High resolution chemical footprinting and cross-linking experiments have provided a basis for elucidating the overall architecture of the complex between the core DNA binding domain of p53 (p53DBD, amino acids 98–309) and the p21/waf1/cip1 DNA response element implicated in the G1/S phase cell cycle checkpoint. These studies complement both a crystal structure and earlier biophysical studies and provide the first direct experimental evidence that four subunits of p53DBD bind to the response element in a regular staggered array having pseudodyad symmetry. The invariant guanosines in the highly conserved C(A/T)(T/A)G parts of the consensus half-sites are critical to the p53DBD-DNA binding. Molecular modeling of the complex using the observed peptide-DNA contacts shows that when four subunits of p53DBD bind the response element, the DNA has to bend ~30° to relieve steric clashes among different subunits, consistent with recent DNA cyclization studies. The overall lateral arrangement of the four p53 subunits with respect to the DNA loop comprises a novel nucleoprotein assembly that has not been reported previously in other complexes. We suggest that this kind of nucleoprotein superstructure may be important for p53 binding to response elements packed in chromatin and for subsequent transactivation of p53-mediated genes.

Wild type p53 is a widely distributed phosphoprotein that has become fundamental in cancer research (1–3). It functions as a tumor suppressor and is an essential component in the overall architecture of the complex between the core DNA binding domain of p53 (p53DBD, amino acids 98–309) and the p21/waf1/cip1 DNA response element implicated in the G1/S phase cell cycle checkpoint. These studies complement both a crystal structure and earlier biophysical studies and provide the first direct experimental evidence that four subunits of p53DBD bind to the response element in a regular staggered array having pseudodyad symmetry. The invariant guanosines in the highly conserved C(A/T)(T/A)G parts of the consensus half-sites are critical to the p53DBD-DNA binding. Molecular modeling of the complex using the observed peptide-DNA contacts shows that when four subunits of p53DBD bind the response element, the DNA has to bend ~30° to relieve steric clashes among different subunits, consistent with recent DNA cyclization studies. The overall lateral arrangement of the four p53 subunits with respect to the DNA loop comprises a novel nucleoprotein assembly that has not been reported previously in other complexes. We suggest that this kind of nucleoprotein superstructure may be important for p53 binding to response elements packed in chromatin and for subsequent transactivation of p53-mediated genes.

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nural model for the complex of four human p53DBD peptides with an important functional response element: the p21/waf1/cip1 binding site. This model goes considerably beyond that provided by the earlier crystallographic study and is able to rationalize a number of earlier observations including the requirement for DNA bending in the full tetramer complex. It also provides unique insights into possible roles of DNA flexibility in the sequence specificity of p53 binding and suggests possible relationships between the relative orientation of a tetrameric p53 complex on response element DNA and p53 transactivation function.

The model is based on the results of several experiments sensitive to base-specific nucleoprotein contacts between the p53DBD peptides and response element DNA including hydroxyl radical footprinting, missing nucleoside analyses, and methylation and ethylation interference assays. Studies were conducted on a 65-bp oligonucleotide that includes the p21/waf1/cip1 response element; this response element contains both a consensus (Fig. 1A, boxes 1 and 2) and a nonconsensus (boxes 3 and 4) half-site. To facilitate comparison of the present work with the crystallographically determined contact sites (18), we also studied a 67-bp oligonucleotide containing the same 20-bp binding sequence used in the cocrystal structure, designated in this work as the Cho sequence (Fig. 1B).

Our solution results generally agree with the crystallographic contacts for the Cho sequence, although we find evidence for a second binding site of reverse orientation within this sequence that was not observed in the cocrystal. Our results for the p21/waf1/cip1 site show unequivocally that four p53DBD peptides bind this response element in a staggered array and that each consensus pentanucleotide of the p21/waf1/cip1 site makes specific contacts with the p53DBD and its invariant guanosine nucleotide playing a critical role. Hydroxyl radical footprinting demonstrates structural microheterogeneity in the consensus binding sites, suggesting that sequence-dependent structural variability of response elements plays a critical role in the binding of p53 with DNA. Model building of the p53DBD-p21/waf1/cip1 nucleoprotein complex using the results of various chemical probes and footprinting shows that the four bound p53DBD peptides bend the response element by ~50° to relieve the steric clashes among the bound subunits. This model is in quantitative agreement with recent T4-DNA ligase-mediated cyclization studies (19) and with circular permutation gel retardation assays of the p53DBD-p21/waf1/cip1 complex (20).

MATERIALS AND METHODS

Oligonucleotide Synthesis and Labeling—Oligonucleotides used in the study (Fig. 1), were synthesized and were purified on a 15% denaturing polyacrylamide gel. Equimolar amounts of complementary sin-

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Ethylation Interference Assay—Labeled oligonucleotides (1 × 10^6 cpms/100 μl of 50 μm sodium cacodylate, pH 7.0, 1 μM EDTA containing sonicated salmon sperm DNA (1 μg) and (2) were incubated with 0.5 μM of dimethyl sulfate at room temperature for 2 min. The methylation reactions were terminated by adding 50 μl of 1.5 M sodium acetate, 1 μg of mercaptoethanol, 100 μg/ml tRNA and 750 μg of ethanol. The DNA was precipitated twice with ethanol, dissolved in 20 μl of 0.3 M sodium acetate containing 0.2 M EDTA and was precipitated with 500 μl of ethanol. The pellet was washed with 70% ethanol, eluted, and dissolved in 20 μl of binding buffer. Purified p53DBD (4 μg) was added, and the complex was allowed to form for 30 min on ice. Samples were loaded on a mobility shift gel as described above, and the bound and unbound fractions were eluted from the gel, precipitated with ethanol, washed with 70% ethanol, and analyzed on a sequencing gel. Control DNA was also cleaved under identical conditions.

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bated in the presence of labeled oligonucleotides containing the p21/waf1/cip1 and Cho sequences (Fig. 1, A and B) for 40 min on ice in 20 μl of DNA binding buffer. Increasing concentrations of freshly diluted glutaraldehyde were added for 15 min on ice. Partially cross-linked samples were dissociated by boiling in SDS and were loaded on an 8% SDS-polyacrylamide gel. The gel was run at 10 V/cm for 2 h and silver-stained to locate the cross-linked protein-DNA complexes. The same gel was gelled and autoradiographed, and the DNA bands corresponding to the cross-linked species were located by superimposing the autoradiogram on the gel. p53DBD, used as a control in the experiment, was also treated under identical conditions. The protein molecular weight marker was heat-denatured in SDS and loaded as a control.

Electrophoretic Mobility Shift Assay—Labeled oligonucleotides of known concentration were mixed with poly(dI·dC) (200 ng) and incubated with varying amounts of p53DBD in DNA binding buffer at 4 °C for 40 min. The samples were electrophoresed on a 7% nondenaturing polyacrylamide gel in 0.25 × TBE at 4 °C. Identical unbound oligonucleotide bands were run in parallel. The bands were quantitated using a PhosphorImager (Bio-Rad), and the DNA binding affinities of the samples were determined as described by Carey et al. (22). The same gels were later used to determine the stoichiometry of p53DBD binding with the p21/waf1/cip1 and Cho sequences (19).

DNase I Footprinting—p21/waf1/cip1 and Cho duplexes singly end-labeled at the 5'-end of the bottom strand (5 × 10⁶ cpm, ~200 ng) were incubated with 12 μg of p53DBD in DNA binding buffer. The complex was allowed to incubate for 40 min at 4 °C. An aliquot of the sample was checked on a nondenaturing polyacrylamide gel to ensure complete saturation of the DNA binding sites with p53DBD. The samples were mixed with 5 mM MgCl₂ and 10 mM CaCl₂ (50 μl) of a stop solution (0.2 M NaCl, 30 mM EDTA, 1% SDS, and 100 μg/ml tRNA). The samples were extracted with phenol:chloroform, precipitated with ethanol, washed with 70% ethanol, dried, electrophoresed on a 12% denaturing polyacrylamide gel, and autoradiographed.

G and G + A Cleavage, Gel Electrophoresis, and Densitometric Scanning—The control G and G + A cleavage reactions were carried out using the now standard protocol reported by Maxam and Gilbert (23). Sequencing gels used to analyze the DNA samples contained 12% acrylamide:bisacrylamide) and 8 M urea. The samples were extracted with phenol:chloroform, precipitated with ethanol, washed with 70% ethanol, dried, electrophoresed on a 12% denaturing polyacrylamide gel, and autoradiographed.

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Model Building—To generate stereochemically acceptable DNA structures, the program DNAminiCarlo was used (25, 26). The generalized coordinates of bases and deoxyriboses served as independent parameters, and the sugar-phosphate chain was closed so that the bond lengths and angles had standard values. Various straight and bent DNA conformations were considered, among them (i) a 20-mer from the p53-DNA complex (18); (ii) regular uniform B-DNA with identical dihedral step parameters averaged over ~40 B-DNA crystal structures (26); (iii) nonuniform B-like DNA with the p21/waf1/cip1 sequence, the dimeric steps having sequence-dependent conformations corresponding to the averages taken from ~40 protein-DNA complexes (26); and (iv and v) bent DNA modeled similarly as in conformation iii but with roll angles in the CA:GT dimers occurring at the junctions between the adjacent pentamers. Specifically, the CA roll angle was 6° in the structure modeled in conformation iii and either 15° (iv) or 20° (v). In the cocrystal structure (18), this roll angle was 4°, and it is 0° in uniform B-DNA. To account for the rigidity of the sugar-phosphate backbone and the correlation between roll and twist, the CA twist in the latter two structures was reduced from 36° (conformation iii) to 31 and 28° in conformations iv and v, respectively (26). The protein domains were positioned with respect to the p21/waf1/cip1 response element DNA in the following way. The p53 domain, together with the pentamer GGCGA:TGCC from the cocrystal structure to which it is bound specifically in that study (18), was superimposed on a pentamer quarter-site from the p21/waf1/cip1 response element. For this purpose, the C⁵ atoms were used; the r.m.s. deviation was never more than 0.6–0.7 Å. Following this procedure, the p53-DNA complexes were analyzed to evaluate the closest peptide-peptide contacts reported here. It was found that, in all cases when p53 domains were bound to unbent DNAs (i.e. with unadjusted CA roll angles as modeled in conformations i–iii), unacceptable steric clashes occurred. By contrast, all clashes vanished in the bent DNA models (iv and v).

RESULTS

Hydroxyl Radical Footprinting of p53DBD with p21/waf1/cip1 and Cho Sequences—Hydroxyl radical cleavage (27) was used to footprint p53DBD complexed with the p21/waf1/cip1 and Cho sequences (Fig. 1, A and B). Fig. 2, A and B, shows hydroxyl radical footprinting data for the p53DBD-p21/waf1/cip1 and p53DBD-Cho complexes, respectively. Densitometric plots of the various lanes are shown in Fig. 2, E and F. The top strand of the naked p21/waf1/cip1 response element (as presented in Fig. 1A) shows reduced cleavage at CATG and TGTT base sequences within the 20-bp consensuses binding site, with clear minima at TG sequence elements (marked as arrows in

FIG. 1. A, 65-mer oligonucleotide containing the 20-bp p21/waf1/cip1 response element (boldface type and boxed). B, 67-mer oligonucleotide containing the 20-bp Cho (18) sequence. Half-sites are separated by solid lines, and quarter-sites are separated by dashed lines.
The unique hydroxyl radical cleavage profiles suggest that the p21/waf1/cip1 response element has narrowed minor grooves at CATG and TGTT sequences and a relatively wider minor groove at the CCCAA sequence. p53DBD binding further narrows the minor grooves involving ATGT and TGTT bases in the two half-sites, compresses the major groove at the GTTGGG bases, and shields their sugar-phosphate backbone from the minor groove side, leading to their reduced cleavage. This may, in turn, further expose the sugar-phosphate backbone of the complementary CCCAA sequence from the minor groove side, making it more susceptible to hydroxyl radical cleavage.

The higher cleavage at the ACAT and CAT bases in the top strand as compared with the ATGT and TGTT sequence at the top strand probably indicates asymmetric distortion of the double helix in this region, leading to differential exposure of the sugar-phosphate backbone of the two strands to hydroxyl radicals. The relatively AT-rich regions of the consensus binding site, which have narrow minor grooves, are spaced at integral helical periodicity and occur on the same face of the double
helix (Fig. 5A). It is also of interest that GGCC sequences, previously shown to have major groove-directed bending (29–31), occur in the central region of many functionally important p53 response elements. The helically phased CA/TG sequence elements in the highly conserved region of the consensus binding site have been shown to be kinked in the CAP nucleoprotein complex (32) and may be similarly kinked in other regulatory complexes (33). Thus, it is possible that intrinsic flexibility in these sequence elements may promote the formation of a more stable p53-DNA complex by facilitating specific protein-DNA and protein-protein interactions. A recent study from this laboratory has shown that p53DBD binds the p21/waf1/cip1 response element as a tetrapeptide with high cooperativity and that the DNA is bent by ∼50–60° in solution (19). Thus, it is likely that a region of compressed major groove, located between two regions of narrow minor groove, as suggested by the present hydroxyl radical footprinting, may provide the structural basis for such a bending. We also observe that there is an inherent asymmetry in the footprinting patterns for the two half-sites, which otherwise might be expected to be identical. We believe that such an inherent asymmetry in the complex may play a crucial physiological role in terms of bending directionality, as in the case of the TATA-binding protein-TATA complex (34).

The hydroxyl radical cleavage patterns for the Cho sequence (Fig. 1B) and its complex with p53DBD are shown in Fig. 2B, and densitometric plots are shown in Fig. 2F. The top strand of the unbound Cho sequence shows reduced cleavage at the TCT and the CA sequence elements within the consensus half-site, while a higher cleavage frequency occurs at the GGG bases (marked with dashed brackets). The cleavage frequency is also markedly reduced at a TT element outside this half-site (Fig. 2B, lane C; Fig. 2F, plot a). Binding with p53DBD further diminishes the cleavage frequency of the TCT and TT sequences, while it enhances the cleavage frequency in the GGG bases (Fig. 2B, lane F; Fig. 2F, plot b). However, cleavage in the bottom strand is enhanced in the complementary AGAC and AA sequences and reduced in the TTGC bases. Binding with p53DBD further reduces the cleavage pattern in the TTGC sequence. The data clearly point to a narrow minor groove in the TCT sequence and a relatively compressed major groove in the GGCC region. These footprinting data are in general agreement with the crystallographic results (18), in which a narrowed minor groove was observed in the TCTAG sequence element due to high propeller twisting. However, the cleavage pattern in the GGCCAA region demonstrates a relatively compressed and shielded major groove and indicates binding of p53DBD in this region, consistent with the crystallographic results (18).

**Missing Nucleoside Experiments**—Specific base contacts for both complexes were determined using missing nucleoside experiments (35). Fig. 2, C and D, show the missing nucleoside data for the p53DBD-p21/waf1/cip1 and p53DBD-Cho complexes respectively; corresponding densitometric plots are shown in Fig. 2, G and H. The bound fraction of DNA in the top strand of p53DBD-p21/waf1/cip1 complex (Fig. 1A) leads to weaker bands for bases in the two ATGG sequence elements in each half-site and intense bands for bases in the central CCCAAC sequence (Fig. 2C, lane B; Fig. 2G, plot a). The opposite pattern is observed in the unbound fraction, i.e. bands corresponding to bases in the central CCAA region are weak, whereas bands for bases ATGG in both of the half-sites are more intense (Fig. 2C, lane U; Fig. 2G, plot b). In the bottom strand, the protein-bound DNA shows weaker bands for bases in ATGG in both half-sites, whereas in the unbound fraction these bases show intense bands (Fig. 2C, lanes B and U; Fig. 2G, plots c and d). These data clearly show that ATGG sequence elements in both half-sites of the top and bottom strands make important contacts with the bound protein. The modification or absence of either of these base contacts greatly reduces the affinity of p53DBD with DNA. It is significant that these bases show reduced cleavage in direct hydroxyl radical footprinting (Fig. 2, A, B–E, and F), suggesting a narrow minor groove in these regions and further substantiating their direct involvement in the stability of the nucleoprotein complex.

Missing nucleoside data for the p53DBD-Cho complex are shown in Fig. 2D with corresponding densitometric plots in Fig. 2H. The top strand of the bound fraction shows relatively weak bands in the GGGC and CTA sequence elements (Fig. 2D, lane B; Fig. 2H, plot a). In the unbound fraction, strong bands are observed in this sequence element, indicating direct contact of these bases with the bound p53DBD (Fig. 2D, lane U; Fig. 2H, plot b). The bottom strand in the bound fraction shows weak bands in the TGCC element, while in the unbound fraction these bases exhibit much stronger bands (Fig. 2D, lanes B and U; Fig. 2H, plots c and d). Thus, the data clearly demonstrate that bases in the GGGC element in the top strand and in the TGCC element in the bottom strand make critical contacts with the bound peptide. Most of the contact sites probed by hydroxyl radical footprinting and missing nucleoside probing in the p53DBD-Cho complex are also observed in the crystal structure data (18).

**Methylation Interference Assays**—Methylation interference assays have been widely used to identify contacts between bound proteins and methylated guanosines in the major groove of the DNA (36). Fig. 3A shows the methylation interference results for the p53DBD-p21/waf1/cip1 complex. Corresponding densitometric plots of the different lanes are shown in Fig. 3E. The bound DNA fraction of the top strand (Fig. 1A) shows reduced cleavage (greater interference) at the guanosines in the two TG sequence elements (G7 and G17 in Fig. 1A) compared with more intense cleavage at these sites in the unbound fraction (Fig. 3A, lanes B and U; Fig. 3E, plots a and b). This suggests that these guanosines are in direct contact with p53DBD in the major groove. In the bottom strand, the bound DNA fraction shows reduced cleavage of guanosines at the two TG elements (G14 and G14') compared with much stronger bands in the unbound DNA fraction (Fig. 3A, lanes B and U; Fig. 3E, plots c and d). The residues in the central GGG region of the bottom strand show reduced cleavage in the bound DNA fraction as compared with the control DNA (lane C), but these guanosine signals are missing entirely from the unbound fraction.

These data clearly show that the central GGG guanosines in the bottom strand contact the p53DBD differently from those in the two TG elements. Methylation of the former does not affect p53DBD binding, whereas the guanosines in the TG doublets evidently make structurally important contacts with the protein, probably at the N-7 position, and when these sites are modified by methylation, p53DBD does not bind to DNA. It is important to note that TG guanosines are present in each pentamer quarter-site and constitute the invariant base in most of the high affinity p53 binding sites reported so far. All four guanosines in the TG base doublets (G7, G17, G14 and G14') are tandemly arrayed in the alternate major groove of the double helix, suggesting that all four subunits of p53DBD bind in the major groove.

The methylation interference data for the p53DBD-Cho complex are shown in Fig. 3B with corresponding densitometric plots in Fig. 3F. In the top strand, the p53DBD-bound fraction shows relatively weaker bands for the GGG (G7, G8, G9) sequence and G13 compared with the unbound fraction (Fig. 3B, lanes B and U; Fig. 3F, plots a and b). In the bottom strand,
both the bound and unbound fractions show relatively intense bands for guanosine G^{10}, with the stronger band observed in the unbound DNA fraction. The data clearly indicate that the bases G{7}, G{8}, G{9}, and G{13} in the top strand and G{10} in the bottom strand make direct contact with the bound peptide, which is consistent with the cocrystal structure (18) in which Arg{283} and Lys{120} are in direct contact with G{7} and G{8}, while Arg{280} contacts G{10}. G{13} (top strand) either makes direct contact with p53DBD at its N7 position or is in very close proximity with the peptide in the complex. This observation is also consistent with the cocrystal structure in which the sugar-phosphate backbone of G{13} and T{14} makes direct contact with Arg{248}. We do not observe contacts between p53DBD and bases in the GGA sequence element at the end of the top strand, i.e. G{18}, G{19}, and A{20}, as observed in the cocrystal structure.

On the other hand, bases in the TG elements appear as highly intense bands in the unbound fraction (Fig. 3C, lane U; Fig. 3G, plot d). These data clearly indicate that the DNA backbone at the ATGT sequences in the two half-sites is in contact with the p53DBD in the complex, which is consistent with the hydroxyl radical and missing nucleoside data. Particularly, the sugar-phosphate backbone at G{7}, G{17}, G{4}, and G{14} makes very critical contacts with p53DBD, and ethylation of phosphates at these residues greatly reduces the binding affinity of the p53DBD-p21/waf1/cip1 complex.

Ethylation Interference Assays—Sugar-phosphate contacts were probed by ethylation interference (37). Results for the p53DBD-p21/waf1/cip1 complex are shown in Fig. 3C with corresponding densitometric plots of the bound and unbound fractions shown in Fig. 3G. For the top strand (Fig. 1A), the bound fraction shows weaker bands for bases in the ATGT sequence elements in the two half-sites with clear minima at the two TG base doublets (Fig. 3C, lane B; Fig. 3G, plot a), whereas in the unbound fraction, these two guanosine residues show significantly more intense bands (Fig. 3C, lane U; Fig. 3G, plot b). The AA sequence elements at the center of the binding site also appear as bands of medium intensity. For the bottom strand, the bound fraction (Fig. 3C, lane B; Fig. 3G, plot c) shows weaker bands in TGT sequence elements in both half-sites, with clear minima at the G residues (marked as arrows).

On the other hand, bases in the TG elements appear as highly intense bands in the unbound fraction (Fig. 3C, lane U; Fig. 3G, plot d). These data clearly indicate that the DNA backbone at the ATGT sequences in the two half-sites is in contact with the p53DBD in the complex, which is consistent with the hydroxyl radical and missing nucleoside data. Particularly, the sugar-phosphate backbone at G{7}, G{17}, and G{13} makes very critical contacts with p53DBD, and ethylation of phosphates at these residues greatly reduces the binding affinity of the p53DBD-p21/waf1/cip1 complex.
of p53DBD bound to the p21/waf1/cip1 and Cho response elements. Fig. 4A shows an SDS-polyacrylamide gel electrophoresis analysis of the cross-linked species of the p53DBD-p21/waf1/cip1 complex (Fig. 4A, lanes 6, 7, and 8) and the p53DBD-Cho complex (lanes 3, 4, and 5) at three glutaraldehyde concentrations. The p53DBD-p21/waf1/cip1 complex shows four bands of 23, 46, 69, and 92 kDa, representing monomer, dimer, trimer, and tetramer, respectively. Direct autoradiography confirms that each band is associated with its corresponding DNA fragment (Fig. 4B, lanes 6, 7, and 8). This demonstrates clearly that the p53DBD peptide associates with the full p21/waf1/cip1 response element as a tetramer, even in the absence of the wild type p53 oligomerization domain, in agreement with gel band shift and ultracentrifugation results (19). With the p53DBD-Cho half-site, cross-linked species include trimers and tetramers in the SDS-polyacrylamide gel (Fig. 4A, lanes 3–5) but only monomers and dimers in the autoradiography (Fig. 4B, lanes 3–5). This indicates that p53DBD associates with the Cho sequence primarily as a monomer or dimer. The p53DBD peptide cross-linked in the absence of response elements can also weakly tetramerize, but the primary cross-linking products are monomers and dimers (Fig. 4A, lane 2). In Fig. 4, A and B, the DNA and protein bands move as doublets in the gel. An explanation for this commonly observed phenomenon may be that the faster band in each doublet arises from additional intrapeptide cross-linking and incomplete denaturation of the protein in SDS, leading to a more compact structure for the denatured complex and therefore slightly higher mobility (39).

A gel electrophoretic mobility shift assay was used to determine the binding affinity and stoichiometry of the p53DBD with the p21/waf1/cip1 and Cho sequences and is shown in Fig. 4C. It is evident that p53DBD binds the p21/waf1/cip1 response element only as a tetramer with high cooperativity. We assign the minor bands of slightly lower mobility than the tetramer complex to the nonspecific binding of p53DBD with the single-stranded duplex overhangs (40, 41). No intermediate bands, e.g. dimer or trimer, are evident. We estimate a mean equilibrium dissociation constant, $K_d$, of $4.2 \times 10^{-9}$ M for the p53DBD-p21/waf1/cip1 complex, which is in satisfactory agreement with the value ($8.3 \pm 1.4 \times 10^{-9}$ M) obtained by analytical ultracentrifugation (19), since the $n$-fold difference most probably results from a lower dissociation rate of the complex in the gel (42). In the case of the Cho sequence, p53DBD binds as a dimer with an estimated mean equilibrium dissociation constant $K_d$ of $7.8 \times 10^{-7}$ M. This level of binding affinity is not much greater than that for many nonspecific nucleoprotein complexes. However, there is no evidence in Fig. 4, B, C, and E, for the association of a third p53DBD monomer as was observed in the cocrystal (18).

Fig. 4, D and E, show DNase I footprinting results for p53DBD binding to the p21/waf1/cip1 and Cho sequences, respectively, at saturating protein concentrations. With the p21/waf1/cip1, the footprint is extremely tight, spanning all 20 bp of the response element and exhibiting absolutely no evidence of nonspecific binding (Fig. 4D, lane F). This footprint is similar to that observed for the wild type p53 protein complexed with ribosomal gene cluster response element (43). These data suggest that p53DBD is tightly associated with the response element, thereby excluding completely the possibility of digestion of the DNA by the enzyme. A tight footprint is also seen with the Cho sequence under identical conditions, which covers completely the 10-bp half-site (42). In the case of the Cho sequence, p53DBD cross-linked in the absence of DNA shows four bands of 23, 46, 69, and 92 kDa, representing monomer, dimer, trimer, and tetramer, respectively. Direct autoradiography confirms that each band is associated with its corresponding DNA fragment (Fig. 4B, lanes 6, 7, and 8). This demonstrates clearly that the p53DBD peptide associates with the full p21/waf1/cip1 response element as a tetramer, even in the absence of the wild type p53 oligomerization domain.

**DISCUSSION**

### A Comparison of the Footprinting Data for p53DBD Complexed with the p21/waf1/cip1 and Cho Sequences

We have summarized the above results on the p53DBD-p21/waf1/cip1 and p53DBD-Cho complexes in Figs. 5, panels A and B, respectively. For the p53DBD-p21/waf1/cip1 complex, certain structurally related characteristics become immediately apparent. (i) Most of the contact signals occur in the major groove of the DNA in a staggered array along the two helical turns of the response element in which each of the four bound p53DBD peptides occupies a single pentanucleotide quarter-site and faces outward, away from the DNA, in the same direction. The data indicate that each p53DBD binds to the major groove in agreement with the cocrystal structural study (18). (ii) Four
guanosine bases, G7 and G17 in the top strand and G4' and G14' in the bottom strand, which are part of an invariant sequence motif in each pentamer quarter-site, are identified as contacts in all of the four footprinting techniques used. These bases are in phase along the helix with a separation of 10 bp and are located in the major groove of the DNA. (iii) Most of the observed contact points are clustered in the major groove with relatively few contacts in the minor groove also in agreement with the specifically bound p53DBD in the crystal structure (18). (iv) The GTTG sequence of the bottom strand shows higher protection from hydroxyl radical cleavage than does the complementary CAAC sequence on the opposite strand, suggesting that it is shielded by bound peptide from the minor groove side, whereas the complementary CAAC sequence is exposed.

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sequence in opposite orientations along the DNA. One is to the GGCGA quarter-site element in the upper strand (Fig. 1B; Fig. 5B, c), and the second is to the TGCCC element in the lower strand. Contacts in the first of these are consistent with those observed in the cocrystal structure (Fig. 5B, a) (18), and since this is the only specific binding site observed crystallographically, it is likely that it is a site of higher binding affinity. A direct repeat of TGCT, having close homology with the TGCCC element in the Cho sequence, has previously been shown to be a functionally important p53 binding site (44, 45).

The relatively lower quality of the footprinting data for the p53DBD-Cho complex compared with the p21/waf1/cip1 complex suggest that, in solution, a dynamic equilibrium may exist between complexes involving these two sites. In the p21/waf1/cip1 response element, all the four invariant guanosines occur in the Cho sequence. By contrast, in the Cho sequence, only one guanosine occurs in the TG element, whereas the other occurs in an AG element. Since we observe intense contact signals for the guanosines in the p21/waf1/cip1 TG elements but not in the Cho half-site AG, this suggests that AG elements are very poor contact sites for p53 binding. Again, these results are consistent with earlier observations (18, 46).

Stereochemical Model for the p53-DNA Complex—To rationalize our footprinting and cross-linking data presented above, we have developed a computer-assisted structural model for the p53DBD-p21/waf1/cip1 complex. In this model, each of the four bound p53DBD peptides is attached to a single pentameric quarter-site as suggested by the present footprinting results. The p53DBD coordinates are taken from the cocrystal structure; in that study, no changes in the p53DBD structure were observed upon DNA binding (18). Docking of the peptides to the DNA is determined by the specific contacts found here (Fig.
The model requires that, to avoid severe interpeptide steric clashes, the four p53DBD peptides must induce bending of the response element DNA by 50° (Fig. 8), a result in close agreement with recent cyclization studies (19) and with circular permutation gel electrophoresis assays (20). Specifically, we find two sets of steric clashes in the complex between four p53 domains and "straight" DNA. The first of these occurs between the zinc-binding H1 helices (18) of peptides bound to two adjacent pentamers (Figs. 6A and 7A); these two p53 domains are bound to opposite sides of the duplex in opposite orientations and "embrace" the DNA. The second clash occurs between the two peptides in similar orientation and bound to the same side of the helix; they are separated by a single helical turn (Fig. 6C). It is important to note that these clashes were observed in all complexes involving unbent DNA that were analyzed (see "Materials and Methods").

The above steric clashes are relieved if the p21/waf1/cip1 DNA is bent by positive rolling through 15° toward the major grooves in the CA:TG dimers indicated by chemical points: GAACzAuTzGTCCCAACzAuTzGTTG. Such axial bending does not interfere with the specific interaction of the p53DBD peptides with their cognate pentamers. The two adjacent rolls in the

**Fig. 6.** Diagrams showing how molecular modeling determines steric clashes between the four p53DBD peptides bound to the p21/waf1/cip1 response element as directed by the chemical probe results and how these clashes are relieved by bending the DNA by a roll at the CA:TG sequence elements. Nomenclature for p53DBD structural elements is from Cho et al. (18). The zinc-binding H1 helices are shown as ellipses. The H2 recognition helices are shown as sharply pointed regions buried in the major grooves. The four pentamer response element quarter-sites are numbered 1–4. Pentamers 1 and 3 and pentamers 2 and 4 are in a parallel orientation, while pentamers 1 and 2 and pentamers 3 and 4 are antiparallel. A, in unbent DNA, binding of two p53DBD peptides to pentamers 1 and 2 (antiparallel orientation) is accompanied by steric hindrance between the H1 helices (overlapped ellipses; see legend to Fig. 7A for details). B, this clash is relieved by bending the DNA toward the major groove along the dyad axis by a positive roll at the TG:CA sequence elements as discussed under "Discussion" (see legend to Fig. 7B for details). C, in unbent DNA, binding of two p53DBD peptides to pentamers 1 and 3 (parallel orientation) is accompanied by steric hindrance. The darkened overlap area represents steric clash between residues 99, 167, 170, and 210 from one peptide and residues 224, 140, 199, and 201 from the other; a steric clash is presumed if the distance between two heavy atoms in a residue pair is less than 2.4 Å. D, this clash is relieved by bending the DNA toward the major grooves along the dyad axes at two points separated by 10 bp. This bending is the same as in B and is affected by a positive roll at the TG:CA sequence elements as discussed under "Discussion."

5A). The model requires that, to avoid severe interpeptide steric clashes, the four p53DBD peptides must induce bending of the response element DNA by 50° (Fig. 8), a result in close agreement with recent cyclization studies (19) and with circular permutation gel electrophoresis assays (20). Specifically, we find two sets of steric clashes in the complex between four p53 domains and "straight" DNA. The first of these occurs between the zinc-binding H1 helices (18) of peptides bound to two adjacent pentamers (Figs. 6A and 7A); these two p53 domains are

**Fig. 7.** Interactions between the H1 helices and L3 loops in the p53-DNA complexes for unbent DNA (18) (A) and bent DNA (B). A, the H2 helix from the left domain (green) interacts specifically with the pentamer GGGCA (also shown in green) (18). The right domain is located symmetrically, so that its H2 helix (blue) interacts with the pentamer AGACT shown in blue (for details, see "Materials and Methods"). Inset, the H1 helices interdigitate, producing unacceptable steric clashes (the heavy atoms in the protein are shown as spheres of 50% van der Waals radii). The zinc atoms are shown as large red spheres. The clashes specifically involve Arg181 and Pro177. Similar clashes occur using other unbent DNA models including canonical B-form DNA (see "Materials and Methods"). B, bending the DNA toward the major groove at the junction between the green and blue pentamers relieves the H1-H1 clash (the bend is directed away from the viewer). The important spatial complementarity between the two H1-L3 moieties is shown in the inset; in this view, the protruding residues are Arg181 (in the H1 helix) and Met243 (in the L3 loop).
CA:TG dimers induce a bend toward the major groove as indicated in Fig. 6, B and D. It is important to note that the same DNA bends relieve both types of clashes: antiparallel, as in Figs. 6A and 7A, and parallel, as in Fig. 6C. The putative rolls in the CA:TG steps are consistent with the well known flexibility of these dinucleotides (33), demonstrated in numerous x-ray structures of pure B-DNA and protein-DNA complexes (26, 32, 47), as well as by gel electrophoresis (48). According to energy calculations, these dinucleotides can also bend anisotropically toward the major groove (25).

This direction of DNA bending is entirely consistent with the consensus p53-binding sequence. All four possible tetramers allowed by the consensus, i.e. CA:TG, CT:AG, CA:AG, and CT:AG, contain dinucleotide elements that can flex toward the major groove. In addition to CA:TG, the CT:AG element also prefers bending toward the major groove (26, 49, 50). AA:TT or AT:AT dinucleotides are the central elements in three of the tetranucleotides. Both dinucleotides prefer a negative or zero roll angle (26); thus, the minor groove is expected to be relatively narrow, which is desirable for interactions with the highly conserved Arg248 (18). The CT:AG tetranucleotide contains the TA:TA element, which can assume roll angles between -6.4° in free DNA (51) to +12° in the trp repressor complex (52) and appears, in addition, to possess unusual torsional flexibility (31, 32). However, the CT:AG element is reported to be extremely rare in functional genomic p53 response elements (15).

In the detailed model presented in Fig. 8, the roll angles for CA:TG are taken as 15°. This leads to an overall bend of 50°, which is consistent both with the present experimental results and with the previous cyclization studies (19). CA:TG roll angles between 15 and 20° are sufficient to relieve all peptide-peptide clashes; 15° seems preferable, however, since this leads to a structure in which the p53DBD peptides are in close proximity to one another (Figs. 7b and 8) and hence can interact to produce the observed binding cooperativity (19).

According to this model, shown in Fig. 8, the C-terminal residues of p53 are located on the inside of the DNA curve. The
C-terminal domains are involved in oligomerization, protein-protein interactions, and the modulation of DNA binding (53). This arrangement may be advantageous for the stabilization of the entire superstructure in the binding of the wild type protein. On the other hand, the N-terminal region, which contains the transactivational domain, is located on the outside of the loop, where it may be more accessible to other proteins. The model presented in Fig. 8 suggests that the intrinsic bendability of p53 response elements favors the same direction of DNA bending as in the p53 nucleoprotein complex. Thus, p53 response elements may be wrapped in chromatin in such a way as to facilitate the approach and binding of the protein. In other words, p53 may prefer to bind response element DNA from the side that is normally exposed in chromatin structures. This is consistent with a transcriptional role for p53 that may be mediated by the architectural organization of chromatin (54, 55) and the looping of DNA (56).

Conclusions—The p21/waf1/cip1 site is one of the most important functional sites for p53 binding presently known (57). The full site consists of a consensus (16) and a nonconsensus half-site, making it generally representative of a broad class of p53 response elements (15). The data presented here provide more complete picture than previously available for the overall spatial arrangement of the peptides on the p21/waf1/cip1 response element DNA along with critical p53DBD-DNA contacts and important information on the conformation of the binding site DNA. The footprinting and contact data show that a p53DBD is bound in the major groove on each of the four pentameric quarter-sites of the p21/waf1/cip1 response element with the peptides oriented in the same direction. Molecular modeling shows that relief of steric clashes in the bound peptides requires the DNA to bend at flexible TG:CA sequences to the same extent as in the p53 DNA nucleoprotein complexes. This is consistent with earlier studies that demonstrated substantial DNA bending in several p53DBD-DNA complexes (19) and suggests a possible role for the variation observed in the sequences of known naturally occurring functional p53 response elements (15). The present findings support the concept that both specificity and stability of p53 DNA-nucleoprotein complexes are controlled by a complex interplay of specific sequence contacts as well as inherent structural features of the DNA. In addition to deformation of the DNA and the sequence-directed requirement of the two narrow minor grooves and a compressed major groove as found in this study, these effects must include a (possibly variable) set of specific protein-DNA and intra-p53 protein-protein contacts and may also include specific or nonspecific inter-p53DBD interactions.

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REFERENCES
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