclobutane pyrimidine dimers, thus further supporting the conclusion that wild-type p53 is central to NER activity in human fibroblasts [12].

As in apoptosis, wild-type p53 was also suggested to transinactivate DNA repair-associated genes such as GADD-45 [21]. Furthermore, expression of the DNA repair protein O^6-methylguanine-DNA methyltransferase induced by ionizing irradiation was shown to be directly facilitated by transcriptional activation mediated by wild-type p53 protein [73].

Another link between p53 and DNA repair is manifested by the fact that wild-type p53 was found to be directly phosphorylated by the ATM kinase [74–76], a gene product of the ATM gene that is defective in patients with ataxia telangiectasia, a syndrome characterized by pleiotropic phenotypes including extreme sensitivity to ionizing irradiation.

p53 ts mutants were extensively used in in vivo cellular models. In all these studies it clearly appeared that temperature shifting of cells armed the p53 molecule with the protein conformation to induce wild-type p53-dependent cell growth arrest and apoptosis [6,47,49]. Our present data show that acquisition of wild-type activity of both murine and human ts p53 may also occur under in vitro conditions following temperature shift. The p53 ts mutants enhanced DNA repair synthesis as well as specific DNA binding following maintenance in vitro of nuclear extracts at the permissive temperature. Gain of wild-type conformation in vitro was also observed with other ts proteins such as VPg-pU and VPg-pUpU of enterovirus 70, which formed only at 33°C but not at 39°C [59]; ts mutant of SV40 large T-antigen, which replicates DNA only at 39°C [58]; a ts pol2 DNA repair enzyme of *Saccharomyces cerevisiae* [60] and the β-subunit of DNA polymerase III [56], which are active only at the permissive temperature.

Using an in vitro experimental system we found that expression of p53 protein with a wild-type conformation induced BER activity in a cell-free system. It is most likely that under in vitro conditions, using cell extracts, the apoptotic pathways are shut off and thus the involvement of p53 in DNA repair can be seen. Interestingly, the mutant p53 protein not only did not induce BER activity but rather seemed to reduce the p53-independent BER activity measured in our assay. This mutant-associated activity may indicate a negative 'gain of function' resembling our previous observations where mutant p53 exerted a blocking activity in p53-independent apoptotic pathways [48,77].

The plasticity of the p53 molecule has repeatedly been suggested. Wild-type p53 protein may exhibit several conformations. Through allosteric activation, p53 may turn from a 'latent' form to an 'activated' form, not necessarily following

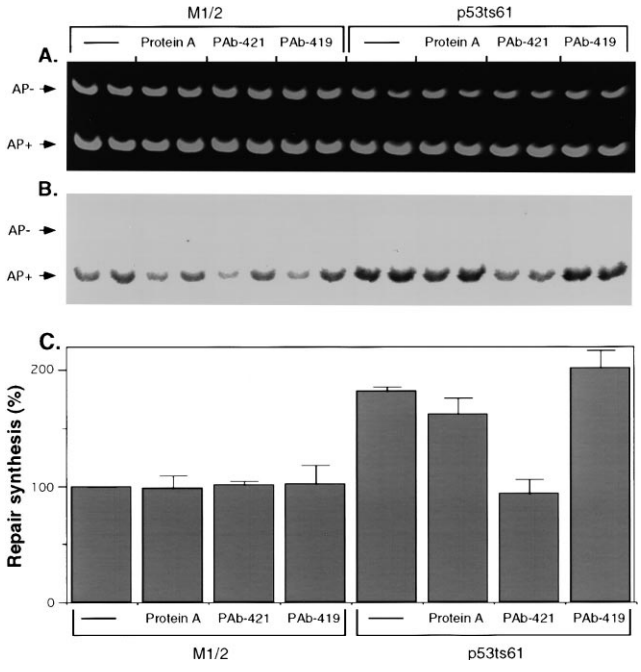

Fig. 5. Reduction in the p53-dependent DNA repair synthesis following treatment with anti-p53 antibodies. Nuclear cell extracts of the M1/2 and p53ts61 cell lines were treated with anti-p53 monoclonal antibodies and the immunocomplexes were removed by absorption onto Sepharose-protein A beads (PAb-421). Controls of cell extracts treated with Sepharose-protein A only (protein A) or a non-relevant antibody anti-large T antigen (PAb-419). Non-treated extracts (−). A, B, C see legend to Fig. 1.

re-folding of 'denatured' p53 [78]. Likewise, a 'tensed' state of p53 with low affinity to DNA has been suggested to turn into a 'relaxed' form with high affinity to DNA [79]. The temperature sensitivity for conformation was suggested to be an intrinsic property of wild-type p53 [57].

In conclusion, our present experiments indicate for the first time that p53 is directly involved in base excision repair. Under in vitro conditions, murine and human ts p53 mutant proteins were found to gain wild-type activity which accelerates base excision repair following shift to the permissive temperature. This suggests that p53 is central to the DNA repair machinery and is involved in both the NER- and the BER-specific pathways.

Acknowledgements: This work was supported in part by grants from the Israel-USA Binational Science Foundation (BSF) and the German Israeli Foundation for Scientific Research and Development (GIF) (V.R.) and the Israel Cancer Research Fund (to V.R. and Z.L.). V.R. is the incumbent of the Norman and Helen Asher Professorial Chair in Cancer Research at the Weizmann Institute. Z.L. is the incumbent of the Maxwell Ellis Professorial Chair in Biomedical Research.

References

[1] Lane, D.P. (1992) Nature 358, 15–16.
[2] Kastan, M.B., Onyekwere, O., Sidransky, D., Vogelstein, B. and Craig, R.W. (1991) Cancer Res. 51, 6304–6311.
[3] Kuerbitz, S.J., Plunkett, B.S., Walsh, W.V. and Kastan, M.B. (1992) Proc. Natl. Acad. Sci. USA 89, 7491–7495.
[4] Lu, X. and Lane, D.P. (1993) Cell 75, 765–778.
[5] Nelson, W.G. and Kastan, M.B. (1994) Mol. Cell. Biol. 14, 1815–1823.
[6] Yonish-Rouach, E., Resnitzky, K., Lotem, J., Sachs, L., Kimchi, A. and Oren, M. (1991) Nature 352, 345–347.
[7] Lowe, S.W., Schmitt, E.M., Smith, S.W., Osborne, B.A. and Jacks, T. (1993) Nature 362, 847–853.
[8] Symonds, H., Krall, L., Remington, L., Saenz-Robles, M., Lowe, S., Jacks, T. and Van Dyke, T. (1994) Cell 78, 703–711.
[9] Clarke, A.R., Purdie, C.A., Harrison, D.J., Morris, R.G., Bird, C.C., Hooper, M.L. and Wyllie, A.H. (1993) Nature 362, 849–852.
[10] Clarke, A.R., Gledhill, S., Hooper, L.M., Bird, C.C. and Wyllie, H.A. (1994) Oncogene 9, 1767–1773.
[11] Strasser, A., Harris, A.W., Jacks, T. and Cory, S. (1994) Cell 79, 329–339.
[12] Ford, J.M. and Hanawalt, P.C. (1997) J. Biol. Chem. 272, 28073–28080.
[13] Maltzman, W. and Czyzyk, L. (1984) Mol. Cell. Biol. 4, 1689–1694.
[14] Kastan, M.B. et al. (1992) Cell 71, 587–597.
[15] Livingstone, L.R., White, A., Sprouse, J., Livanos, E., Jacks, T. and Tlsty, T.D. (1992) Cell 70, 923–935.
[16] Yonish-Rouach, E., Grunwald, D., Wilder, S., Kimchi, A., May, E., Lawrence, J.J., May, P. and Oren, M. (1993) Mol. Cell. Biol. 13, 1415–1423.
[17] Oren, M. (1994) Semin. Cancer Biol. 5, 221–227.
[18] Wyllie, A.H. (1995) Curr. Opin. Genet. Dev. 5, 97–104.
[19] Williams, G.T. (1991) Cell 65, 1097–1098.
[20] Oltvai, Z.N. and Korsmeyer, S.J. (1994) Cell 79, 189–192.
[21] Dutta, A., Ruppert, J.M., Aster, J.C. and Wincherester, E. (1993) Nature 365, 79–82.
[22] Smith, M.L. et al. (1994) Science 266, 1376–1378.
[23] Miller, S.D., Moses, K., Jayaraman, L. and Prives, C. (1997) Mol. Cell. Biol. 17, 2194–2201.
[24] Ford, J.M. and Hanawalt, P.C. (1995) Proc. Natl. Acad. Sci. USA 92, 8876–8890.
[25] Wang, X.W., Forrester, K., Yeh, H., Feitelson, M.A., Gu, J. and Harris, C. (1994) Proc. Natl. Acad. Sci. USA 91, 2230–2234.
[26] Wu, L., Bayle, H., Elenbaas, B., Pavletich, N.P. and Levine, A.J. (1995) Mol. Cell. Biol. 15, 497–504.
[27] Jayaraman, L. and Prives, C. (1995) Cell 81, 1021–1029.
[28] Lee, S., Elenbaas, B., Levine, A. and Griffith, J. (1995) Cell 81, 1013–1020.
[29] Reed, M., Woelker, B., Wang, P., Wang, Y., Anderson, M.E. and Tegtmeyer, P. (1995) Proc. Natl. Acad. Sci. USA 92, 9455–9459.
[30] Wang, X.W. et al. (1995) Nature Genet. 10, 188–195.
[31] Mirzayans, R., Enns, L., Dietrich, K., Barley, R.D.C. and Paterson, M.C. (1996) Carcinogenesis 17, 691–698.
[32] Yokote, H. et al. (1998) Mutat. Res. 409, 147–162.
[33] Mummenbrauer, T., Janus, F., Muller, B., Wiesmuller, L., Deppert, W. and Grosse, F. (1996) Cell 85, 1089–1099.
[34] Janus, F., Albrechtsen, N., Dornreiter, I., Wiesmuller, L., Grosse, F. and Deppert, W. (1999) Cell. Mol. Life Sci. 55, 12–27.
[35] Janus, F., Albrechtsen, N., Knippschild, U., Wiesmüller, L., Grosse, F. and Deppert, W. (1999) Mol. Cell. Biol. 19, 2155–2168.
[36] Sturzbecher, H.-W., Donzelmann, B., Henning, W., Knippschild, U. and Buchhop, S. (1996) EMBO J. 15, 1992–2002.
[37] Buchhop, S., Gibson, M.K., Wang, X.W., Wagner, P., Sturzbecher, H.-W. and Harris, C.C. (1997) Nucleic Acid Res. 25, 3868–3874.
[38] Wang, X.W. et al. (1996) Genes Dev. 10, 1219–1232.
[39] Bakalkin, G. et al. (1995) Nucleic Acids Res. 23, 362–369.
[40] Bakalkin, G. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 413–417.
[41] Brain, R. and Jenkins, J.R. (1994) Oncogene 9, 1775–1780.
[42] Friedberg, E.C., Walker, G.C. and Siede, W. (1995) ASM Press, Washington, DC.
[43] Sancar, A. (1996) Annu. Rev. Biochem. 65, 43–81.
[44] Aboussekhra, A. and Wood, R.D. (1994) Curr. Opin. Genet. Dev. 4, 212–220.
[45] Seeberg, E., Eide, L. and Bjoras, M. (1995) Trends Biochem. Sci. 20, 391–397.
[46] Lindahl, T. (1993) Nature 362, 709–715.
[47] Michalovitz, D., Halevy, O. and Oren, M. (1990) Cell 62, 671–680.
[48] Peled, A., Zipori, D. and Rotter, V. (1996) Cancer Res. 56, 2148–2156.
[49] Zhang, W., Guo, X.Y., Hu, G.Y., Liu, W.B., Shay, J.W. and Deisseroth, A.B. (1994) EMBO J. 13, 2535–2544.
[50] Yee, J.K., Friedmann, T. and Burns, J.C. (1994) Methods Cell Biol. 43, 99–112.
[51] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1474–1489.
[52] Wolkowicz, R., Elkind, N.B., Ronen, D. and Rotter, V. (1995) Oncogene 10, 1167–1174.
[53] Harlow, E., Crawford, L.V., Pim, D.C. and Williamson, N.M. (1981) J. Virol. 39, 861–869.
[54] Wang, Z., Svejstrup, J.Q., Feaver, W.J., Wu, X., Kornberg, R.D. and Friedberg, E.C. (1994) Nature 368, 74–76.
[55] Frosina, G. et al. (1996) J. Biol. Chem. 271, 9573–9578.
[56] Burgers, P.M.J., Kornberg, A. and Sakakibara, Y. (1981) Proc. Natl. Acad. Sci. USA 78, 5391–5395.
[57] Hainaut, P., Butcher, S. and Milner, J. (1995) Br. J. Cancer 71, 227–231.
[58] Ray, S., Anderson, M.E., Loeber, G., McVey, D. and Tegtmeyer, P. (1992) J. Virol. 66, 6509–6516.
[59] Takeda, N., Miyamura, K., Takegami, T. and Yamazaki, S. (1988) Virus Genes 2, 347–355.
[60] Wang, Z., Wu, X. and Friedberg, E.C. (1993) Mol. Cell. Biol. 13, 1051–1058.
[61] Miller, M.R. and Chinault, D.N. (1982) J. Biol. Chem. 257, 46–49.
[62] Bargonetti, J., Friedman, P.N., Kern, S.E., Vogelstein, B. and Prives, C. (1991) Cell 65, 1083–1901.
[63] El-Deiry, W., Kern, S.E., Pietenpol, J.A., Kinzler, K.W. and Vogelstein, B. (1992) Nature Genet. 1, 45–49.
[64] Li, G. and Ho, V.C. (1998) Br. J. Dermatol. 139, 3–10.
[65] Gottlieb, T.M. and Oren, M. (1999) Semin. Cancer Biol. 8, 359–368.
[66] Miyashita, T. and Reed, J.C. (1995) Cell 80, 293–299.
[67] Reinke, V. and Lozano, G. (1997) Oncogene 15, 1527–1534.
[68] Owen-Schaub, L.B. et al. (1995) Mol. Cell. Biol. 15, 3032–3040.
[69] Muller, M. et al. (1998) J. Exp. Med. 188, 2033–2045.

[70] Bennet, M., Macdonald, K., Chan, S.-W., Luzio, J.P., Simari, R. and Weissberg, P. (1998) Science 282, 290–293.
[71] Gottlieb, E., Lindner, S. and Oren, M. (1996) Cell Growth Differ. 7, 301–310.
[72] Smith, M.L., Chen, I.T., Zhan, Q., O'Connor, P.M. and Fornace Jr., A.J. (1995) Oncogene 10, 1053–1059.
[73] Grombacher, T., Eichhorn, U. and Kaina, B. (1998) Oncogene 17, 845–851.
[74] Banin, S. et al. (1998) Science 281, 1674–1677.
[75] Canman, C.E. et al. (1998) Science 281, 1677–1679.
[76] Siliciano, J.D., Canman, C.E., Taya, Y., Sakaguchi, K., Appella, E. and Kastan, M.B. (1997) Genes Dev. 11, 3471–3481.
[77] Li, R. et al. (1998) Oncogene 16, 3269–3277.
[78] Hupp, T.R. and Lane, D.P. (1994) Curr. Biol. 4, 865–875.
[79] Halazonetis, T.D. and Kandil, A.N. (1993) EMBO J. 12, 1021–1028.