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Cognate DNA Stabilizes the Tumor Suppressor p53 and Prevents Misfolding and Aggregation†

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ABSTRACT: The tumor suppressor protein p53 is a nuclear protein that serves as an important transcription factor. The region responsible for sequence-specific DNA interaction is located in its core domain (p53C). Although full-length p53 binds to DNA as a tetramer, p53C binds as a monomer since it lacks the oligomerization domain. It has been previously demonstrated that two core domains have a dimerization interface and undergo conformational change when bound to DNA. Here we demonstrate that the interaction with a consensus DNA sequence provides the core domain of p53 with enhanced conformational stability at physiological salt concentrations (0.15 M). This stability could be either increased or abolished at low (0.01 M) or high (0.3 M) salt concentrations, respectively. In addition, interaction with the cognate sequence prevents aggregation of p53C into an amyloid-like structure, whereas binding to a nonconsensus DNA sequence has no effect on p53C stability, even at low ionic strength. Strikingly, sequence-specific DNA binding also resulted in a large stabilization of full-length p53, whereas nonspecific sequence binding led to no stabilization. The effects of cognate DNA could be mimicked by high concentrations of osmolytes such as glycerol, which implies that the stabilization is caused by the exclusion of water. Taken together, our results show an enhancement in protein stability driven by specific DNA recognition. When cognate DNA was added to misfolded protein obtained after a pressurization cycle, the original conformation was mostly recovered. Our results may aid the development of therapeutic approaches to prevent misfolded species of p53.

The tumor suppressor protein p53 is ubiquitously expressed in mammalian cells at almost undetectable levels, but it plays a central role in cell cycle control. In cells subjected to stressful conditions, p53 presents an important function as a transcription factor that results in cell cycle arrest or apoptosis (for reviews, see refs (*1*) and (*2*)). It is the failure of these responses that results in an uncontrolled cell cycle, one of the main characteristics of tumor cells. In fact, mutations in the *p53* gene are the most frequent genetic alterations found in human cancers, and nonfunctional p53 is found in >50% of human tumors. Thus, p53 represents an important target for cancer-related therapies (*3*). Wild-type p53 binds as a tetramer to response elements containing the consensus sequence PuPuPuC(A/T)(T/A)GPyPyPy, where Pu and Py are purines and pyrimidines, respectively (*4*, *5*). Several studies have shown that p53 binds to more than 100 different genes but with distinguishable binding affinities (*6*). In addition, the p53−DNA interaction has been shown to involve DNA bending, and it has been suggested that the energetics of DNA bending may contribute to the described differential affinities (*7*). Interestingly, in addition to binding to double-stranded sequence-specific DNA, p53 has been also described to bind several nonspecific sequences of RNA (*8*), short single-stranded DNA (*9*), double-stranded DNA containing loops (*10*), or insertion/deletion lesions (*11*) and Holliday junctions (*12*).

The central or core domain of p53 (p53C), which is comprised of residues 94−312, is responsible for specific DNA interactions, but it has also been described to be involved in nonspecific DNA binding (*13*). Although the tetramerization domain resides at the C-terminus of p53, some studies have demonstrated that four or even two p53C monomers are able to bind consensus sequences in a cooperative manner (*14*, *15*). Two independent research groups have identified a dimerization interface for DNA-bound p53C by nuclear magnetic resonance techniques (*14*, *15*). Both groups worked with a consensus DNA sequence bound by two core domains and showed that p53C undergoes

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significant conformational changes in this state, which is in accordance with the findings based on the crystal structure (*16*).

Given the fundamental importance of p53's DNA-binding ability for its tumor suppression activity, it is essential to understand the dynamics involved in p53C−DNA binding. However, few studies have focused on the stability of the protein upon binding to a DNA sequence (*17*). DNA-induced stabilization of p53 is important since some therapeutic approaches using stabilizing compounds, such as peptides, depend on the transfer of a misfolding-prone protein from the stabilizing effect of the peptide to a putative stabilizing effect induced by DNA binding (*18*). In addition, the use of nucleic acid aptamers is an emerging therapy that could be equally used for misfolded p53. Because aptamers specifically bind to misfolded or mutant proteins, they prevent the aggregation of the proteins that are prone to aggregate, allowing them to recover the correctly folded conformation (*19*−*21*).

Formation of amyloid-like aggregates has been described for the core domain (*22*), for the tetramerization domain (*23*, *24*), and for the transactivation domain (*25*) of p53. We found that the wild-type p53 core domain (p53C) can form fibrillar aggregates after a mild high-pressure treatment (*22*). Aggregates could be obtained by a cycle of compression and decompression at 37 °C, and their fibrillar character was demonstrated by electron and atomic force microscopies, by binding of thioflavin T, and by circular dichroism. An intermediate oligomer of p53C was also observed during equilibrium and kinetic folding/unfolding transitions induced by guanidinium chloride (*26*). Several carcinomas exhibit abnormal accumulation of wild-type tumor suppressor protein p53 either in the cytoplasm or in the nucleus of the cell (*27*). Therefore, the fibrillogenesis of p53 could theoretically contribute to the loss of function of p53 and to the accumulation of a conformationally altered protein in malignant cells. The search for molecules that preclude the formation of the misfolded conformation, which may ultimately lead to the prevention of tumor development, is a major goal in cancer research (*18*, *28*).

Here we describe that binding to a consensus DNA sequence enhances p53C stability and prevents the formation of the misfolded conformation, an effect that is related to DNA-binding affinity and appears to be mimicked by high concentrations of glycerol. Interference with DNA-binding affinity by altering ionic strength either increased or completely abolished the observed stability of the protein. The finding that full-length p53 was also dramatically stabilized by the binding of a cognate sequence is of particular biological and medical interest. This increase in DNA-driven protein stability may be relevant for therapeutic approaches based on strategies for rescue of misfolded wild-type p53C or denatured p53C mutants. In fact, we find that cognate DNA was capable of rescuing most of the native conformation of a misfolded protein.

EXPERIMENTAL PROCEDURES

Chemicals. All reagents were of analytical grade. The probe 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid, dipotassium salt (bis-ANS), was purchased from Molecular Probes (Eugene, OR). Distilled water was deionized and filtered through a Millipore water purification system before use. All solutions were prepared just before use.

DNA Preparation. Double-stranded oligonucleotides were prepared by mixing equimolar amounts of the complementary single-stranded oligonucleotides, poly(GC) DNA (5′ ATAATT-**GCGCGCGCGCGC**AGGAAA 3′) (purchased from DNAgency, Malvern, PA) or consensus DNA (5′ TTTCCT**AGACATGCCT**A ATTA 3′) (purchased from Invitrogen, Carlsbad, CA), in 50 mM Tris-HCl, pH 7.2, containing 250 mM NaCl. This mixture was incubated at 96 °C for 5 min, and the temperature was slowly reduced to 25 °C.

p53C Subcloning, Expression, and Purification. The core domain of the tumor suppressor human p53 protein (p53C) was obtained as previously described (*17*, *22*). The purity of all protein preparations was checked by SDS−PAGE and gel filtration chromatography. Detailed procedures for purification and purity of p53C are described in Supporting Information Figure 1. Full-length p53 was purchased from Sigma Chemical Co. (St. Louis, MO).

Fluorescence Measurements. All experiments monitored by intrinsic fluorescence spectra had an excitation wavelength at 278 nm, and emission was collected from 295 to 415 nm. Changes in fluorescence spectra at pressure p were evaluated by the changes in spectral center of mass, $\langle \nu p \rangle$:

$$\langle \nu p \rangle = \sum \nu_i F_i / \sum F_i$$

where F_i stands for the fluorescence emitted at wavenumber ν_i and the summation is carried out over the range of appreciable values of F. Experiments followed by light scattering data had an excitation wavelength at 320 nm, and the emission was collected from 300 to 340 nm. Bis-ANS fluorescence emission was recorded with excitation at 360 nm and emission from 400 to 600 nm. For pressure experiments, the high-pressure system was previously described (*29*) and was purchased from ISS (Champaign, IL). All experiments were performed at 25 °C, and intervals of 10 min were taken before each measurement. p53C at 5 μM in 50 mM Tris-HCl, pH 7.2, 5 mM dithiothreitol (DTT), and 5% glycerol was used throughout this paper, and the NaCl concentrations are as indicated in the figure legends. Experiments were done with different protein preparations and were highly repetitive. For each experiment shown, the data point was the average of three measurements.

Electrophoretic Mobility Shift Assay (EMSA). DNA-binding experiments were performed with either poly(GC) DNA or consensus DNA (5′ TTTCCT**AGACATGCCT**AATTA 3′). Both double-stranded oligonucleotides were end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (New England BioLabs), and 120 nM consensus or 60 nM poly(GC) was incubated with 600 nM p53C in the presence of different NaCl concentrations as indicated. In Supporting Information Figure 2, a representative EMSA gel shows the typical shift of the DNA band upon binding to p53C. The reactions were carried out in 50 mM Tris-HCl, pH 7.2, 5 mM DTT, 5% glycerol, with varying amounts of NaCl for 60 min at room temperature after addition of 70000 cpm of the labeled DNA to p53C. Samples were loaded onto a 6% nondenaturant polyacrylamide gel and separated by electrophoresis in 45 mM Tris−borate (pH 8.0) and 1 mM EDTA at 180 V at 4 °C. After electrophoresis, gels were dried and analyzed by autoradiography. Films were scanned and analyzed with NIH Image Software Version 4.0 β (Research Services Branch, National Institutes of Health).

Analysis of Salt-Dissociation Isotherms of p53−DNA Complexes. The effect of ionic strength on p53−DNA complexes was interpreted according to the Wyman linkage (*30*−*32*):

$$\mathrm{d} \ln K_\mathrm{d} / \mathrm{d} \ln a_\mathrm{NaCl} = \Delta N_\mathrm{NaCl} \quad (1)$$

The slope of ln K_d versus the log of salt activity gives ΔN_{NaCl}, the difference between the number of NaCl associated with complex formation. In the absence of protein−salt interactions, the amount of salt molecules obtained from this analysis corresponds to the number of electrostatic contacts involved in complex formation. According to Lima et al. (*32*), for the equilibrium condition:

$$\alpha = (K_d/4 \times 2 \times D)((1 + 8 \times 2 \times D/K_d)^{0.5} - 1) \quad (2)$$

where α is the fraction of remaining, undissociated, p53−DNA complex and D is the maximum p53−DNA complex concentration. Combining eqs 2 and 3 results in

$$\alpha = ((K_d \exp(\Delta N_{NaCl} \ln a_{NaCl}))((1 + (16D) / (K_d \exp(\Delta N_{NaCl} \ln a_{NaCl}))))^{0.5} - 1)/(8D) \quad (3)$$

Equation 4 was used for direct analysis of the salt-induced isotherm of p53−DNA complexes by nonlinear least-squares fitting of raw data using SigmaPlot (Jandel Scientific).

RESULTS

Binding to Consensus DNA Enhances the Stability of p53C. In order to analyze the properties of p53C in the presence of double-stranded DNA, we followed its conformation by intrinsic fluorescence (*33*). The intrinsic fluorescence of p53C comes from the excitation of eight tyrosines and a single tryptophan (Trp146). Trp146 is located in a region away from the DNA-binding site (Figure 1A). It is partially exposed to the solvent but is in close contact with several amino acid residues that lead to quenching of the fluorescence and prevent solvent relaxation by interaction with the solvent. This explains the highly blue-shifted emission spectrum. Therefore, any change in conformation of the protein leading to changes in the environment where Trp146 is located results in spectra shifts and/or changes in intensity. To quantify the shift in tryptophan emission, we use the average energy of the emission (center of spectral mass) to follow the changes in the tertiary structure of the protein. Therefore, from spectral emission shifts, reflected as changes in the center of spectral mass, we can estimate changes in protein conformation. Figure 1B shows the fluorescence emission spectra at atmospheric pressure at 25 °C of free p53C (black line), poly(GC)-bound p53C (red line), and consensus-bound p53C (blue line). It should be pointed out that for both DNAs the concentrations (protein and DNA) and other solution conditions used ensured that p53C was complexed to DNA, as determined by fluorescence polarization (Supporting Information Figure 3) and by EMSA (not shown). One can observe that the spectra of both free and poly(GC)-bound p53C are superimposed, while the maximum peak of the consensus-bound p53C is slightly shifted to higher wavelengths. The changes in the spectra emission upon binding of the cognate DNA indicate that there are overall changes in the conformation of the protein. The fluorescence of the extrinsic probe bis-ANS was used to further distinguish between the changes in conformation induced by cognate and noncognate DNA sequences (Figure 2). The data clearly demonstrate that binding of the cognate sequence (squares) induces a dramatic increase in fluorescence, whereas the noncognate sequence (triangles) leads to a small decrease, corroborating the results obtained by intrinsic fluorescence.

It can be inferred that the lower affinity of p53C for nonspecific DNA could result in a less stable DNA−p53C complex.

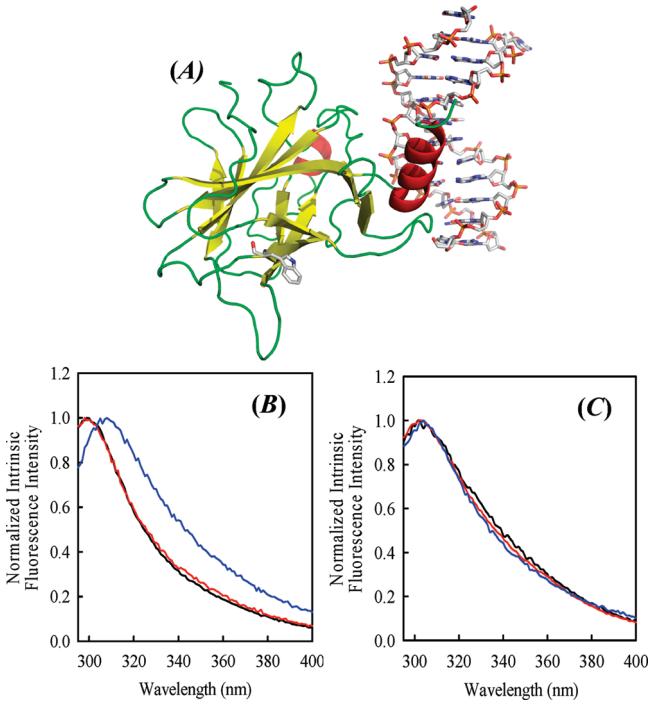

FIGURE 1: Spectroscopic and conformational properties of p53C. (A) Trp146 in the structure of p53C bound to DNA (figure generated with PyMol using the crystal structure from PDB entry 2ABY, showing one p53 monomer). (B, C) p53C spectrum at 5 μM (black line); p53C in the presence of consensus DNA (2:1) (blue line); p53C in the presence of poly(GC) DNA (2:1) (red line). All samples were diluted in 50 mM Tris-HCl, pH 7.2, 5 mM DTT, and 5% glycerol, and measurements were performed at 25 °C and at atmospheric pressure (B) in the presence of 150 mM NaCl and (C) in the presence of 300 mM NaCl.

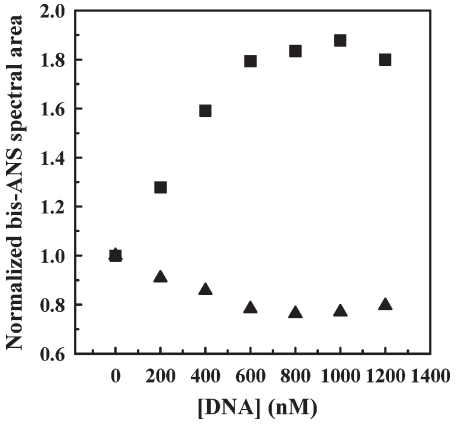

FIGURE 2: Long-range conformational change in p53C induced by cognate DNA binding revealed by bis-ANS fluorescence emission. Consensus DNA (squares) or poly(GC) DNA (triangles) was titrated in a solution containing 1 μM p53C bound to 2 μM bis-ANS. Bis-ANS fluorescence emission was recorded (excitation, 360 nm; emission, from 400 to 600 nm). The plots shown were normalized to the initial condition (p53C + bis-ANS without DNA addition). Measurements were performed at 25 °C in 50 mM Tris, 150 mM NaCl, 5 mM DTT, and 5% glycerol (pH 7.2).

Consequently, this protein might behave as a free protein when subjected to denaturing conditions with minor free energy contributions from poly(GC) DNA. Conversely, the shift in the spectrum of the consensus-bound p53C indicates that this protein undergoes conformational changes in this complex, as also observed by NMR studies (*14*, *15*).

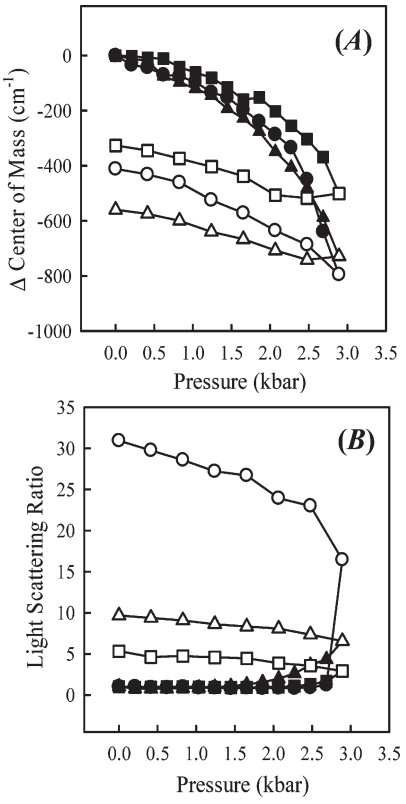

FIGURE 3: DNA-binding contributions to p53C behavior under pressure. Increasing hydrostatic pressure values (1 bar to 2.9 kbar) were applied to the samples at 25 °C: free p53C (circles), poly(GC)-bound p53C (triangles), and consensus-bound p53C (squares). Samples were analyzed in 50 mM Tris-HCl, pH 7.2, 5 mM DTT, 150 mM NaCl, and 5% glycerol. Black symbols correspond to values during the compression cycle, and white symbols correspond to decompression. Data were analyzed as (A) center of mass and (B) light scattering. Each data point is the average of three measurements, and the error bars are smaller than the symbols.

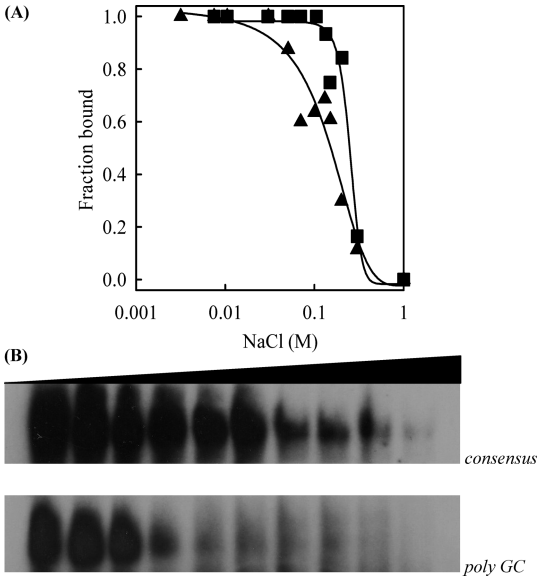

FIGURE 4: Specific DNA binding evaluated by salt dissociation isotherms. EMSA was performed as described in the Experimental Procedures section. (A) Quantitative densitometric analysis of the p53C−DNA complex was plotted as a function of ionic strength for (squares) consensus DNA and (triangles) poly(GC) DNA. Equation 3 was adjusted to the data in order to calculate dissociation constants and the salt stoichiometric coefficient, as described in the text. (B) Polyacrylamide gel electrophoresis (PAGE) showing representative results for the salt dissociation isotherms of p53C in complex with consensus (upper PAGE) and poly(GC) (lower PAGE) DNA. Binding buffer was supplemented with varying NaCl concentrations as follows (from left to the right): 8, 10, 30, 50, 70, 105, 130, 150, 205, and 300 mM. Bands correspond to p53C bound to DNA. Densitometric analysis and curve fitting for dissociation constants are shown in Figure 4, as described in the Experimental Procedures section.

To evaluate the contributions of DNA binding to p53C stability, we investigated its denaturation profile by measuring high-pressure effects on p53C conformation. Hydrostatic pressure has been widely used to assess the dissociation and denaturation of folded proteins, protein−nucleic acid complexes, and amyloid aggregates (*34*−*40*). In Figure 3, we measured the pressure effects on p53C in the absence of DNA (circles) or in the presence of consensus (squares) or nonspecific (poly(GC)) (triangles) sequences. As shown by the fluorescence emission spectra (Figure 1B), both free and poly(GC)-bound p53C denature with increasing pressure (Δ of center of mass = 790 cm^{-1} for the free form and 730 cm^{-1} for the nonspecific DNA-bound form) (Figure 3A). In addition to the red shift, there is an increase in the total emitted fluorescence when p53C denatures (not shown). On the other hand, when p53C is incubated with the consensus DNA sequence, the pressure-induced denaturation is milder (shifted to higher pressures). Therefore, the higher affinity to consensus sequences not only provides higher stability of the complex of p53C with the consensus DNA but also confers higher stability to the protein itself. Indeed, p53C in the presence of the consensus DNA does not reach complete denaturation when pressurized up to 3 kbar (Δ of center of mass = 500 cm^{-1}).

Interestingly, when following light scattering data, i.e., monitoring the appearance of large particles in solution, we noticed that the presence of the consensus DNA prevented the drastic aggregation that occurred upon pressure denaturation of p53C in the absence of DNA (Figure 3B). The noncognate sequence had a partial effect on precluding aggregation. This suppression in aggregation is probably due to the prevention of major changes in the conformation of the protein into a misfolded state.

Ionic Strength Interferes with the DNA-Induced Stability of p53C. p53C has been described to bind with high affinity to both specific and nonspecific DNA sequences. However, only the specific binding of DNA to p53C seems to promote significant changes in protein conformation and overall stability. To test the reversibility of this event and evaluate the driving forces of the p53C−DNA interaction, we measured salt dissociation isotherms (*31*, *32*) of the complexes with poly(GC) (nonspecific) and consensus (specific) DNA sequences. Figure 4A shows salt dissociation isotherms for DNA-bound p53C derived from electrophoretic mobility shift assay (EMSA) (Figure 4B). From the EMSA densitometry analysis, one can observe the difference in salt dependence behavior of the p53C complexes with consensus (squares) and poly(GC) (triangles) DNAs. Both the midpoint transitions and the steepness are dissimilar, indicating different affinities and electrostatic contacts, respectively. Application of a Wyman linkage function (eq 4; see Experimental Procedures section for details) for salt isotherms of the complex allowed us to estimate the number of counterions recruited for shielding charges from the dissociated species, which is a measure of the number of ionic contacts. Interestingly, from these analyses we could determine that 9.8 ± 0.3 ionic contacts are involved in the p53C−consensus DNA complex while only 2.8 ± 0.9 are involved in the p53C−poly(GC) complex. These data give

strong evidence that p53C−DNA complexes present different consolidated interfaces, as judged by the different number of electrostatic contacts, which might be critical for DNA recognition specificity and thus p53 function.

The analysis of the salt dissociation isotherms allowed us to determine the apparent dissociation constants at different salt concentrations. At 150 mM NaCl, the dissociation constants of p53C with consensus and poly(GC) DNAs are 0.8 and 50 nM, respectively, which are compatible to those values described in the literature (*41*).

From Figure 1B, one can expect that the fluorescence emission spectrum of the poly(GC) bound to p53C at low salt concentrations, for instance, 10 mM NaCl, would be shifted to higher wavelengths as observed for consensus-bound p53C at physiological salt concentrations (0.15 M). Moreover, one can also predict that at this low ionic strength, a nonspecific DNA molecule would be able to protect p53C against denaturation. Surprisingly, when we observed the fluorescence emission spectra of free p53C or p53C bound to poly(GC) or consensus DNA at 10 mM NaCl, we could not differentiate the free protein from the one bound to the poly(GC) sequence (data not shown). Once again, the consensus-bound p53C spectrum was shifted to higher wavelengths. When we subjected these samples to high hydrostatic pressures, the poly(GC)-bound p53C was as susceptible to denaturation as the free protein (Figure 5A). Interestingly, proteins in both conditions underwent higher unfolding extension compared to those observed at 150 mM NaCl. However, the consensus-bound p53C complex was much more stable at low ionic strength.

The data from Figure 5 clearly suggest that at low salt concentrations the consensus DNA−p53C complex is more stable and this increased affinity induces enhanced stability of the p53C itself. If this is true, as observed in our salt-dissociation isotherms (Figure 4), one could anticipate that high salt concentrations would be able to disrupt the DNA−protein complex, leaving this free protein as vulnerable to the denaturing effects of high pressure as p53C alone. In fact, Figure 1C shows that the fluorescence emission spectrum of consensus-bound p53C is similar to both free and poly(GC)-bound forms of p53C. At high ionic strength (300 mM NaCl), the protective effect of the cognate DNA also disappeared, probably due to the salt dissociation of the complex (Figure 5B).

Cognate DNA Stabilizes Full-Length p53. The full-length p53 protein is tetrameric, and each monomer contains a transactivation domain, a connecting proline-rich segment, the DNA-binding domain, and a tetramerization domain (*41*). As pointed out by Joerger and Fersht (*42*), there are many exciting structural features of the function of p53 that remain to be solved. The main question related to the experiments that we report above with the DNA-binding domain (p53C) is whether a similar stabilization would occur with the full-length protein. The full-length p53 monomer contains three tryptophans in the intrinsically disordered N-terminal domain, in addition to Trp146 partially buried in the hydrophobic core of the DNA-binding domain (Figure 1A). In contrast to Trp146, the other tryptophans are exposed to the solvent and contribute to the shift in the emission spectrum to red wavelengths (Figure 6). However, because Trp146 is highly quenched in the native state, it is still possible to follow the unfolding of the protein by the changes in the total emission intensity, as can be visualized in Figure 6A. In the denatured state, the total fluorescence intensity is 60% higher than in the native state.

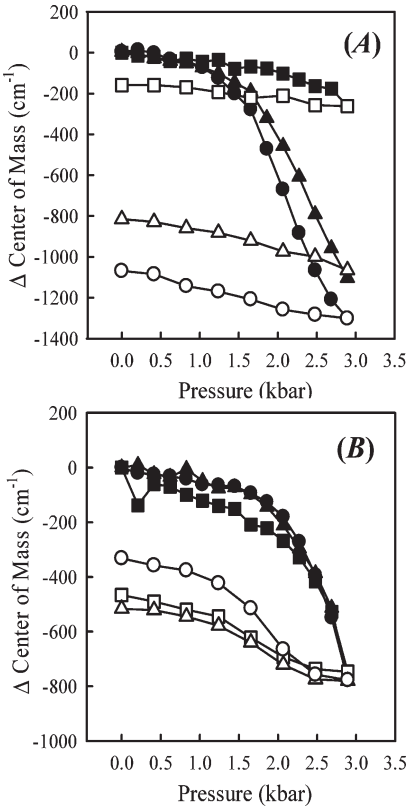

FIGURE 5: High-pressure effects on p53C in the absence or presence of DNA at low and high salt concentrations. Free or DNA-bound p53C was diluted in 50 mM Tris-HCl, pH 7.2, 5 mM DTT, and 5% glycerol. Increasing hydrostatic pressures (1 bar to 2.9 kbar) were applied to the samples at 25 °C: free p53C (circles), poly(GC)-bound p53C (triangles), and consensus-bound p53C (squares). Black symbols correspond to the values obtained during the compression cycle and white symbols to the values during decompression. Data were analyzed as the center of mass (A) in the presence of 10 mM NaCl and (B) in the presence of 300 mM NaCl. Each data point is the average of three measurements, and the error bars are smaller than the symbols.

Full-length p53 is as sensitive to pressure as the core domain (p53C) (Figure 6C). The modular arrangement of the domains might explain why the tetrameric structure does not increase the stability of the protein. Binding of full-length p53 to the cognate DNA resulted in a dramatic stabilization (Figure 6B,C, squares). In contrast, binding of the nonspecific sequence poly (GC) did not result in stabilization of p53 (Figure 6C, triangles). A small red shift was observed when the protein bound the cognate DNA, as was expected from the experiment with p53C. The stabilization can be pictured by the small changes in the spectra of p53 bound to the cognate sequence as a function of pressure, as well as by the complete reversibility when the pressure was returned to 1 bar. These results clearly show that full-length p53 can be highly stabilized by sequence-dependent nucleic acid binding.

Exclusion of Water as a General Stabilizing Mechanism of p53C. The interaction of a protein with DNA depends on forces similar to those involved in protein−protein interactions, such as electrostatic forces, hydrogen bonds, and hydrophobic interactions. The exclusion of water molecules contributes to generate a tight and relatively dry complex. The stabilization produced by the binding of a cognate DNA on the complex could be mimicked by the use of relatively high concentrations of glycerol (above 10%). This can be verified in Figure 7, where the pressure-induced unfolding of p53C was monitored in the

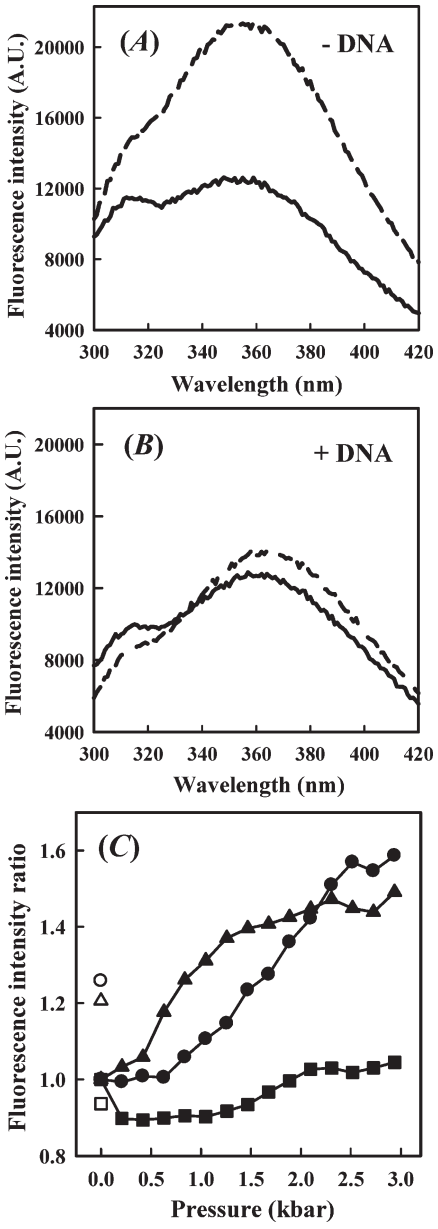

FIGURE 6: Full-length p53 is stabilized against pressure denaturation upon sequence-specific DNA binding. Full-length p53 (0.4 μM) was diluted in 50 mM Tris-HCl, pH 7.2, 5 mM DTT, 10 mM NaCl, and 5% glycerol in the absence or presence of consensus DNA. (A) Intrinsic fluorescence emission spectrum of free p53 or (B) in the presence of consensus DNA at atmospheric pressure (solid line) and at 2.9 kbar (dashed line). (C) Fluorescence intensity ratio of free p53 (circles), consensus-bound p53 (squares), and poly(GC)-bound p53 (triangles) subjected to hydrostatic pressure (1 bar to 2.9 kbar) at 25 °C. Black symbols correspond to the values obtained during the compression cycle and white symbols to the values after decompression to atmospheric pressure.

presence of glycerol (Figure 7A). Like consensus DNA, glycerol also protected p53C from pressure-induced aggregation (Figure 7B).

Cognate DNA Induces Partial Recovery of the Misfolded Conformation. We decided to verify whether cognate DNA would be able to rescue the conformation even after misfolding and aggregation had occurred (Figure 8). Figure 8 shows that cognate DNA is capable of restoring most of the original tryptophan fluorescence spectra after the p53C had been subjected to a cycle of compression and decompression. Not only is the tertiary conformation significantly restored but the protein

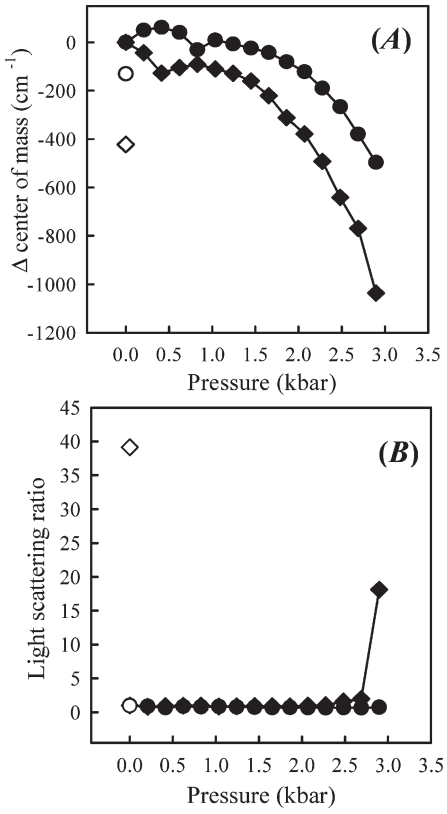

FIGURE 7: Glycerol and high-pressure effects on p53C. Hydrostatic pressure (1 bar to 2.9 kbar) was applied to the samples containing p53C at 5 μM at 25 °C. Measurements were performed with 5% (◆) and 20% (●) glycerol and analyzed as the center of mass (A) and as light scattering (B). Open symbols correspond to fluorescence or LS values after return to atmospheric pressure. Each data point is the average of three measurements, and the error bars are smaller than the symbols.

becomes resistant to denaturation and aggregation after a second cycle of pressure (Figure 8B).

DISCUSSION

The interaction between a protein and a DNA leads into conformational changes in both partners (*43*, *44*). Here, we show that the interaction between a consensus DNA sequence and p53 promotes a substantial increase in p53 stability. These results were obtained for the isolated DNA-binding domain (p53C) as well as for the full-length p53. Recent studies have shown that p53C undergoes substantial conformational changes upon sequence-specific DNA binding (*14*, *15*, *45*, *46*). The shifts in intrinsic fluorescence spectra and the increase in bis-ANS fluorescence induced by cognate DNA (Figures 1 and 2), but not by noncognate DNA, reveal the extraordinary changes in the tertiary conformation of p53 that are related to DNA recognition. NMR studies have shown that significant changes in p53C chemical shifts between free and DNA-bound forms occur not only at residues of the DNA-binding region, some of which have been determined by the crystal structure (*16*), but also at residues located in the β-sheet region of the core domain, indicating that the conformational changes induced by DNA binding are propagated throughout the protein (*14*). Recently, Tidow et al. (*46*) compared the structures of the free protein and p53 bound to DNA by a diversity of methods and noted a very interesting pattern: while the p53−DNA complex had a relatively rigid structure, the free protein had a structure of loosely tethered

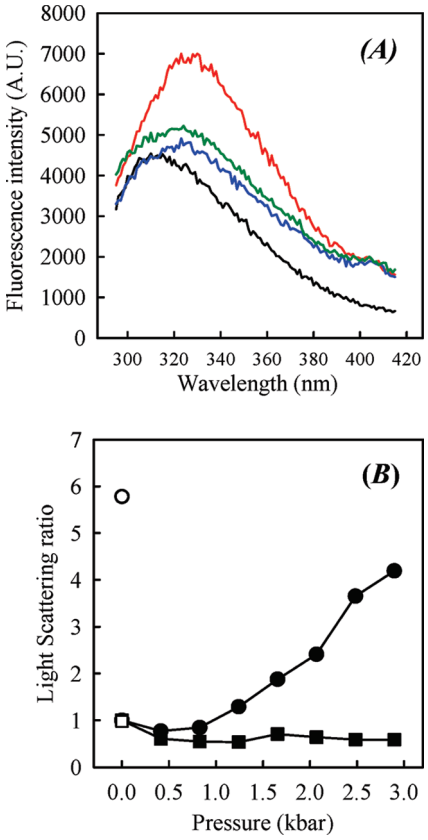

FIGURE 8: Cognate DNA rescues the native conformation of p53C after pressure-induced misfolding and aggregation. WT p53C at 5 μM in the absence of DNA was compressed up to 3 kbar, and after return to atmospheric pressure, consensus DNA was added at 2.5 μM final concentration, and the sample was repressurized. Samples were analyzed in 50 mM Tris-HCl, pH 7.2, 5 mM DTT, 10 mM NaCl, 0.5 M urea, and 5% glycerol at 25 °C. (A) Intrinsic fluorescence emission spectrum of wt p53C at atmospheric pressure (solid black line), after the first cycle of compression and decompression in the absence of DNA (red line), after DNA addition at atmospheric pressure (blue line), and after the second compression and decompression cycle in the presence of DNA (green line). (B) Light scattering ratio for the same experiment in (A) in the absence of DNA (circles) and after compression/decompression after addition of consensus DNA (squares). Open symbols correspond to LS values after return to atmospheric pressure. The small concentration of urea (0.5 M) was used to prevent extensive aggregation.

pairs of core domains, indicating large conformational changes upon DNA binding. Accordingly, Boeckler et al. (*47*) demonstrated the rescue of the Y220C p53 mutant by the drug PhiKan083. Interestingly, the mutant Y220C does not affect DNA-binding residues. In fact, it creates a cavity on the surface of the protein in a region that is conformationally distant from the DNA-binding site; still, these changes are significant enough to affect the protein stability and its functions. As a result, the drug PhiKan083 binds to the cavity created by the Y220C mutant, rescuing the stability of the p53 protein, indicating that conformational changes in distinct parts of the protein contribute to p53's DNA-binding function. Therefore, it is interesting to observe that DNA binding also generates large-scale conformational changes throughout the protein.

As the stability of a protein is intrinsically related to its conformation, changes in p53C conformation due to DNA binding should interfere with the protein's stability. Our results demonstrate that binding to consensus DNA indeed enhances the stability of the p53 core domain (Figures 3 and 5A) and of full-length p53 (Figure 6). The dimer of the LexA repressor, a bacterial protein, was observed to tighten upon binding to specific DNA, while nonspecific DNA did not present stabilizing effects (*48*). Interestingly, it has been suggested that p53C might undergo DNA-induced stabilization in the crystal-obtained conformation through a strong attraction between the β-sheets (*16*), restricting its flexible conformation described in the unbound state (*49*) and providing intramolecular bonds that could stabilize p53 in the DNA-bound form (*16*). Additionally, the enhanced stability of DNA-bound p53C could be a consequence of protein−protein interactions. Klein et al. (*14*) and Rippin et al. (*15*) described a putative dimerization interface for p53 core domains in the DNA-bound state, which is probably related to p53 regulation (*15*). At this moment we are not able to prove one alternative over the other. However, our finding that the tetrameric full-length protein is equally sensitive to pressure denaturation in the absence of DNA indicates that oligomerization by itself does not stabilize p53. Alternatively, Veprintsev and Fersht (*50*) analyzed the contribution of individual nucleotides within the p53 binding site on the DNA to the affinity of p53−DNA interactions and constructed a binding predictor. However, the authors described that the spread of the K_ds by itself was not sufficient to explain the activation of different genes by changes in p53 concentration alone.

The biological importance of our study can also be seen in the light of a recent notable work from Tafvizi et al. (*51*), where they suggested that p53 moves along a free energy landscape with low activation barriers. The authors demonstrated that p53 is capable of sliding−moving along DNA while in continuous contact with the duplex, rather than through a series of hops between nearby bases. Therefore, it is reasonable to envision that in order for a protein to recognize and stably interact with its target binding site two different but complementary forces come to play: affinity of the protein for the DNA as well as increased protein stability generated by this interaction. Moreover, Kitayner et al. (*52*) recently demonstrated that differential binding affinities of the p53 tetramer were correlated to sequence-specific variations in the protein−DNA contact geometry. As a result, one could predict that variations in the protein−DNA geometry would also induce variations in the protein's stability, suggesting that the gain in protein stability may play a role in the stability of the protein−DNA complex.

Depending on the salt condition, the protection against p53C denaturation upon DNA-specific binding was incomplete. Even though apolar surfaces might be buried during protein−DNA complex formation, high pressure still exerts its effects on p53C (Figures 3 and 5). Since pressure affects hydrophobic as well as van der Waals bonds (*36*, *37*, *53*), it is more likely that it operates through the described hydrophobic core domain present in the site opposite to the DNA-binding region (*16*). In other words, our data suggest that p53C might undergo denaturation in a state still bound to the DNA molecule, as suggested for the E2c protein (*32*), which may also explain the absence of an increase in the average size of the complex, in contrast to the observed aggregation of the free protein when subjected to the same pressure range (Figure 3B). To obtain more information about the nature of the stabilizing effect of cognate DNA on pressure-induced unfolding of p53C, we calculated the volume changes associated to the transitions for all conditions at low salt concentration (Table 1). In Table 1, also depicted are the pressure values necessary to achieve 50% of the transition ($P_{1/2}$ values). Pressure curves were performed at 25 °C, pH 7.2, and 10 mM

Table 1: Volume Changes and $P_{1/2}$ Values for p53C Pressure-Induced Unfolding Transitions

	ΔV (mL/mol)	$P_{1/2}$ (kbar)
without DNA	68.4 ± 2.5	2.2
+poly(GC)	57.8 ± 1.1	2.4
+consensus	29.7 ± 4.0	4.2

NaCl. The curves were fitted assuming a two-state transition, and all transitions were fixed for ΔCM of 1470 cm^{-1}. Binding to a specific DNA (consensus) increases the pressure stability of p53C, along with a diminished volume change, whereas binding to a nonspecific DNA (poly(GC)) had minor effects. The stabilization induced by cognate DNA binding with a concomitant decrease in the volume change can be explained by a decrease in the changes in the accessible surface area when the protein is bound to the specific DNA sequence.

It has been demonstrated that some p53 mutants do not lose their transcriptional activity but instead activate distinct genes (*54*−*57*). Alternatively, it has been shown that the majority of p53 mutants have lower stability (*17*, *58*). For instance, Bullock et al. (*58*) demonstrated that the hot-spot mutant R282W is globally denatured, being 38% folded at 37 °C, but presenting DNA-binding ability under subphysiological temperatures (under which the mutant is largely folded). Therefore, it is reasonable to hypothesize that an increase in protein stability induced by DNA binding could compensate for the intrinsic lower stability of mutant proteins and account for the maintenance of transcriptional activity of some mutant proteins. Moreover, it has been suggested that the intrinsic low thermodynamic stability of p53C is important for the rapid turnover of inactive unfolded p53 protein (*59*). Inversely, the higher stability of DNA-bound p53C could be important for its lower turnover rate in stimulated cells. Our finding that sequence-specific DNA binding also stabilizes full-length p53 (Figure 6) corroborates the idea that nucleic acids might play a protective role in the protein structure in addition to being the target for p53 binding. The recent reports that p53 core (*22*, *26*), C-terminal (*23*, *24*), and transactivation (*25*) domains can undergo misfolding and aggregation make the search for stabilizers a crucial goal. As we previously proposed (*22*), aggregation could act as a sink to sequester native protein in the inactive conformation. Our findings that specific nucleic acid binding stabilizes both the p53 core and full-length p53 point to the delicate balance between the processes of folding, DNA binding, aggregation, and misfolding. Nucleic acids have been recently recognized as important players in amyloid aggregation (*21*).

Many currently investigated therapeutic strategies for cancer treatments are based on peptide binding-induced stability of p53C (*60*, *61*). Such stabilization would shift the equilibrium toward the native conformation of p53C, as opposed to misfolded states. In addition, this strategy is based on a peptide or a drug that acts as a chaperone, i.e., that maintains the protein in a native conformation until its transfer to a consensus DNA sequence, which would also keep it in a stable conformation. Similarly, the transient binding to a therapeutic nucleic acid could have the same effect. In summary, our observations that p53C and full-length p53 present higher stability when bound to a consensus DNA sequence broaden our molecular understanding of the forces involved in protein−DNA complexes. More specifically, it is an important factor to be considered when one is searching for compounds that reactivate and stabilize wild-type as well as mutant p53 *in vivo*, i.e., drug therapies for cancer. In the past few years, the unique binding properties of nucleic acids have been explored for the development of new therapeutic strategies (*19*, *20*, *62*). The studies reported here shed light on the great potential of using high-affinity nucleic acid sequences to rescue misfolded p53. The potential therapeutic use of aptameric nucleic acids is corroborated by our findings showing that cognate DNA is capable of recovering most of the native conformation of p53C from pressure-induced misfolded protein (Figure 8). Because nucleic acids are susceptible to various modifications, which alter *in vivo* processing without affecting target discrimination, our study paves the way for the application of aptameric nucleic acids in cancer.

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SUPPORTING INFORMATION AVAILABLE

Figure 1 showing the analysis of purity of purified p53C by gel filtration chromatography, Figure 2 showing a typical gel shift assay to measure binding of DNA to p53C, and Figure 3 showing the binding curves to cognate and nonspecific DNAs determined by fluorescence anisotropy. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Prives, C., and Hall, P. A. (1999) The p53 pathway. *J. Pathol. 187*, 112–126.
2. Vogelstein, B., Lane, D., and Levine, A. J. (2000) Surfing the p53 network. *Nature 408*, 307–310.
3. Levesque, A. A., and Eastman, A. (2007) p53-based cancer therapies: Is defective p53 the Achilles heel of the tumor?. *Carcinogenesis 28*, 13–20.
4. el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B. (1992) Definition of a consensus binding site for p53. *Nat. Genet. 1*, 45–49.
5. Vogelstein, B., and Kinzler, K. W. (1992) p53 function and dysfunction. *Cell 70*, 523–526.
6. Meek, D. W. (2004) The p53 response to DNA damage. *DNA Repair (Amsterdam) 3*, 1049–1056.
7. Nagaich, A. K., Appella, E., and Harrington, R. E. (1997) DNA bending is essential for the site-specific recognition of DNA response elements by the DNA binding domain of the tumor suppressor protein p53. *J. Biol. Chem. 272*, 14842–14849.
8. Riley, K. J., Cassiday, L. A., Kumar, A., and Maher, L. J., 3rd (2006) Recognition of RNA by the p53 tumor suppressor protein in the yeast three-hybrid system. *RNA 12*, 620–630.
9. Oberosler, P., Hloch, P., Ramsperger, U., and Stahl, H. (1993) p53-catalyzed annealing of complementary single-stranded nucleic acids. *EMBO J. 12*, 2389–2396.
10. Stros, M., Muselikova-Polanska, E., Pospisilova, S., and Strauss, F. (2004) High-affinity binding of tumor-suppressor protein p53 and HMGB1 to hemicatenated DNA loops. *Biochemistry 43*, 7215–7225.
11. Szak, S. T., and Pietenpol, J. A. (1999) High affinity insertion/deletion lesion binding by p53. Evidence for a role of the p53 central domain. *J. Biol. Chem. 274*, 3904–3909.
12. Lee, S., Cavallo, L., and Griffith, J. (1997) Human p53 binds Holliday junctions strongly and facilitates their cleavage. *J. Biol. Chem. 272*, 7532–7539.
13. Wolcke, J., Reimann, M., Klumpp, M., Gohler, T., Kim, E., and Deppert, W. (2003) Analysis of p53 "latency" and "activation" by fluorescence correlation spectroscopy. Evidence for different modes of high affinity DNA binding. *J. Biol. Chem. 278*, 32587–32595.
14. Klein, C., Planker, E., Diercks, T., Kessler, H., Kunkele, K. P., Lang, K., Hansen, S., and Schwaiger, M. (2001) NMR spectroscopy reveals the solution dimerization interface of p53 core domains bound to their consensus DNA. *J. Biol. Chem. 276*, 49020–49027.

15. Rippin, T. M., Freund, S. M., Veprintsev, D. B., and Fersht, A. R. (2002) Recognition of DNA by p53 core domain and location of intermolecular contacts of cooperative binding. *J. Mol. Biol. 319*, 351–358.
16. 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.
17. Bullock, A. N., Henckel, J., DeDecker, B. S., Johnson, C. M., Nikolova, P. V., Proctor, M. R., Lane, D. P., and Fersht, A. R. (1997) Thermodynamic stability of wild-type and mutant p53 core domain. *Proc. Natl. Acad. Sci. U.S.A. 94*, 14338–14342.
18. Joerger, A. C., and Fersht, A. R. (2007) Structure-function-rescue: the diverse nature of common p53 cancer mutants. *Oncogene 26*, 2226–2242.
19. Kocisko, D. A., Vaillant, A., Lee, K. S., Arnold, K. M., Bertholet, N., Race, R. E., Olsen, E. A., Juteau, J. M., and Caughey, B. (2006) Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. *Antimicrob. Agents Chemother. 50*, 1034–1044.
20. King, D. J., Safar, J. G., Legname, G., and Prusiner, S. B. (2007) Thioaptamer interactions with prion proteins: sequence-specific and non-specific binding sites. *J. Mol. Biol. 369*, 1001–1014.
21. Silva, J. L., Lima, L. M., Foguel, D., and Cordeiro, Y. (2008) Intriguing nucleic-acid-binding features of mammalian prion protein. *Trends Biochem. Sci. 33*, 132–140.
22. Ishimaru, D., Andrade, L. R., Teixeira, L. S., Quesado, P. A., Maiolino, L. M., Lopez, P. M., Cordeiro, Y., Costa, L. T., Heckl, W. M., Weissmuller, G., Foguel, D., and Silva, J. L. (2003) Fibrillar aggregates of the tumor suppressor p53 core domain. *Biochemistry 42*, 9022–9027.
23. Lee, A. S., Galea, C., DiGiammarino, E. L., Jun, B., Murti, G., Ribeiro, R. C., Zambetti, G., Schultz, C. P., and Kriwacki, R. W. (2003) Reversible amyloid formation by the p53 tetramerization domain and a cancer-associated mutant. *J. Mol. Biol. 327*, 699–709.
24. Higashimoto, Y., Asanomi, Y., Takakusagi, S., Lewis, M. S., Uosaki, K., Durell, S. R., Anderson, C. W., Appella, E., and Sakaguchi, K. (2006) Unfolding, aggregation, and amyloid formation by the tetramerization domain from mutant p53 associated with lung cancer. *Biochemistry 45*, 1608–1619.
25. Rigacci, S., Bucciantini, M., Relini, A., Pesce, A., Gliozzi, A., Berti, A., and Stefani, M. (2008) The (1−63) region of the p53 transactivation domain aggregates in vitro into cytotoxic amyloid assemblies. *Biophys. J. 94*, 3635–3646.
26. Ishimaru, D., Lima, L. M., Maia, L. F., Lopez, P. M., Ano Bom, A. P., Valente, A. P., and Silva, J. L. (2004) Reversible aggregation plays a crucial role on the folding landscape of p53 core domain. *Biophys. J. 87*, 2691–2700.
27. Moll, U. M., Ostermeyer, A. G., Haladay, R., Winkfield, B., Frazier, M., and Zambetti, G. (1996) Cytoplasmic sequestration of wild-type p53 protein impairs the G1 checkpoint after DNA damage. *Mol. Cell. Biol. 16*, 1126–1137.
28. Selivanova, G., and Wiman, K. G. (2007) Reactivation of mutant p53: molecular mechanisms and therapeutic potential. *Oncogene 26*, 2243–2254.
29. Paladini, A. A., and Weber, G. (1981) Absolute measurements of fluorescence polarization at high pressures. *Rev. Sci. Instrum. 52*, 419–427.
30. Wyman, J. Jr. (1964) Linked functions and reciprocal effects in hemoglobin: a second look. *Adv. Protein Chem. 19*, 223–286.
31. Record, M. T. Jr., Lohman, M. L., and De Haseth, P. (1976) Ion effects on ligand-nucleic acid interactions. *J. Mol. Biol. 107*, 145–158.
32. Lima, L. M., and Silva, J. L. (2004) Positive contribution of hydration on DNA binding by E2c protein from papillomavirus. *J. Biol. Chem. 279*, 47968–47974.
33. Ishimaru, D., Maia, L. F., Maiolino, L. M., Quesado, P. A., Lopez, P. C., Almeida, F. C., Valente, A. P., and Silva, J. L. (2003) Conversion of wild-type p53 core domain into a conformation that mimics a hot-spot mutant. *J. Mol. Biol. 333*, 443–451.
34. Lima, L. M., Foguel, D., and Silva, J. L. (2000) DNA tightens the dimeric DNA-binding domain of human papillomavirus E2 protein without changes in volume. *Proc. Natl. Acad. Sci. U.S.A. 97*, 14289–14294.
35. Da Poian, A. T., Johnson, J. E., and Silva, J. L. (1994) Differences in pressure stability of the three components of cowpea mosaic virus: implications for virus assembly and disassembly. *Biochemistry 33*, 8339–8346.
36. Mozhaev, V. V., Heremans, K., Frank, J., Masson, P., and Balny, C. (1996) High pressure effects on protein structure and function. *Proteins 24*, 81–91.
37. Silva, J. L., Foguel, D., and Royer, C. A. (2001) Pressure provides new insights into protein folding, dynamics and structure. *Trends Biochem. Sci. 26*, 612–618.
38. Foguel, D., and Silva, J. L. (2004) New insights into the mechanisms of protein misfolding and aggregation in amyloidogenic diseases derived from pressure studies. *Biochemistry 43*, 11361–11370.
39. Akasaka, K. (2006) Probing conformational fluctuation of proteins by pressure perturbation. *Chem. Rev. 106*, 1814–1835.
40. Senear, D. F., Tretyachenko-Ladokhina, V., Opel, M. L., Aeling, K. A., Hatfield, G. W., Franklin, L. M., Darlington, R. C., and Alexander Ross, J. B. (2007) Pressure dissociation of integration host factor-DNA complexes reveals flexibility-dependent structural variation at the protein-DNA interface. *Nucleic Acids Res. 35*, 1761–1772.
41. Weinberg, R. L., Veprintsev, D. B., Bycroft, M., and Fersht, A. R. (2005) Comparative binding of p53 to its promoter and DNA recognition elements. *J. Mol. Biol. 348*, 589–596.
42. Joerger, A. C., and Fersht, A. R. (2008) Structural biology of the tumor suppressor p53. *Annu. Rev. Biochem. 77*, 557–582.
43. von Hippel, P. H. (1994) Protein-DNA recognition: new perspectives and underlying themes. *Science 263*, 769–770.
44. von Hippel, P. H. (2007) From "simple" DNA-protein interactions to the macromolecular machines of gene expression. *Annu. Rev. Biophys. Biomol. Struct. 36*, 79–105.
45. Nichols, N. M., and Matthews, K. S. (2001) Protein-DNA binding correlates with structural thermostability for the full-length human p53 protein. *Biochemistry 40*, 3847–3858.
46. Tidow, H., Melero, R., Mylonas, E., Freund, S. M., Grossmann, J. G., Carazo, J. M., Svergun, D. I., Valle, M., and Fersht, A. R. (2007) Quaternary structures of tumor suppressor p53 and a specific p53 DNA complex. *Proc. Natl. Acad. Sci. U.S.A. 104*, 12324–12329.
47. Boeckler, F. M., Joerger, A. C., Jaggi, G., Rutherford, T. J., Veprintsev, D. B., and Fersht, A. R. (2008) Targeted rescue of a destabilized mutant of p53 by an in silico screened drug. *Proc. Natl. Acad. Sci. U.S.A. 105*, 10360–10365.
48. Mohana-Borges, R., Pacheco, A. B., Sousa, F. J., Foguel, D., Almeida, D. F., and Silva, J. L. (2000) LexA repressor forms stable dimers in solution. The role of specific dna in tightening protein-protein interactions. *J. Biol. Chem. 275*, 4708–4712.
49. Bell, S., Klein, C., Muller, L., Hansen, S., and Buchner, J. (2002) p53 contains large unstructured regions in its native state. *J. Mol. Biol. 322*, 917–927.
50. Veprintsev, D. B., and Fersht, A. R. (2008) Algorithm for prediction of tumour suppressor p53 affinity for binding sites in DNA. *Nucleic Acids Res. 36*, 1589–1598.
51. Tafvizi, A., Huang, F., Leith, J. S., Fersht, A. R., Mirny, L. A., and van Oijen, A. M. (2008) Tumor suppressor p53 slides on DNA with low friction and high stability. *Biophys. J. 95*, L01–L03.
52. Kitayner, M., Rozenberg, H., Kessler, N., Rabinovich, D., Shaulov, L., Haran, T. E., and Shakked, Z. (2006) Structural basis of DNA recognition by p53 tetramers. *Mol. Cell 22*, 741–753.
53. Daniel, I., Oger, P., and Winter, R. (2006) Origins of life and biochemistry under high-pressure conditions. *Chem. Soc. Rev. 35*, 858–875.
54. 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. *Nat. Genet. 4*, 42–46.
55. Lang, G. A., Iwakuma, T., Suh, Y. A., Liu, G., Rao, V. A., Parant, J. M., Valentin-Vega, Y. A., Terzian, T., Caldwell, L. C., Strong, L. C., El-Naggar, A. K., and Lozano, G. (2004) Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell 119*, 861–872.
56. Olive, K. P., Tuveson, D. A., Ruhe, Z. C., Yin, B., Willis, N. A., Bronson, R. T., Crowley, D., and Jacks, T. (2004) Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell 119*, 847–860.
57. Mizuarai, S., Yamanaka, K., and Kotani, H. (2006) Mutant p53 induces the GEF-H1 oncogene, a guanine nucleotide exchange factor-H1 for RhoA, resulting in accelerated cell proliferation in tumor cells. *Cancer Res. 66*, 6319–6326.
58. Bullock, A. N., Henckel, J., and Fersht, A. R. (2000) Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: definition of mutant states for rescue in cancer therapy. *Oncogene 19*, 1245–1256.
59. Klein, C., Georges, G., Kunkele, K. P., Huber, R., Engh, R. A., and Hansen, S. (2001) High thermostability and lack of cooperative DNA binding distinguish the p63 core domain from the homologous tumor suppressor p53. *J. Biol. Chem. 276*, 37390–37401.
60. Friedler, A., Hansson, L. O., Veprintsev, D. B., Freund, S. M., Rippin, T. M., Nikolova, P. V., Proctor, M. R., Rudiger, S., and

Fersht, A. R. (2002) A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. *Proc. Natl. Acad. Sci. U.S.A. 99*, 937–942.
61. Issaeva, N., Friedler, A., Bozko, P., Wiman, K. G., Fersht, A. R., and Selivanova, G. (2003) Rescue of mutants of the tumor suppressor p53 in cancer cells by a designed peptide. *Proc. Natl. Acad. Sci. U.S.A. 100*, 13303–13307.
62. Proske, D., Blank, M., Buhmann, R., and Resch, A. (2005) Aptamers—basic research, drug development, and clinical applications. *Appl. Microbiol. Biotechnol. 69*, 367–374.