Oncogene (2001) 20, 4891–4898

www.nature.com/onc

The C-terminus of mutant p53 is necessary for its ability to interfere with growth arrest or apoptosis

Alex Sigal[1], Devorah Matas[1], Nava Almog[1], Naomi Goldfinger[1] and Varda Rotter*,[1]

[1]*Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, 76100, Israel*

The ability to suppress wild type p53-independent apoptosis may play an important role in the oncogenicity of p53 mutant proteins. However, structural elements necessary for this activity are unknown. Furthermore, it is unclear whether this mutant p53 mediated inhibition is specific to the apoptotic pathway or a more general suppression of the cellular response to stress. We observed that an unmodified C-terminus was required for the suppression of apoptosis by the p53 135(Ala to Val) oncogenic p53 mutant. It was also required for the novel activity of G_2 arrest suppression, the predominant response at low levels of genotoxic stress. These observations are consistent with a model whereby mutant p53 suppressive activity is not specific to the apoptotic pathway, but rather increases the threshold of genotoxic stress needed for a DNA damage response to occur. Furthermore, these observations indicate that it may be possible to selectively kill mutant p53 expressing cells based on the lower sensitivity of their growth arrest response. *Oncogene* (2001) **20,** 4891–4898.

Keywords: mutant p53; gain of function; apoptosis; growth arrest; G_2

The p53 tumor suppressor gene responds to cellular stress such as DNA damage through initiating temporary growth arrest and DNA repair, permanent growth arrest, terminal differentiation, or apoptosis, depending on cell type, the nature of the stress, and its degree (Ko and Prives, 1996; Levine, 1997; Zhao *et al*., 2000). It is mutated in over 50% of human cancers and 74% of these mutations are missense, which result in the expression of full length, albeit mutant, p53 proteins. This fraction of missense mutations is much higher than in other tumor suppresser genes (Hussain and Harris, 1998), and implies that p53 mutant proteins confer some selective advantage in carcinogenesis. About three quarters of missense mutations occur in the DNA binding core domain of p53, where they often occur in one of six hotspots (Hussain and Harris, 1998). Such missense mutants may lose wild type p53 function. In addition, some exhibit oncogenic activity (reviewed in Sigal and Rotter, 2000). This oncogenic activity is partly the result of mutant p53 interference with the function of the remaining wild type p53 protein by a oligomerization domain dependent dominant negative mechanism (Chene, 1998; Kern *et al*., 1992; Milner and Medcalf, 1991; Shaulian *et al*., 1992; Srivastava *et al*., 1993; Unger *et al*., 1993). However, even in the absence of wild type p53, p53 mutant proteins have oncogenic effects. This gain of function includes increased tumorigenicity (Dittmer *et al*., 1993; Hsiao *et al*., 1994; Lanyi *et al*., 1998; Wolf *et al*., 1984), increased mutation frequency following irradiation (Iwamoto *et al*., 1996), genomic instability (Murphy *et al*., 2000), augmented metastatic potential (Crook and Vousden, 1992; Hsiao *et al*., 1994), interference with differentiation (Shaulsky *et al*., 1991), suppression of apoptosis (Blandino *et al*., 1999; Li *et al*., 1998; Murphy *et al*., 2000; Peled *et al*., 1996), and enhanced long term survival (Blandino *et al*., 1999).

The importance of functional regions outside the DNA binding core domain tends to be conserved between wild type and mutant p53. Thus, the N-terminal transactivation domain (Frazier *et al*., 1998; Lin *et al*., 1995), the oligomerization domain (Chene, 1998; Unger *et al*., 1993), and the main nuclear localization signal (Shaulsky *et al*., 1990), exhibit similar functions in both proteins. The role of the extreme C-terminus (approximately the last 30 residues) in the gain of oncogenic function of p53 mutants is less clear. In wild type p53, the extreme C-terminus has a negative regulatory function that keeps non-activated wild type p53 in its cryptic form until its inhibition is removed by post-translational modifications during activation (Anderson *et al*., 1997; Gu and Roeder, 1997; Hupp and Lane, 1994; Hupp *et al*., 1992, 1995; Marston *et al*., 1998; Mundt *et al*., 1997; Wolkowicz *et al*., 1998). Surprisingly therefore, the C terminus of the wild type p53, encoded by the regular spliced p53 mRNA, plays a positive role in the induction of apoptosis (Almog *et al*., 1997, 2000). When the extreme C-terminus of p53 mutants was modified, some oncogenic p53 mutants regained DNA binding to wild type p53 specific elements, and some even regained the ability to transactivate wild type p53 target genes (for review see Selivanova *et al*., 1998). When the extreme C-terminus was truncated in the murine p53 135(Ala to Val) mutant, this protein

*Correspondence: V Rotter, E-mail: varda.rotter@weizmann.ac.il
Received 6 June 2000; revised 26 April 2001; accepted 14 June 2001

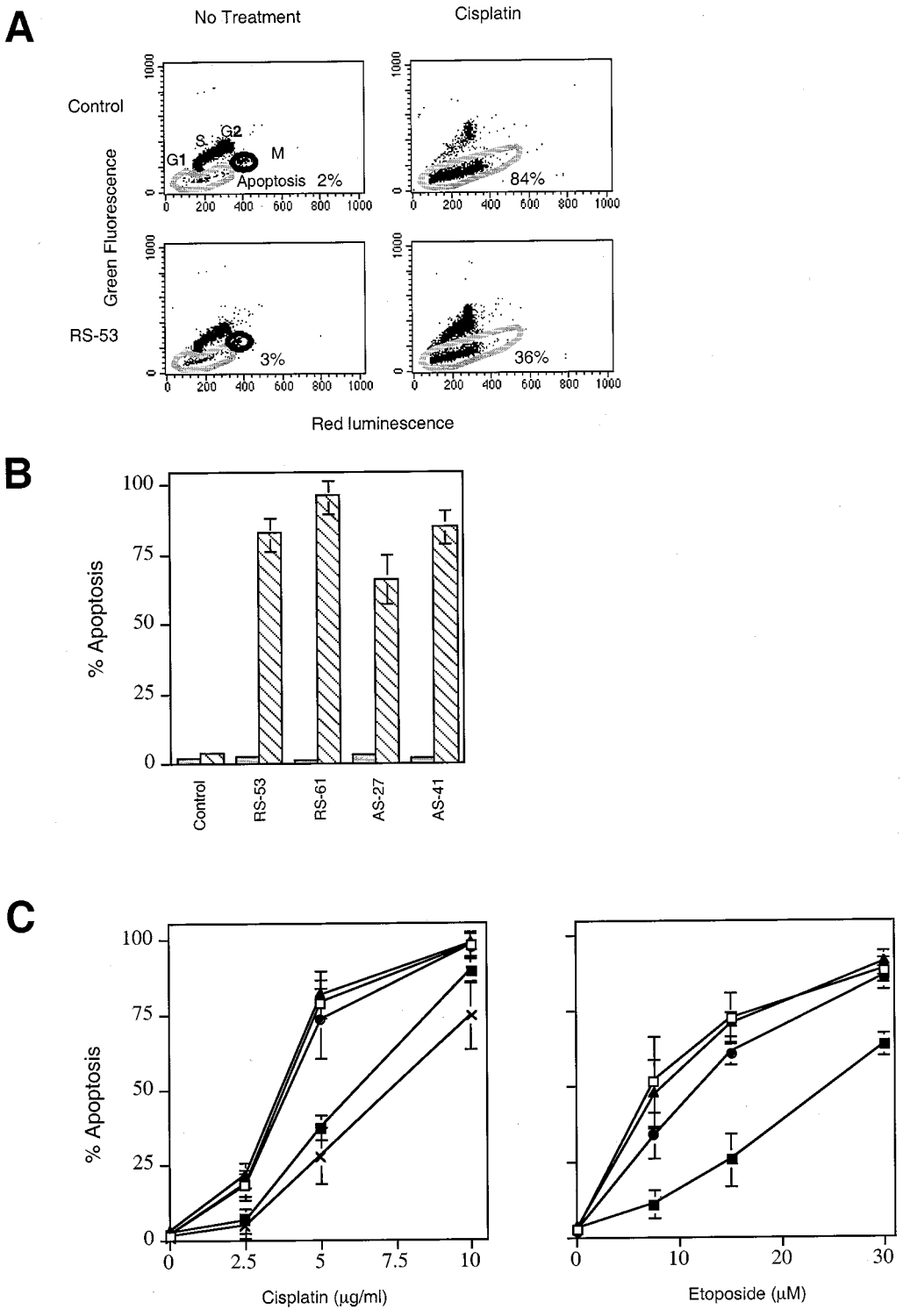

Figure 1 A regular C-terminus is necessary for the p53 Val 135(Ala to Val) mediated suppression of apoptosis induced by genotoxic stress. Clones were generated from murine p53 null M1/2 myeloid cells infected with the vector pLXSN (control clone), and pLXSN with an insert containing either p53 135(Ala to Val) with a regularly spliced (RS) C-terminus (clones RS-53 and RS-61), or alternatively spliced (AS) C-terminus (clones AS-27 and AS-41). Clones were treated with the genotoxic agents cisplatin (Abic) or etoposide (Sigma), and apoptosis measured by the acridine orange (AO) assay for DNA condensation described previously for these cells (Li *et al*., 1998). Briefly, cells were fixed in 80% EtOH, and on the day of the assay incubated with RNase (Sigma) and treated with 0.1 M HCl. After 1 min, the acid denaturation was quenched by the addition of a solution of 90% citric acid, 10% Na_2HPO_4, and 0.06% acridine orange (Molecular Probes). Cells were analysed in a FACSort flow cytometer (Becton Dickinson) with an excitation wavelength of 488 nm. (**a**) shows a representative AO data set after 28 h at 37°C with no treatment or 5 μg/ml cisplatin. Cells with condensed DNA are either apoptotic (circled in gray), or mitotic (circled in black). The percentage of apoptotic cells is indicated in the lower part of each plot. Upon cisplatin treatment, the mitotic population disappeared, and there was a sharp

became reactive with PAb246 and PAb1620, core domain antibodies specific for the wild type conformation (Milner and Medcalf, 1991). Functionally, truncation of the extreme C-terminus resulted in a loss of mutant p53 transactivation potential in the 281(Asp to Gly) mutant (Frazier *et al*., 1998) and truncation of a section that included the extreme C-terminus and the oligomerization domain led to decreased the tumorgenicity of this mutant (Lanyi *et al*., 1998).

p53 core domain mutants were found to interfere with p53-independent apoptosis induced by growth factor removal, serum starvation (Lassus *et al*., 1999), the combination of HBV-X expression and TNF-α (Lee *et al*., 2000), and various types of DNA damage in p53 null M1/2 myeloid and H1299 lung adenocarcinoma cells (Blandino *et al*., 1999; Li *et al*., 1998; Peled *et al*., 1996). p53 mutants also interfered with apoptosis and growth suppression induced by p73 (Di Como *et al*., 1999; Marin *et al*., 2000; Strano *et al*., 2000), which gives rise to the hypothesis that the mechanism behind mutant p53 gain of function is dominant negative inhibition of p53 family members. The oligomerization domains of p53 and p73 have been shown not to associate (Davison *et al*., 1999), though binding does occur through the DNA binding domains (Strano *et al*., 2000).

In this study we characterized a novel activity of the p53 135(Ala to Val) oncogenic mutant, the desensitization of cells to G_2 arrest induction, which required a concentration of etoposide two orders of magnitude lower than apoptosis. We observed that at high levels of genotoxic stress, expression of the p53 135(Ala to Val) mutant downgraded the DNA damage response from apoptosis to G_2 arrest, while it increased the threshold for G_2 arrest at low levels of genotoxic stress. Both effects of p53 135(Ala to Val) were dependent on an intact C-terminus. Thus, cells expressing mutant p53 may be protected against apoptosis at high levels of genotoxic stress. However, it may be possible to kill such cells based on their lack of growth arrest entry at low levels of genotoxic stress, by using agents that only damage cells when they enter mitosis.

The p53 135(Ala to Val) mutant raises the levels of genotoxic stress required to initiate the apoptotic response

To confirm the ability of mutant p53 to interfere with wild type p53-independent apoptosis, we used the p53-null M1/2 murine myeloblastic cell line, retrovirally infected with the p53 135(Ala to Val) mutant (Almog

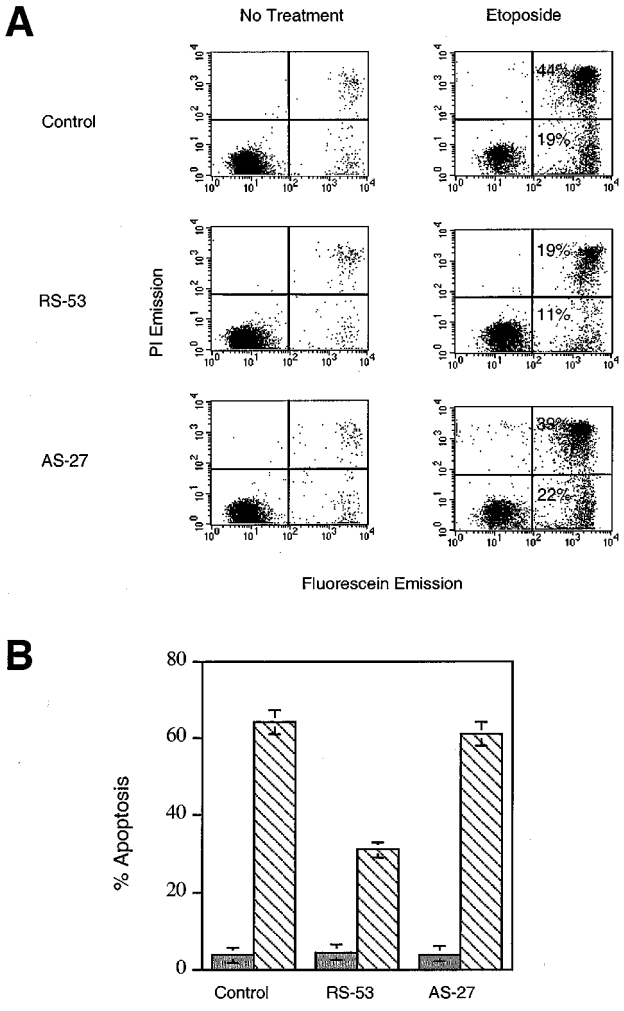

Figure 2 Alternative splicing of the C-terminus abolishes the apoptotic suppression mediated by p53 135(Ala to Val): analysis by Annexin-V. Clones RS-53, AS-27 and control were left untreated or treated with 30 μM etoposide for 24 h at 37°C, and apoptosis was measured by staining unfixed cells with Annexin-V and propidium iodide (PI) as follows: cells were incubated in 10 mM HEPES pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$ containing 2% fluorescein labeled Annexin-V stock solution (Boehringer Mannheim), and PI was pulsed at a concentration of 4 μg/ml immediately before FACS analysis. (**a**) shows a representative data set. The fractions of cells in the lower right (early apoptosis) and upper right (late apoptosis) quadrants after etoposide treatment are shown as a percentage of total cells. (**b**) is a representative experiment with bars representing the sum of early and late apoptosis in untreated (filled bars) and treated (hatched bars) cells. Average$\pm$s.d. of triplicates

increase in the apoptotic population. Cells expressing the p53 135(Ala to Val) mutant with an RS C-terminus were protected from the cisplatin induced apoptosis. To ensure clones expressed functional p53 135(Ala to Val), untreated cells were shifted to 32°C, the permissive temperature for the wild type p53 conformation of this mutant, and apoptosis assayed by AO (**b**). After 36 h at 32°C, clones expressing both AS and RS p53 135(Ala to Val) showed extensive apoptosis while the control clone remained unaffected. Average$\pm$s.d. of three independent experiments. The role of the C-terminus in the suppression of apoptosis mediated by p53 135(Ala to Val) at 37°C, the temperature permissive for the mutant conformation, was examined in (**c**). Clones RS-53 (■), RS-61 (x), AS-27 (▲), AS-41 (●), and the control clone (□) were treated with cisplatin at concentrations of 0, 2.5, 5, and 10 μg/ml for 28 h or etoposide at concentrations of 0, 7.5, 15, and 30 μM for 38 h, and apoptosis assayed by AO. Clones that expressed p53 135(Ala to Val) with a regularly spliced C-terminus had a markedly lower apoptotic profile relative to the control clone, while clones expressing the AS form were similar in their response to the control. Average$\pm$s.d. of triplicates of independent representative experiments

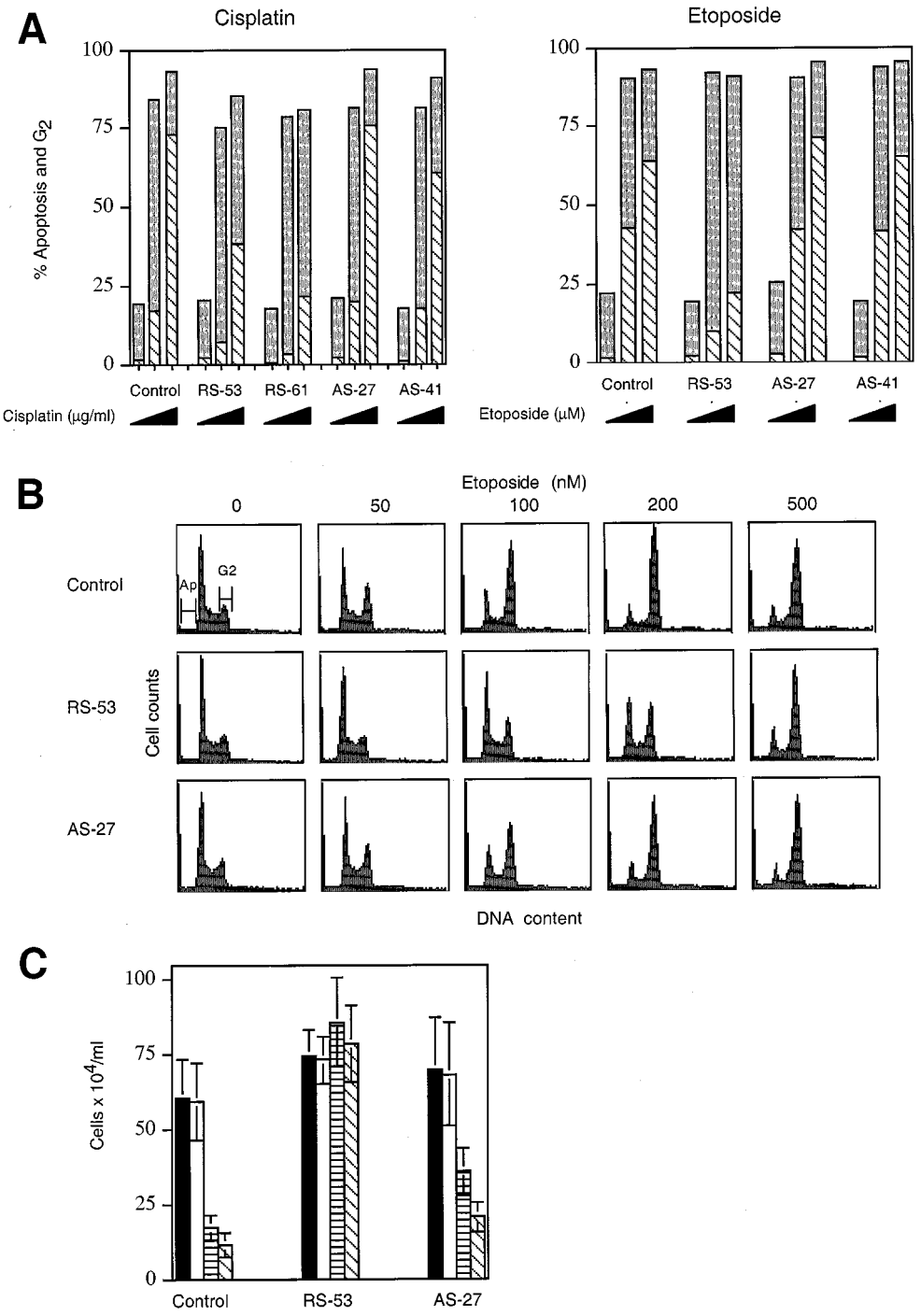

Figure 3 The p53 135(Ala to Val) mutant decreases the sensitivity of genotoxic stress responses. (**a**) apoptotic (hatched bars) and G_2 arrest responses (filled bars) of clones treated at 37°C with 0, 2.5, and 5 μg/ml cisplatin for 28 h or 0. 7.5, and 15 μM etoposide for 38 h, and assayed by AO. Each stacked bar represents the fraction of apoptotic and arrested cells in the same sample. Clones expressing RS p53 135(Ala to Val) undergo G_2 arrest, a response to lower levels of genotoxic stress at the expense of the apoptotic response. A representative experiment. Sensitivity of clones to G_2 arrest inducing levels of etoposide is shown in (**b**). Cells were treated with the concentrations of etoposide shown for 24 h and assayed by propidium iodide (Sigma) for DNA content. The sub G_1 apoptotic (Ap) and G_2 regions were gated as shown in the upper left plot. At these low levels of genotoxic stress after 24 h, G_2 arrest was the primary response and no increase in apoptosis was seen. At 100 nM etoposide, the G_2 arrest was well developed in both the control and AS-27, but not in RS-53. This growth arrest was also reflected in the cell numbers (**c**). Clones were seeded at a density of 5×10^4/ml and left untreated or treated with 100 nM etoposide for 68 h. Cell numbers after 68 h were estimated by counting, and their viability by Tryphan Blue (Sigma) exclusion. Total cells in untreated (filled bars) and treated (striped bars) samples, and viable untreated (unfilled bars) and treated (hatched bars) samples are shown. The control and AS-27 underwent growth arrest after etoposide treatment, while RS-53 did not. Average $\pm$ s.d. of six fields of a representative experiment

et al., 1997). Stable clones were subjected to apoptosis inducing levels of genotoxic stress. We detected apoptosis based on its hallmark of DNA condensation measured by the acridine orange (AO) DNA binding dichromatic stain. After denaturation with weak acid, condensed DNA does not re-aneal and the single stranded DNA is detected by AO as a shift from green to red luminescence (Darzynkiewicz, 1994a,b). Upon treatment with the chemotherapeutic agent cisplatin, which causes DNA damage by intrastrand and interstrand crosslinking, we observed that the control clone infected with the retroviral vector without the mutant p53 coding insert, exhibited more than twice the level of apoptosis relative to the clone that expressed the p53 135(Ala to Val) mutant (Figure 1a). This pattern was also confirmed by the sub-G1 fractions obtained with propidium iodide (data not shown).

Having established the anti-apoptotic gain of function of the p53 135(Ala to Val) mutant, we now asked whether the extreme C-terminus was involved in this effect. As a model for extreme C-terminal modification, we used an alternatively spliced (AS) form of p53, a naturally occurring murine modification that changes the last 26 amino acids of the C-terminus relative to the regularly spliced (RS) form (Arai *et al*., 1986). This modification is similar in its effects in wild type p53 to the truncation of the C-terminus or modification by the binding of C-terminal antibody PAb421 (Wolkowicz *et al*., 1995). To exclude clonal variations, we used two clones expressing AS and RS p53 135(Ala to Val) in several assays. To ensure that the clones were indeed expressing p53 135(Ala to Val), we took advantage of the conformational sensitivity of this mutant to temperature. At 32°C p53 135(Ala to Val) shifts to the wild type p53 conformation and cells expressing it enter apoptosis (Michalovitz *et al*., 1990). Figure 1b shows that after 36 h at 32°C, cells that expressed either the AS or RS forms of p53 135 (Ala to Val) underwent extensive apoptosis, while the control clone did not.

When we examined the level of cisplatin and etoposide induced apoptosis in clonal populations at the temperature permissive for the mutant conformation (37°C), clones AS-27 and AS-41 which expressed AS p53 135(Ala to Val), had a similar apoptotic profile to the control clone and differed markedly from clones RS-53 and RS-61 which expressed the RS p53 135(Ala to Val) mutant (Figure 1c). However, treatments with the highest concentrations of etoposide or cisplatin used resulted in high apoptotic levels in clones expressing RS p53 135(Ala to Val), and this was especially evident with cisplatin treatment at 10 μg/ml. Thus, RS p53 135(Ala to Val) did not completely block apoptosis, but rather increased the threshold of genotoxic stress needed to initiate it.

To confirm that RS p53 135(Ala to Val) mediated suppression of apoptosis while AS p53 135(Ala to Val) did not, we used the Annexin-V assay. The results showed a similar pattern as obtained with AO, where the clone RS-53 exhibited a markedly lower proportion of apoptotic cells relative to the control or AS-27, while AS-27 did not differ from the control in its response (Figure 2a,b).

The p53 135(Ala to Val) raises the threshold of genotoxic stress for the initiation of G_2 arrest

We next examined whether the p53 135(Ala to Val) mutant can interfere with other responses to DNA damage, or whether its interference is specific to the apoptotic pathway. One response distinct from apoptosis seen in this system is G_2 arrest. Cells not undergoing apoptosis were found mostly in G_2 after treatment with cisplatin or etoposide (Figure 3a). Cells that expressed RS p53 135(Ala to Val) had lower apoptosis levels and higher G_2 arrest (Figure 3a). To determine whether p53 135(Ala to Val) caused cells preferentially to enter G_2 arrest, we examined the sensitivity of cells to G_2 entry. We used low etoposide concentrations to obtain G_2 arrest, and propidium iodide staining as a measure of DNA content, where G_2 arrest was indicated by an increase in the number of cells having a DNA content of 4N (Figure 3b). We

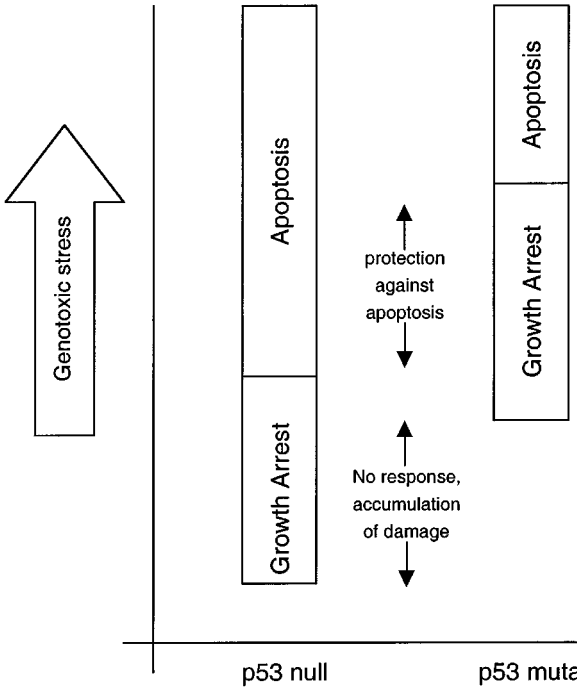

Figure 4 Possible consequences of the decrease of genotoxic stress response sensitivity mediated by mutant p53. Mutant p53 expression causes increased thresholds of DNA damage for the initiation of either the growth arrest or apoptosis responses. Thus, at high levels of DNA damage mutant p53 expressing cells will enter growth arrest instead of apoptosis, and at low levels of DNA damage they will continue cycling instead of entering growth arrest. This predicts that they will continue to accumulate damage while cells without mutant p53 have arrested, initiated repair, and stopped damage accumulation, if the genotoxic agents are cell cycle dependent. If enough DNA damage is accumulated during this window of no response, it is possible that cells with mutant p53 will in the long-term growth arrest or die, while cells without mutant p53 will be less affected

observed that G_2 arrest was the primary response at these low levels of genotoxic stress, as opposed to apoptosis which occurred at drug concentrations two orders of magnitude higher (compare etoposide concentrations in Figure 3b to Figures 1 and 2). At these low levels of genotoxic stress, no significant increase in apoptosis (measured as cells with a sub-G_1 DNA content) was detected relative to untreated cells (Figure 3b). However, a pronounced G_2 arrest was observed. This arrest was reversible and cells that were treated with 100 nM etoposide for 68 h and then washed, were able to re-enter the cell cycle and showed no signs of impaired viability for the 2 weeks we observed them (data not shown). Clones AS-27 and the control showed a pronounced G_2 arrest after treatment with 100 nM etoposide, but RS-53 did not (Figure 3b). These differences gradually disappeared as progressively higher concentrations of etoposide were used (Figure 3b). Clone RS-61 showed a similar pattern to clone RS-53 (data not shown). Thus, expression of the p53 135(Ala to Val) mutant caused a shift from an apoptotic to a growth arrest response, consistent with the cells recognizing apoptosis inducing levels of genotoxic stress as lower, growth arrest inducing levels. At low, growth arrest inducing levels of genotoxic stress, cells expressing p53 135(Ala to Val) did not arrest, indicating that the genotoxic stress signal given was below threshold for initiating the G_2 response in these cells. Significantly, the clone expressing the AS p53 135(Ala to Val) mutant had a very similar response profile to the control (Figure 3b), indicating that the C-terminus is necessary for this mutant p53 activity. To confirm the presence of growth arrest after low etoposide treatment, we seeded clones at a density of 5×10^4/ml, and counted the number of cells present after 68 h of 100 nM etoposide treatment. We observed no significant difference between the number of cells of RS-53 with treatment versus without treatment, while the control clone and AS-27 clearly entered growth arrest after treatment (Figure 3c). The arrest was less pronounced in clone AS-27 relative to the control, and matched the slightly lower G_2 entry of AS-27 at this etoposide concentration relative to control (Figure 3b), indicative of some residual mutant p53 gain of function present in AS p53 135(Ala to Val).

The requirement for a regularly spliced C-terminus for mutant p53 gain of function activity is consistent with a role for this domain in the stabilization of the mutant conformation, previously shown as the appearance of wild type p53 epitopes with C-terminal truncation (Milner and Medcalf, 1991). Based on evidence of the role of the extreme C-terminus as an allosteric negative regulatory domain in wild type p53, it has been proposed that oncogenic core domain mutations stabilize the contact that occurs between the DNA binding domain and the C-terminus in latent p53, so that this allosteric contact cannot be broken by post-translational modifications. Hence, activation of p53 mutant proteins cannot occur by the normal physiological mechanisms (Hupp *et al*., 1993; Selivanova *et al*., 1998). Interestingly, wild type p53, when expressed at low levels, was shown to protect from apoptosis in a growth arrest independent manner. This protection from apoptosis was dependent on the presence of the last 38 amino acids of the C-terminus of human p53 (Lassus *et al*., 1996, 1999), perhaps hinting at some link between wild type p53 physiological activities and mutant p53 gain of function. The same group showed that DD, a p53 fragment with a Δ15-301 deletion that retains the oligomerization and the extreme C-terminus, can protect cells against apoptosis (Lassus *et al*., 1999).

We observed that mutant p53 can interfere with the two discrete outcomes of DNA damage: apoptosis and growth arrest. Such inhibition can either occur on each pathway separately, or it can occur at a common initiation point. The latter possibility is more probable: if the probability of mutant p53 having one gain of function A is P_A, where $0 < P < 1$, then the probability of it having two independent functions B and C is $P_B \times P_C$. Since the probability of one given protein physiologically interacting with another given protein is small, all the P values here are small as well. Therefore, if the P values are not hugely different from each other, then $P_A >>> P_B \times P_C$. It can be argued that mutant p53 retains the wild type p53 ability to bind many targets, but this seems unlikely in the p53 135(Ala to Val) mutant due to the conformational shift.

Candidates for the initiation point of both growth arrest and apoptosis in cells lacking wild type p53 are p53 family members such as p73. p73 has been shown to induce apoptosis in response to DNA damage (Agami *et al*., 1999; Wang, 2000; Yuan *et al*., 1999). Furthermore, p53 mutants have been shown to associate with p73 (Strano *et al*., 2000) and block p73 function (Di Como *et al*., 1999; Marin *et al*., 2000; Strano *et al*., 2000). Since we observed that the C-terminus of the p53 135(Ala to Val) mutant is necessary for its gain of function, an indication of p73 involvement would be to examine whether the ability of mutant p53 to inhibit p73 depends on the mutant p53 C-terminus. In addition, p73 must be shown to mediate the wider spectrum of responses, such as apoptosis resulting from growth factor deprivation (Peled *et al*., 1996) and the combination of HBV-X expression and TNF-α (Lee *et al*., 2000), which mutant p53 has been shown to inhibit. It was shown that the maintenance of G_2 arrest requires 14-3-3 (Chan *et al*., 1999), and it would be interesting to examine whether mutant p53 can interfere with this effect.

Previously, it was observed that co-expression of mutant p53 protein, with wild type p53 protein, deregulates the control of the DNA-damage-dependent G_2 checkpoint (Aloni-Grinstein *et al*., 1995; Delia *et al*., 1997; Goi *et al*., 1997; Powell *et al*., 1995; Schwartz and Rotter, 1998). Data presented here indicates that this may not, or not only, be the results of dominant negative inhibition of wild type p53.

The suppression of apoptosis and G_2 arrest by RS p53 135(Ala to Val) was not a total block, but an increase in the thresholds of genotoxic stress needed to

initiate these responses. We propose that this shift upwards of both thresholds results in two windows being created relative to cells null for p53 (Figure 4). In one, cells that express mutant p53 experience sufficient genotoxic stress to enter growth arrest but not apoptosis, and so are protected from apoptosis and in some cases from further genotoxic stress (see below). A second window occurs where the genotoxic stress is sub-threshold for growth arrest initiation for mutant p53 expressing cells but not p53 null cells. Growth arrest is expected to be protective against damaging agents such as etoposide (a topoisomerase II inhibitor, and therefore damages DNA during replication) and also has been shown to be anti-apoptotic by allowing time for DNA repair before mitosis entry (Chan *et al*., 1999; Zhou and Elledge, 2000).

The increased accumulation of DNA damage in cells expressing mutant p53 in the low genotoxic stress window may lead to several outcomes, depending on whether cells expressing p53 mutants are also more tolerant of genetic aberrations. If they are, then they will exit the growth arrest with more DNA lesions and their tumorgenicity will likely increase. If they have similarly stringent repair requirements as cells not expressing mutant p53 or if the amount of damage is not compatible with further growth, they will in the long term die or growth arrest while cells not expressing mutant p53 may recover. A chemotherapeutic approach can be devised that takes better advantage of the lack of G_2 arrest at low etoposide concentrations. This may involve first arresting cells with low etoposide, and killing the cycling mutant p53 expressing cells with microtubule-active drugs such as paclitaxel or vinblastine. This approach has met with some success in differentially killing cells that do not enter growth arrest due to a lack of wild type p53 or p21, or EGF withdrawal insensitivity (Blagosklonny *et al*., 2000a,b). Therefore, it may be possible to use the window of insensitivity to DNA damage induced growth arrest to selectively target cells expressing oncogenic p53 mutants.

Acknowledgments
A Sigal wishes to thank Ayala Sharp and Eitan Ariel for lessons in flow cytometry. This work was supported in part by grants from the Israel-USA Binational Science Foundation (BSF) the DIP (Deutsch-Israelische Projektkooperation) and the Kadoori Foundation. V Rotter is the incumbent of the Norman and Helen Asher Professorial Chair in Cancer Research at the Weizmann Institute.

References

Agami R, Blandino G, Oren M and Shaul Y. (1999). *Nature,* **399,** 809–813.
Almog N, Goldfinger N and Rotter V. (2000). *Oncogene,* **19,** 3395–3403.
Almog N, Li R, Peled A, Schwartz D, Wolkowicz R, Goldfinger N, Pei H and Rotter V. (1997). *Mol. Cell. Biol.,* **17,** 713–722.
Aloni-Grinstein R, Schwartz D and Rotter V. (1995). *EMBO J.,* **14,** 1392–1401.
Anderson ME, Woelker B, Reed M, Wang P and Tegtmeyer P. (1997). *Mol. Cell. Biol.,* **17,** 6255–6264.
Arai N, Nomura D, Yokota K, Wolf D, Brill E, Shohat O and Rotter V. (1986). *Mol. Cell. Biol.,* **6,** 3232–3239.
Blagosklonny MV, Bishop PC, Robey R, Fojo T and Bates SE. (2000a). *Cancer Res.,* **60,** 3425–3428.
Blagosklonny MV, Robey R, Bates S and Fojo T. (2000b). *J. Clin. Invest.,* **105,** 533–539.
Blandino G, Levine AJ and Oren M. (1999). *Oncogene,* **18,** 477–485.
Chan TA, Hermeking H, Lengauer C, Kinzler KW and Vogelstein B. (1999). *Nature,* **401,** 616–620.
Chene P. (1998). *J. Mol. Biol.,* **281,** 205–209.
Crook T and Vousden KH. (1992). *EMBO J.,* **11,** 3935–3940.
Darzynkiewicz Z. (1994a). *Methods Cell Biol.,* **41,** 427–541.
Darzynkiewicz Z. (1994b). *Cell Biol. Lab. Handb.,* **1,** 261–271.
Davison TS, Vagner C, Kaghad M, Ayed A, Caput D and Arrowsmith CH. (1999). *J. Biol. Chem.,* **274,** 18709–18714.
Delia D, Goi K, Mizutani S, Yamada T, Aiello A, Fontanella E, Lamorte G, Iwata S, Ishioka C, Krajewski S, Reed JC and Pierotti MA. (1997). *Oncogene,* **14,** 2137–2147.
Di Como CJ, Gaiddon C and Prives C. (1999). *Mol. Cell. Biol.,* **19,** 1438–1449.
Dittmer D, Pati S, Zambetti G, Chu S, Teresky A, Moore M, Finlay C and Levine A. (1993). *Nature Genet.,* **4,** 42–46.
Frazier MW, He X, Wang J, Gu Z, Cleveland JL and Zambetti GP. (1998). *Mol. Cell. Biol.,* **18,** 3735–3743.
Goi K, Takagi M, Iwata S, Delia D, Asada M, Donghi R, Tsunematsu Y, Nakazawa S, Yamamoto H, Yokota J, Tamura K, Saeki Y, Utsunomiya J, Takahashi T, Ueda R, Ishioka C, Eguchi M, Kamata N and Mizutani S. (1997). *Cancer Res.,* **57,** 1895–1902.
Gu W and Roeder RG. (1997). *Cell,* **90,** 595–606.
Hsiao M, Low J, Dorn E, Ku D, Pattengale P, Yeargin J and Haas M. (1994). *Am. J. Pathol.,* **145,** 702–714.
Hupp TR and Lane DP. (1994). *Curr. Biol.,* **4,** 865–875.
Hupp TR, Meek DW, Midgley CA and Lane DP. (1992). *Cell,* **71,** 875–886.
Hupp TR, Meek DW, Midgley CA and Lane DP. (1993). *Nucleic Acids Res.,* **21,** 3167–3174.
Hupp TR, Sparks A and Lane DP. (1995). *Cell,* **83,** 237–245.
Hussain SP and Harris CC. (1998). *Cancer Res.,* **58,** 4023–4037.
Iwamoto KS, Mizuno T, Ito T, Tsuyama N, Kyoizumi S and Seyama T. (1996). *Cancer Res.,* **56,** 3862–3865.
Kern SE, Pietenpol JA, Thiagalingam S, Seymor A, Kinzler KW and Vogelstein B. (1992). *Science,* **256,** 827–830.
Ko JL and Prives C. (1996). *Genes Dev.,* **10,** 1054–1072.
Lanyi A, Deb D, Seymour RC, Ludes-Meyers JH, Subler MA and Deb S. (1998). *Oncogene,* **16,** 3169–3176.
Lassus P, Bertrand C, Zugasti O, Chambon JP, Soussi T, Mathieu-Mahul D and Hibner U. (1999). *Oncogene,* **18,** 4699–4709.

Lassus P, Ferlin M, Piette J and Hibner U. (1996). *EMBO J.,* **15,** 4566 – 4573.
Lee YI, Lee S, Das GC, Park US, Park SM and Lee YI. (2000). *Oncogene,* **19,** 3717 – 3726.
Levine JA. (1997). *Cell,* **88,** 323 – 331.
Li R, Sutphin PD, Schwartz D, Matas D, Almog N, Wolkowicz R, Goldfinger N, Pei H, Prokocimer M and Rotter V. (1998). *Oncogene,* **16,** 3269 – 3277.
Lin J, Teresky AK and Levine AJ. (1995). *Oncogene,* **10,** 2387 – 2390.
Marin MC, Jost CA, Brooks LA, Irwin MS, O'Nions J, Tidy JA, James N, McGregor JM, Harwood CA, Yulug IG, Vousden KH, Allday MJ, Gusterson B, Ikawa S, Hinds PW, Crook T and Kaelin WG. (2000). *Nature Genet.,* **25,** 47 – 54.
Marston NJ, Ludwig RL and Vousden KH. (1998). *Oncogene,* **16,** 3132 – 3131.
Michalovitz D, Halevy O and Oren M. (1990). *Cell,* **62,** 671 – 680.
Milner J and Medcalf EA. (1991). *Cell,* **65,** 765 – 774.
Mundt M, Hupp T, Fritsche M, Merkle C, Hansen S, Lane D and Groner B. (1997). *Oncogene,* **15,** 237 – 244.
Murphy KL, Dennis AP and Rosen JM. (2000). *FASEB J.,* **14,** 2291 – 2302.
Peled A, Zipori D and Rotter V. (1996). *Cancer Res.,* **56,** 2148 – 2156.
Powell S, Defrank J, Connell P, Eogan M, Preffer F, Dombkowski D, Tang W and Friend S. (1995). *Cancer Res.,* **55,** 1643 – 1648.
Schwartz D and Rotter V. (1998). *Semin. Cancer Biol.,* **8,** 325 – 336.
Selivanova G, Kawasaki T, Ryabchenko L and Wiman KG. (1998). *Semin. Cancer Biol.,* **8,** 369 – 378.
Shaulian E, Zauberman A, Ginsberg D and Oren M. (1992). *Mol. Cell. Biol.,* **12,** 5581 – 5592.
Shaulsky G, Goldfinger N, Ben-Ze'ev A and Rotter V. (1990). *Mol. Cell. Biol.,* **10,** 6565 – 6577.
Shaulsky G, Goldfinger N and Rotter V. (1991). *Cancer Res.,* **51,** 5232 – 5237.
Sigal A and Rotter V. (2000). *Cancer Res.,* **60,** 6788 – 6793.
Srivastava S, Wang S, Tong YO, Hao ZM and Chang E. (1993). *Cancer Res.,* **53,** 4452 – 4455.
Strano S, Munarriz E, Rossi M, Cristofanelli B, Shaul Y, Castagnoli L, Levine AJ, Sacchi A, Cesareni G, Oren M and Blandino G. (2000). *J. Biol. Chem.,* **275,** 29503 – 29512.
Unger T, Mietz JA, Scheffner M, Yee CL and Howley PM. (1993). *Mol. Cell. Biol.,* **13,** 5186 – 5194.
Wang JY. (2000). *Oncogene,* **19,** 5643 – 5650.
Wolf D, Harris N and Rotter V. (1984). *Cell,* **38,** 119.
Wolkowicz R, Peled A, Elkind NB and Rotter V. (1995). *Proc. Natl. Acad. Sci. USA,* **92,** 6842 – 6846.
Wolkowicz R, Peled A, Elkind NB and Rotter V. (1998). *Cancer Detect. Prev.,* **22,** 1 – 13.
Yuan ZM, Shioya H, Ishiko T, Sun X, Gu J, Huang YY, Lu H, Kharbanda S, Weichselbaum R and Kufe D. (1999). *Nature,* **399,** 814 – 817.
Zhao R, Gish K, Murphy M, Yin Y, Notterman D, Hoffman WH, Tom E, Mack DH and Levine AJ. (2000). *Genes Dev.,* **14,** 981 – 993.
Zhou BB and Elledge SJ. (2000). *Nature,* **408,** 433 – 439.