p53 Binding to Nucleosomal DNA Depends on the Rotational Positioning of DNA Response Element

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The sequence-specific binding to DNA is crucial for the p53 tumor suppressor function. To investigate the constraints imposed on p53-DNA recognition by nucleosomal organization, we studied binding of the p53 DNA binding domain (p53DBD) and full-length wild-type p53 protein to a single p53 response element (p53RE) placed near the nucleosomal dyad in six rotational settings. We demonstrate that the strongest p53 binding occurs when the p53RE in the nucleosome is bent in the same direction as observed for the p53-DNA complexes in solution and in co-crystals. The p53RE becomes inaccessible, however, if its orientation in the core particle is changed by ~180°. Our observations indicate that the orientation of the binding sites on a nucleosome may play a significant role in the initial p53-DNA recognition and subsequent cofactor recruitment.

The p53 protein is the object of intensive investigation because of its preeminent role as a tumor suppressor in human cancers (1–3). p53 is critically involved in coordinating the cellular response to various metabolic and genotoxic stresses. In response to cellular stress, including DNA damage, ribonucleotide depletion, and hypoxia, cells containing WTp53 undergo cell-cycle arrest or apoptosis depending on the severity of the insult, position in the cell-cycle, and cell lineage. p53 is uniquely positioned at a central node for pathways involving cell-cycle progression, transcription, and DNA repair and is involved in a network of protein-protein and protein-nucleic acid interactions of amazing complexity (4–6).

p53 is a sequence-specific DNA-binding protein that acts as a transcriptional regulator for a wide variety of genes involved in the genotoxic stress response pathway (7, 8). The p53 protein possesses classical features of a eukaryotic transcription factor (TF), including a sequence-specific DNA binding domain (DBD), a transactivation domain at its N terminus, and a tetramerization domain at its C terminus (9, 10). Mutations in the p53 gene are associated with more than 50% of all forms of human malignancies (11). Most tumor-derived p53 mutants were found to be defective in DNA binding, and this finding has instigated an intensive search for the molecular details of p53-DNA recognition.

Numerous studies over the past decade have provided significant insights into the mode of DNA recognition by p53 (12–14). Normally, p53 binds to DNA as a tetramer, recognizing two decamers, RRRWWGGYYY (pseudo-symmetric half-sites), separated by a variable spacer (R stands for purine, Y for pyrimidine, and W for A or T). The decamers are separated by as many as 13–14 bp in the functional p53REs. However, in the strongest p53 binding sites, the spacer is short, 0–1 bp, and the central tetramer is most frequently CATG (15). Upon binding to such sites, p53 induces DNA bending, first observed in ligase-mediated cyclization experiments (16). Remarkably, the degree of p53-induced bending of DNA depends on the sequence of p53RE, and the most pronounced DNA bending correlates with high affinity p53 binding sites (17). According to theoretical predictions and gel electrophoresis data (18, 19), DNA is bent into the major groove in the CATG tetramers. These predictions have been confirmed recently, when the structures of several dimeric p53DBD-DNA complexes were solved by crystallography (20). The general architecture of the tetrameric p53-DNA complex consistent with these results implies that the p53 core domains are positioned on the outer side of the DNA loop, which is potentially important for p53 interaction with nucleosomal DNA. In other words, wrapping DNA around the histone core can facilitate p53 binding by exposing the cognate DNA site in a bent conformation favorable for p53-DNA recognition.

To test this inference, we have systematically analyzed the role of nucleosomal positioning of p53REs in the sequence-specific recognition by p53. We report that purified recombinant human p53 expressed in baculovirus (WTp53) and p53DBD (amino acids 96–308) show preferential binding to nucleosomes when the two half-sites of the RE are bent into the
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major grooves in the central CATG fragments, so that their minor grooves are exposed for the protein binding. Other orientations of the RE significantly diminish or completely abolish p53 binding. Binding of p53 to nucleosomal RE does not destabilize the overall nucleosomal architecture or cause any substantial rearrangement of DNA on the octamer surface. Our data indicate that p53 belongs to a class of transcription factors that can recognize certain binding sites embedded in a nucleosomal structure and that the initial orientation of the binding site may play a significant role in the binding of p53 to chromatin and subsequent cofactor recruitment. We propose that nucleosomal positioning sequences may preset certain p53 binding sites in a more favored rotational frame for rapid recognition by p53 in the chromatin. Alternatively, radiation-induced DNA damage may induce changes in the rotational settings of p53REs, leading to more favorable positions for p53 binding. Our results underscore the importance of nucleosomal organization for the recognition of p53 binding sites in the genome and suggest a putative role for nucleosome dynamics and chromatin remodeling in p53 function.

EXPERIMENTAL PROCEDURES

Construction and Design of the Nucleosome Positioning Sequences—The nucleosome positioning sequences were constructed by subcloning a series of oligonucleotides having two groups of the phased A-tracts, separated by a p53RE and variable spacers, into the EcoRI and BsrGI sites of the plasmid Litmus 39 (New England Biolabs, Ipswich, MA). The positioning sequences are organized as follows: 5' - BsrGI-(A-tract DNA: CGGGCAAAAAACGGGCAAAA|AACGGCAAAAAACGGGCAAA|CGGGCAAAA|AACGGCAAAAAACGGGCAAA)-HindIII-ApaI-variable spacer-(A-tract DNA: CGGGCAAAAAACGGGCAAA|AACGGCAAAAAACGGGCAAA)-HindIII-Apal-3'. The vertical bars denote the center of the p53 binding site and the centers of the A-tract DNA curvature. The length of the fragment S3 between the second and third bars varies from 30 to 42 bp (Fig. 1A). The variable spacers help to change the rotational orientation of the p53RE located close to the center without a significant change in its translational position (Fig. 1B). All of the recombinant plasmids were digested with BsrGI, labeled with [α-32P]dATP and Klenow fragment, and digested with ApaI. The labeled probes were further purified by 10% native PAGE to eliminate free label.

Purification of WTp53 and p53 DNA Binding Domain (p53DBD)—A human p53 cDNA clone encoding amino acid residues 96–308 was amplified by PCR using p53-specific primers 5’-ATATCATATGGTCCTTCCAGAAGACCTAT-3’ and 5’-ATATGGATCTCAGTGCCTGCTATGGTCTC-3’. The amplified product was cloned in the pet12a expression vector (Novagen, Gibbstown, NJ), and the core DNA binding domain was overproduced in Escherichia coli BL21 (DE3) and purified as described (21). Human wild-type p53 containing an HA epitope at the N terminus was purified in baculovirus by infecting S9 cells with human p53 recombinant virus. Cells were harvested 48 h post-infection and extracted, and HA-tagged p53 was immunopurified over a mouse anti-HA monoclonal antibody (12CA5)-conjugated protein A-Sepharose column. Purified proteins were analyzed by SDS-PAGE followed by silver staining. All p53s were greater than 95% homogenous and contained no detectable proteases, DNase, or RNase activity (Fig. 1C).

Nucleosome Reconstitution and Purification—Nucleosomes were reconstituted onto radiolabeled DNA fragments either by exchange from chicken erythrocyte core particles or by dialysis from high salt with purified chicken histones (22). Labeled BsrGI and Apal DNA fragments (50 ng (5 μl)) were suspended in 100 mM Tris-Cl, pH 7.5, 2 mM EDTA (5 μl), 5 mM NaCl (10 μl), sonicated salmon sperm DNA (1 μg/μl (5 μl)), chicken nucleosome core particles (1 μg/μl (5 μl)), and sterile water (20 μl) in a total volume of 50 μl. After every 20 min at 37 °C, the reconstitution reaction was subjected to stepwise dilution with (20, 55, and 375 μl of 10 mM Tris-Cl, pH 7.5, 0.2 mM EDTA). At the final step, 10 mM Tris-Cl, 0.2 mM EDTA (2 ml) was added, and the reaction was spun through a Microcon-30 column (Millipore, Billerica, MA) for 30 min. The reconstituted nucleosomes were electrophoresed for 7 h on a 4.5% polyacrylamide gel (acylamide:bisacylamide, 40:1) using 20 mM HEPEs, pH 7.5, 1 mM EDTA, and 5% glycerol, as a constant recircularization buffer. The labeled nucleosomes were gel-eluted with 20 mM HEPEs, pH 7.5, 1 mM EDTA at 4 °C overnight and spin-dialyzed at 4 °C. More than 50% of the DNA was reconstituted into uniquely positioned nucleosomes (Fig. 1D). The integrity of the purified nucleosomes was verified by examining the ratio of core histones on SDS-PAGE followed by silver staining.

Electrophoretic Mobility Shift and Antibody Supershift Assays—The electrophoretic mobility shift assays with WTp53 and p53DBD were carried out as follows. For WTp53 binding assay, labeled DNA probes and nucleosomes (5 × 10^5 cpm, 2 ng) were mixed with 30 bp of nonspecific competitor DNA (30 ng) and WTp53 (50 ng) in binding buffer (10 mM Tris-Cl, pH 7.5, 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 2 mM MgCl2, 2 mM spermidine, 0.025% Nonidet P-40, and 5% glycerol, 10 μl) at 4 °C for 15 min. The binding reactions were analyzed by electrophoresis on 4% polyacrylamide gels (33 × 42 cm) in 1 × Tris borate-EDTA buffer at 4 °C. For the p53DBD binding assay, labeled DNA probes and nucleosomes (5 × 10^5 cpm, 2 ng) were mixed with (d dC) (8 ng) in binding buffer (50 mM Bistris-propane-HCl, pH 6.8, 1 mM DTT, 100 mM NaCl (10 μl)) and p53DBD (25 ng) in a total volume of 10 μl at 4 °C for 15 min. The binding reactions were analyzed by electrophoresis on 4.5% polyacrylamide gels in 1 × Tris borate-EDTA at 4 °C. The concentration of the WTp53 and p53DBD proteins was adjusted in the binding reactions so that it resulted in ~50% unbound DNA or nucleosomes and 50% WTp53- or p53DBD-nucleosome complexes.

The antibody supershift assays were carried out after incubating WTp53 and p53DBD binding reactions for 15 min followed by the addition of pAb421 (10 ng) and DO1 (10 ng) anti-bodies targeted to C-terminal (amino acids 371–382) and N-terminal (amino acids 20–25) regions of p53 in a total volume of 12 μl. To detect if WTp53 and p53DBD-nucleosome ternary complexes contain full complement of core histones, the binding reactions were scaled up 100-fold. The WTp53 and p53DBD-nucleosome ternary complexes were excised from the polyacrylamide gels and eluted in 20 mM HEPEs, pH 7.5, 1 mM EDTA, 0.5 mM PMSF, and 1× protease inhibitors (Roche Diag-
nastics). The eluted complexes were concentrated on Centri-
con-30 centrifugal filters (Millipore) and analyzed on 18% SDS-
PAGE followed by silver staining followed by gel electro-
phoresis as described above.

Nucleosome Pulldown Assay—P53Con30—42 plasmids were
PCR-amplified using a biotinylated primer, 5′-Biotin-GAG-
GGGCCATTCATGGC-3′ (reverse) and 5′-TGCGTTGA-
CACCAGGAAGACG-3′ (forward) to produce biotinylated
probes. These probes (300 ng each) were resuspended in 1×
nucleosome reconstitution buffer (10 mM HEPES, pH 7.9, 1 mM
EDTA, 0.5 mM PMSE, and 1× protease inhibitor mixture) (6 µl),
chicken nucleosome core particles (6 µg, 1 µg/µl), and 5 M
NaCl (8 µl) in a total volume of 20 µl. The reconstitution mix
was incubated at 37 °C for 15 min. The mix was then serially
diluted with 6.6, 13.4, 10, 7.2, 9.4, 13.4, 20, 60, and 40 µl of 1×
nucleosome reconstitution buffer and incubated at 37 °C for 15
min after each dilution. The mix was further diluted with 10 mM
Tris-Cl, pH 7.5, 1 mM EDTA, 0.1% Nonidet P-40, 5 mM EGTA,
5 mM DTT, 0.5 mM PMSE, and 1× protease inhibitor mixture (100 µl), and incubated at 30 °C for 15 min. The diluted
nucleosomes (400 µl) were bound to 300 µg of streptavidin-
coated paramagnetic beads (Invitrogen) as follows. The beads
were washed 2 times with 10 mM Tris-Cl, pH 7.5, 1 mM
EDTA, 0.1% Nonidet P-40, 5 mM EGTA, 5 mM DTT, 0.5 mM
PMSE, 20% glycerol, and 0.1 mg/ml bovine serum albumin (100 µl), and incubated at 30 °C for 15 min. The bead-immobilized
nucleosomes (400 µl) were added to the beads (400 µl) and rotated for 3 h at
room temperature. The bead-immobilized nucleosomes were
washed once with 20 mM HEPES-NaOH, pH 7.5, 1 mM EDTA,
10% glycerol, 0.5 mM DTT, and 0.5 mM PMSE and stored in 20
mM HEPES-NaOH, pH 7.5, 1 mM EDTA, 10% glycerol, 0.5 mM
DTT, 0.5 mM PMSE, and 1× protease inhibitors (Roche Diag-
nostics) at 4 °C. The integrity of the nucleosomes was verified
by examining ratios of the core histones on 18% SDS-PAGE.

To check for core histone dissociation during WTp53 bind-
ing, the remaining fractions of both supernatant and beads (15
µl each) were resolved on 18% Tris-glycine SDS-polyacryl-
amide gels, blotted on polyvinylidene difluoride membranes, and
probed first with anti-histone H3 antibody (Sigma H-0164),
stained with alkaline phosphatase-conjugated affinity-purified
anti-rabbit IgG, and developed using chemiluminescent sub-
strate. The blots were successively stripped using Restore Plus
Western blot stripping buffer (Thermo Scientific, Rockford, IL)
and reprobed with anti-histone H2A (Millipore, 07-146), anti-
histone H2B (Millipore, 07-371), and anti-histone H4 (Milli-
pore, 05-858) antibodies. The Western blot signals were quan-
tified using images generated by autoradiography followed by
densitometric scanning. The ratios of bead-associated core his-
tones (H3/H4 and H2A/H2B), obtained by three independent
experiments and normalized to a known concentration of core
histones, are shown in the panel b of Fig. 2D.

Hydroxyl Radical Footprinting of Nucleosomes and
Quantitation—The hydroxyl radical (OH-radical) footprinting
was carried out on purified reconstituted nucleosomes. The
amount of WTp53 required to bind the nucleosomes on a pre-
parative scale was determined by stepwise titration and analysis
by electrophoresis on 4.0% polyacrylamide gels. Binding reac-
tions were carried out as described above but were scaled up by
10-fold. Single end-labeled DNA probes, nucleosomes, and
WTp53-nucleosome complexes were suspended in the binding
buffer (8 µl) without glycerol. The OH-radical cleavage reac-
tions were initiated by adding a mixture containing 8 mM FeSO4
(NH4)2SO4, 6H2O and 16 mM EDTA (2 µl), 0.03% H2O2 (2 µl),
and 20 mM sodium ascorbate (2 µl). Reactions were carried out
on ice for 2 min and quenched by adding 0.1 M thiourea (5 µl).
Each sample was mixed with 15% Ficoll (4 µl) and was loaded
on a 4% native polyacrylamide gel containing 20 mM HEPES, pH
8.3, as a running buffer. The gels were run for about 3 h at 8
V/cm to separate p53-bound nucleosomes from unbound
nucleosomes. Both bound and unbound fractions were identi-
fied by autoradiography, gel-eluted in 0.5 M NH4Cl, 0.1 mM
EDTA, extracted with phenol:chloroform, precipitated twice
with ethanol, and analyzed by electrophoresis on a 6% denatur-
ing polyacrylamide gel followed by autoradiography. The con-
tral DNA was also cleaved under identical conditions. Specific
DNA markers were produced by Maxam-Gilbert cleavage at
guanine and guanine plus adenosine bases. The gels were dried
on Whatman No. 3MM paper sheets and autoradiographed
with Eastman Kodak X-Omat AR film. The x-ray films were
digitized using a densitometer, and the bands were quantified using AlphaEaseFC image analysis software (Santa Clara, CA). Briefly, the pixel intensity of individual DNA bands was integrated over a fixed area. The background subtraction was carried out using local background mode of the AlphaEaseFC software that designates the background as the mean of the 10 lowest pixel values in the perimeter of the area defined for quantitation. The integrated pixel intensity values for each band encompassing the p53RE are shown in Figs. 3, C–D.

**Micrococcal Nuclease Mapping of Translational Positioning of Nucleosomes**—The reconstituted nucleosomes p53Con30, p53Con35, and p53Con40 (500 ng, each) were digested with 10 μl of micrococcal nuclease (2.4 units/μl) in 200 μl of 1× micrococcal nuclease digestion buffer (40 mM HEPES, pH 7.3, 6 mM MgCl2, 10 mM β-mercaptoethanol, 2 mM CaCl2) for 25 min on ice. The digestion was stopped by adding 0.5 M EDTA (5 μl), 10% SDS (4 μl), and 3 mM sodium acetate, pH 5.2 (24 μl). The DNA was extracted with phenol:chloroform and precipitated twice with ethanol. The digested DNA was labeled with [γ-32P]ATP and polynucleotide kinase and purified on a 5% native polyacrylamide gel. The unreconstituted control DNA fragments were labeled with [γ-32P]ATP and polynucleotide kinase. Both unreconstituted and micrococcal nuclease-digested nucleosomal DNA were digested with EcoRI and HindIII and analyzed by electrophoresis on 12% native polyacrylamide gels.

To map the translational positioning of nucleosomes at a single nucleotide resolution, DNA fragments derived from micrococcal nuclease-digested p53Con30, -35, and -40 nucleosomes were end-repaired with T4 DNA polymerase and polynucleotide kinase and ligated with a double-stranded ligation-mediated PCR linker, 5’-GGCGTGAACCCGGG-GAGATCTGAATTC-3’ (top strand) and 5’-GAATTCA-GATC-3’ (bottom strand) using T4 DNA ligase. The linker-ligated DNA fragments were linearly amplified by two rounds of PCR using the top strand of the linker as the primer. The amplified DNA was subcloned into a pCR 2.1 TOPO® vector (Invitrogen). Positive clones containing the ligation-mediated PCR fragments were identified by restriction analysis. Several clones of each fragment were sequenced using both forward and reverse primers to determine nucleosomal boundaries.

**RESULTS**

**Incorporation of p53RE in the Nucleosome Affects p53 Binding**—We designed a series of nucleosome-positioning constructs in which the p53 binding site was incorporated near the center of DNA fragments in different orientations relative to the nucleosomal surface (Fig. 1A). To secure the rotational positioning of p53RE, we used the known differential bending of A-tract DNA and GC-cluster DNA (23, 24). Our design is based on the premise that sequence elements with a strong preference for anisotropic bending set the initial frame for nucleosome positioning by assuming their preferred rotational orientations. The remainder of the DNA is “molded” around the histone octamer by exploiting the conformational flexibility of DNA.

A 10.5-bp periodic phasing of A-tract DNA (5’-AAAAAAA-3’) with intervening GC-clusters (5’-CGGGGC-3’) is known to cause macroscopic DNA curvature and direct rotational positioning of DNA in nucleosomes such that the minor groove in the A-tracts faces toward the histone core and in the GC-cluster faces out (25). In our constructs, a p53RE is positioned between the two DNA fragments, each of which contains three A-tracts and four GC-clusters, with the potential to orient the p53RE in different rotational settings in the nucleosome (Fig. 1A).

In all constructs the center of A-tract DNA curvature located at the junction between the S1 and S2 segments and the center of p53-induced DNA bending are separated by an integral number of helical turns; both bends are directed into the minor groove (Fig. 1, A and B) (21). The variable spacer (located in the S3 segment) changes from 0 (S3 = 30 bp) to 12 bp (S3 = 42 bp), which allows the center of bending in the p53RE and the center of A-tract DNA curvature at the S3 and S4 junction to be “in phase” in the p53Con30 and -40 constructs and “out of phase” in the p53Con35 construct. This design is expected to produce a strongly curved “C-shaped” DNA helix trajectory in the p53Con30 and -40 constructs and an extended “S-shaped” helix trajectory in the p53Con35 construct (Fig. 1B). Importantly, all constructs formed stable positioned nucleosomes irrespective of their sequence-dependent three-dimensional shape (Fig. 1D).

Electrophoretic mobility shift analysis shows that both purified WTp53 and p53DBD (Fig. 1C) bind each of the DNA fragments (Fig. 2A, lanes 1–6 and lanes 13–18). The six constructs exhibit different gel electrophoretic mobilities both as free DNA and as p53-DNA complexes, consistent with their sequence-directed intrinsic curvature and p53-induced DNA bending and twisting (19). However, when these sequences are folded into nucleosomes, only the p53Con30, -32, -40, and -42 constructs show binding with WTp53 and the p53DBD (Fig. 2A, lanes 7 and 8 and lanes 11 and 12; lanes 19 and 20 and lanes 23 and 24). Nucleosomes reconstituted on p53Con35 and -37 constructs show no binding with the proteins (Fig. 2A, lanes 9 and 10 and lanes 21 and 22). Thus, our data suggest that the p53RE embedded in nucleosome remains fully accessible for p53 binding only in specific orientations on the nucleosomal surface.

To understand if the N-terminal transactivation and C-terminal basic domains in the nucleosome-bound p53 are free for interactions, we incubated the p53Con30 and p53Con32 nucleosome-p53 complexes with antibodies specific to p53 terminal domains. We found that WTp53-nucleosome complexes were able to bind to the DO1 (epitope, amino acids 11–25) and pAb421 (epitope, amino acids 370–381) antibodies (Fig. 2B, lanes 5–8), suggesting that both these domains remain accessible in the nucleosome-p53 complex. Thus, our data indicate that p53 can bind nucleosomal response element DNA in an orientation that keeps its transactivation and basic domains accessible for the recruitment of co-regulators (26).

**p53 Binding to Nucleosome Occurs without Significant Core histone Dissociation**—We next examined whether the nucleosome complexes with WTp53 and the p53DBD indeed contained the full complement of core histones. The binding reactions were scaled up 50-fold, and the bands corresponding to the WTp53- and p53DBD-nucleosome complexes were excised from the gel and analyzed by SDS-PAGE with silver staining.
As a control, an excised band corresponding to a reconstituted nucleosome was resolved on the same gel (Fig. 2C). We found that all four core histones (H3, H2A, H2B, and H4) remain associated in the WTp53 and p53DBD-nucleosome complexes (Fig. 2C, lanes 1–4 and 5–8). The relative intensity of the bands recovered from the p53-bound nucleosomes is comparable with that in nucleosome used as a control. Thus, our data indicate that binding of WTp53 and p53DBD to nucleosomes occurs without significant disruption in the nucleosome assembly.

To better understand how p53 binding to nucleosomes affects the stability of the histone octamer core, we developed a solid-phase nucleosome binding assay. Briefly, nucleosomes were reconstituted on biotinylated DNA fragments and immobilized on streptavidin-coated paramagnetic beads. The integrity of the bead-immobilized nucleosomes was evaluated by measuring the ratios of core histones separated by SDS-PAGE followed by silver staining and, additionally, by comparing the accessibility of the nucleosomes and free-DNA to restriction enzymes. In our reconstitution conditions, the DNA to histone ratio was adjusted to result in a greater than 90% formation of nucleosomes. WTp53 and p53DBD were incubated with bead-immobilized nucleosomes (p53Con30–42) or the corresponding free DNA fragments under the conditions used in the electrophoretic mobility shift assay. The WTp53 and p53DBD-bound nucleosomes were separated from the supernatant,
washed twice with their respective binding buffers to remove unbound proteins, and analyzed by electrophoresis on SDS-PAGE followed by Western blotting either with DO1 antibody (for WTp53) or FL-393 antibody (for p53DBD). We found that both WTp53 and p53DBD showed a stronger binding to p53Con30, -32, -40, and -42 nucleosomes (Fig. 2D, panels a and b, lanes 3 and 4 and lanes 7 and 8) than to p53Con35 and -37 nucleosomes (Fig. 2D, panels a and b, lanes 5 and 6). The binding of p53 and p53DBD to p53Con35 and -37 nucleosomes was comparable with the p53 binding to a nonspecific positioned nucleosome with a mutated p53RE (Fig. 2D, panels a and b, lanes 2). By contrast, both WTp53 and p53DBD showed much
associated fractions indicates that the nucleosomes remain in the bead and supernatant fractions of WTp53 binding reactions (Fig. 2, panels a and b, lanes 9–15). To test if streptavidin-coated paramagnetic beads show any nonspecific binding to p53, p53DBD, or core histones, we incubated beads with identical amounts of core histones and WTp53 or p53DBD in their respective binding buffers. The samples were processed identically to WTp53 and p53DBD binding reactions to nucleosomes and DNA. We found that beads alone show no binding to either of these proteins (Fig. 2D, panels a and b, lanes B). To check if equal concentrations of reconstituted nucleosome were used in the binding reactions, we stripped and reprobed the blots with anti-histone H3 antibody. Our data suggest equal loading of nucleosomes in both the WTp53 and p53DBD binding reactions (Fig. 2D, panels a and b, rows marked H3). Note that WTp53 and p53DBD binding to the bead-immobilized nucleosomes is consistent with electrophoretic mobility shift assay data, suggesting that immobilization of nucleosomes on magnetic beads retains the initial nucleosome positioning.

To ascertain if p53 binding to nucleosomes leads to dissociation of the histone octamer, the supernatants and beads in the p53 binding reactions were analyzed by electrophoresis on 18% SDS-polyacrylamide gels followed by Western blotting with anti-histone antibodies. The amount of core histones, present in the bead and supernatant fractions of WTp53 binding reaction, are shown in Fig. 2E, panel a. We found that WTp53 binding to the nucleosomes does not cause significant dissociation of the core histones, as indicated by the absence of histones in the supernatant fraction (Fig. 2E, panel a, rows marked S). Conversely, the presence of all the core histones in the bead-associated fractions indicates that the nucleosomes remain intact during p53 binding (Fig. 2E, panel a, rows marked B). To determine whether all the core histones on the beads remain bound in stoichiometric amounts, we quantitated the band intensities of the core histones associated with the beads (rows B) and normalized with a known amount of core histone loaded on the gels (lanes C). The ratios of band intensity of histones (H3/H4 and H2A/H2B) for each nucleosome were calculated using AlphaEaseFC image analysis program. An average ratio of H3/H4 (black bars) and H2A/H2B (gray bars) with ± S.D., estimated using three independent experiments, are plotted in Fig. 2E, panel b. We found that the ratio of histones H3/H4 and H2A/H2B varies between 0.95 and 1.05 for each nucleosome, suggesting that histones H3-H4 and H2A-H2B remain bound with the nucleosomes in stoichiometric ratio. Note that the ratios of H3/H4 and H2A/H2B for each nucleosome show negligible variation, further suggesting that p53 and p53DBD binding to nucleosomes occurs without perturbing the nucleosome assembly.

WTp53 Selectively Binds to Nucleosomes with CATG Tetramers in the Half-sites Bent into the Major Groove—To map the rotational orientation of the p53RE on the nucleosomes, we carried out hydroxyl radical (OH-radical) probing on p53Con35 and -40 DNA and nucleosomes. The OH-radical cleaves the DNA strand by abstracting a hydrogen atom from the sugar-phosphate backbone and provides useful information on the minor-groove width and solvent accessibility of local regions within the DNA (27).

The gel images of the OH-radical cleavage of the p53Con35 and -40 DNA, nucleosomes, and nucleosome-WTp53 complexes with their integrated intensity plots are shown in Fig. 3, A–D. The OH-radical cleavage of the free p53Con35 and -40 DNA constructs (without histones) showed a reduced cleavage at the A-tracks and a slightly higher cleavage at the GC clusters, indicating a narrow minor groove in the A-track and a wide minor groove in the GC-rich regions (Fig. 3, A and B, lanes D, and supplemental Fig. S2, A and B, lanes D, cyan bars) (28). The same procedure applied to the p53Con35 and -40 nucleosomes showed a periodic cutting pattern with significantly reduced cleavage at the A-tracks and an increased cleavage at the GC-clusters, indicating that the minor grooves in the A-tracks are oriented toward the histone core but oriented away from the core in the GC-clusters (Fig. 3, A and B, lanes N).

Note that the OH-radical cleavage patterns of the p53Con35 and -40 nucleosomes demonstrate the same rotational positioning in the ApaI-HindIII (A+H) half of the nucleosomes; i.e., GC-clusters are exposed, whereas A-tracks are protected, suggesting that in both constructs it is the A+H half that determines overall nucleosome positioning (Figs. 3, A and B, and supplemental Fig. S2, A and B, top of lanes N). This in phase periodicity is lost in the EcoRI-BsrG1 (E+B) half of both nucleosomes, suggesting it has a secondary role in rotational positioning (Fig. 3, A and B, bottom of lanes N, supplemental Fig. S2, A and B (lanes N), and C and D (E+B half)). The integrated intensity plots of the bands corresponding to the p53RE show reduced cleavage at the CATG tetramers and increased cleavage of the intervening CCTAGG region in the p53Con35 nucleosome (Fig. 3, A (lane N) and C (plot b)). By contrast, the p53Con40 nucleosome shows increased cleavage at the CATG and reduced cleavage at the CCTAGG region (Fig. 3, B (lane N) and D (plot b)). Our data clearly indicate that minor grooves at the CATG sequences of p53RE are oriented inward in the p53Con35 and outward in the p53Con40 nucleosomes. Note that OH-radical cleavage patterns in the ApaI + HindIII half of both the constructs are identical (top part of the gels), suggesting that S3 and S4 segments determine nucleosome positioning in these constructs.

The OH-radical cleavage pattern of the p53Con40 nucleosome in complex with p53 shows a tight footprint encompassing ~16 bp of the RE (Fig. 3B (lane p53 + N) and 3D (plot c)), whereas no footprint is observed on the p53Con35 nucleosome. However, note that the p53 binding does not alter the OH-radical cleavage pattern in the p53Con40 nucleosome beyond the footprint, suggesting that sequence-specific binding of p53 to its cognate binding sites occurs without significant changes in DNA conformation (Fig. 3, A and B, (lanes p53 + N) and C and D (plots c)). Our data demonstrate that WTP53 preferentially binds to nucleosomes with their minor grooves at the CATG tetramers exposed outward. At the same time, p53 binding is inhibited when the CATG minor grooves face the octamer core.

Mapping Translational Positioning of Reconstituted Nucleosomes—Although the OH-radical footprinting of nucleosomes clearly defines rotational orientation of the DNA on nucleosomes, its utility is limited by the fact that multiple nucleosomal positions, separated by a complete helical turn, could lead to a similar cleavage pattern. Therefore, to detect the
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Boundaries of the nucleosomes, we used an exonuclease mapping technique (29). The reconstituted p53Con30, -35, and -40 nucleosomes were digested with micrococcal nuclease. After deproteinization, DNA from the digested nucleosomes was isolated, radiolabeled, digested with EcoRI and HindIII, and analyzed by electrophoresis on a native PAGE. For comparison, p53Con30, -35, and -40 free DNA were similarly treated and analyzed on the same gel.

The analysis by gel electrophoresis and images of the gels are shown in Fig. 4, A–C, and supplemental Table S1A. The digestion of p53Con30, -35, and -40 nucleosomes with micrococcal nuclease generated a single nucleosome length fragment of \(~147–150\) bp as expected (Fig. 4, A–C, lanes 3). Digestion of nucleosomal fragments with EcoRI and HindIII generated shorter fragments than in the case of control DNA (see the bands marked with red dots, Fig. 4, A–C, lanes 4 and 5 and lanes 6 and 7). The HindIII digestion of p53Con30 and -40 nucleosomal DNA generated two distinct short DNA fragments in the bottom part of the gel, indicating two strong nucleosomal boundaries in the H+A region of the sequence (Fig. 4, A and C, lanes 7, bands marked with red dots). By contrast, HindIII digestion of p53Con35 nucleosomal DNA created a single short fragment, suggesting a single dominant boundary for this nucleosome in the H+A region (Fig. 4B, lane 7). The EcoRI digestion of p53Con30, -35, and -40 nucleosomal DNA generated two fragments, each shorter than the EcoRI-digested control DNA (Fig. 4, A–C, lanes 4 and 5, bands marked with red dots). Further separation of the EcoRI-digested p53Con30 and -40 nucleosomal fragments showed that each band consists of two DNA fragments, indicating two strong boundaries in the B+E region of the sequence (data not shown).

To define nucleosome boundaries at a single nucleotide resolution, the DNA fragments, isolated from the micrococcal nuclease-digested nucleosomes, were end-repaired using T4 DNA polymerase and polynucleotide kinase, ligated with ligation-mediated PCR linkers, and subcloned in a pCR^®2.1 TOPO® vector (Invitrogen). Several clones containing p53Con30, -35, and -40 nucleosomal inserts were sequenced using both forward and reverse primers to determine the boundaries of the nucleosomes (supplemental Table S1B). We observed two sets of clones for the p53Con30 and -40 nucleosomes and a single set of clones for the p53Con35 nucleosome. Specific base pairs defining translational positions of nucleosomes, as determined by micrococcal nuclease mapping and direct sequencing, are provided in supplemental Table S1, A and B and are marked in the bottom panels of Fig. 4, A–C.

In summary, we find that the 3’ boundary of the histone octamers in all the nucleosomes has a major position about 2 bp from the 3’-end (Fig. 4, A–C, shown with solid ellipses in the bottom panel). Nucleosomes p53Con30 and p53Con40 show a secondary position 12 bp from the 3’-end, separated from the primary position by one complete helical turn (Fig. 4, A and C, lower panel, dotted ellipses). By contrast, the p53Con35 nucleosome assembles with a unique translational position (Fig. 4B, bottom panel, solid ellipse). Although our data suggest that

**FIGURE 4.** Micrococcal nuclease mapping of histone octamer positions on nucleosomes. A–C, mapping of the boundaries of p53Con30, -35, and -40 nucleosomes is shown. Lanes 1, labeled marker (Gene Ruler Ultra-low Range, Fermentas, Ontario, Canada); lanes 2, undigested DNA; lanes 3, undigested core particle DNA; lanes 4, control DNA digested with EcoRI; lanes 5, core particle DNA digested with EcoRI; lanes 6, control DNA digested with HindIII; lanes 7, core particle DNA digested with HindIII. The dots in lanes 4 and 6 indicate predominant DNA fragments that together generate 161, 166, and 171 bp DNA (in panels A, B, and C, respectively). The dots in lanes 5 and 7 indicate fragments that together generate \(~146–149\)-bp fragments. The schemes in the lower panel show the boundaries of the nucleosome cores (bold and dotted ellipsoids), mapped to the DNA fragments used for nucleosome reconstitution. The p53Con30-40 nucleosomes show two translational positions, whereas the p53Con35 nucleosome shows a single position. The base pair positions of the boundaries, mapped by gel-mobility analysis of digested fragments and direct sequencing of micrococcal nuclease-digested nucleosomal fragments (supplemental Table S1) are shown next to the ellipsoids. The sites of cleavage by restriction endonucleases BsrGI, EcoRI, HindIII, and ApaI are marked as red dots, red diamond, cyan vertical bars, and red pentagon, respectively.
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p53Con30 and p53Con40 nucleosomes have two translational positions, these positions are separated by one turn of the helix, thereby exposing the same surface of the response element DNA to solvent. By contrast, the p53Con35 nucleosome shows a single strong position 2 bp from the 3'-end, indicating that its p53RE is in the opposite orientation compared with the p53Con30 and -40 nucleosomes, which is consistent with the OH-radical footprinting data.

**Modeling of p53 Tetramer Bound to Nucleosomal DNA**

Based upon data presented above, both constructs p53Con30 and p53Con40 are characterized by two nucleosome positions located 2 and 12 bp from the 3'-end of the constructs. The corresponding positions of p53REs in these two positions are shown in Fig. 5A and supplemental Table S2. In particular, the nucleosome position 22–169 in the p53Con40 construct (Fig. 4C) implies that the p53 half-sites would be separated from the dyad by 5 and 15 bp (supplemental Table S2); their location in the nucleosome is shown by decamers d1 and d2 in Fig. 5A. In the alternative nucleosome position 12–159 (Fig. 4C), the p53 half-sites are located as shown by decamers d2 and d3 (Fig. 5A). Similarly, in the case of the p53Con30 construct, the nucleosome positions are 12–159 and 2–149 (Fig. 4A), and the p53 half-sites are represented either by decamers d2 and d3 or by decamers d3 and d4. The p53 binding to decamers d2 and d3 is common for both constructs (supplemental Table S2). Therefore, we present a model of p53 tetramer bound to these decamers in Fig. 5.

Note that the rotational orientation of the decamers d1–d4 in the nucleosome (Fig. 5A) is such that they are bent into the major groove in their centers, with bending angles of 15–20°, comparable with the angles observed in the crystallographic structures of the dimeric p53DBD-DNA complex (20). In this model the p53 half-sites are properly exposed on the nucleosomal surface, and in principle each of these decamers could be recognized by a p53 dimer.

We next modeled the p53 tetramer bound to nucleosomal DNA using the p53 co-crystal structure with dodecamer aAGGCATGCTt (12) (PDB code 2ATA). In this case the p53 half-site (shown above in capital letters and in Fig. 1A) is identical to the decamers d2 and d3 embedded in our constructs (Fig. 1A, supplemental Table S2). In the co-crystal, the p53 dimer has direct contacts only with the underlined G:C base pairs, whereas the terminal A:T base pairs do not interact with the protein. Therefore, we used the central octamer GGCGATGCC to superimpose the DNA-bound p53 dimers with the decamers d2 and d3 in the nucleosome core particle (31). The superposition was made with PROFIT (32) using atoms C1' and N9 (purine)/N1(pyrimidine) as tethers; overall, $8 \times 4 = 32$ tethers were used; root mean square deviation was $\sim 1 \text{ Å}$. The “junctures” between the nucleosomal DNA and the octamers (where DNA is kinked into the minor groove) were optimized using DNAminicarlo (33) and CHARMM (34).

Only minor adjustments were necessary to optimize the histone-DNA interactions. In particular, the $\sim 0.5$-Å adjustments were made in the side chains of two Arg-45 residues from histones H4 penetrating into the minor groove at the junction between decamers d2 and d3 and decamers d3 and d4. The p53-DNA interactions were not optimized, as they were the same as in the crystal structure (the atomic coordinates are presented in supplemental Table S4). As a result, in the model presented in Fig. 5B, the four p53 subunits interact with DNA in the same way as in the p53DBD-DNA co-crystal (12). In our model there are no steric clashes between p53DBDs and the histone core, whereas p53DBDs retain all sequence-specific contacts with DNA observed in the co-crystal (Fig. 5B and supplemental Fig. S1). Our results suggest that the p53 tetramer can recognize its cognate binding site in the nucleosomal context when the response element is “properly oriented”; that is, when the major grooves of the central tetramer CATG face the histone core. Importantly, the N termini are located on the external side of the DNA loop (Fig. 5B); thus, they would be readily available for interaction with proteins involved in the trans-activation and trans-repression cascades (histone acetyltransferases, e.g. p300, histone deacyetylases, etc.).

**DISCUSSION**

In this study we provide evidence that sequence-specific binding of p53 to the nucleosome is strongly affected by the rotational orientation of response elements on the nucleosomal surface. The strongest p53 binding occurs when the minor grooves in the CATG tetramers in the two half-sites are exposed to solvent. Other orientations significantly decrease...
p53 binding. Our data suggest that the wrapping of p53RE around nucleosome in a “proper” orientation is critical for p53 binding to its target sites in chromatin.

Traditionally, it has been assumed that the nucleosomal organization of binding sites in chromatin is refractory to the TF binding (35, 36). According to this point of view, TFs require various enzymatic activities, including recruitment of ATP-dependent remodeling enzymes (37–39) and histone modifications (40, 41), to gain access to their binding sites in chromatin. On the other hand, it is known that a number of TFs such as GR, FoxA1, Sp1, and Myc/Max can easily bind their cognate sites embedded in nucleosomes (42–45).

TF binding to target sites in the nucleosome often results in the formation of the lower affinity complexes (46, 47). By contrast, p53 binds to the p21 DNA response element, residing in reconstituted chromatin, with higher affinity in comparison to free DNA, indicating a role of nucleosomal architecture in stabilizing the p53-RE interactions (48). Our data suggest that nucleosome positioning elements could play an important role by presenting p53 binding sites in the correct rotational frames for the initial rapid interaction of p53 in response to DNA damage (49).

Our data lend further support to this observation and suggest that nucleosome architecture can effectively modulate the ability of p53 to recognize its binding sites embedded in chromatin. This effect could be both positive and negative, i.e. if the response elements are “exposed” on the nucleosomal surface with the bending direction similar to that found in the p53-DNA co-crystals, binding of p53 is increased. However, if the response elements are arranged in the opposite orientation, the p53 binding is strongly inhibited. We suggest that such inaccessible p53 binding sites in the genome could be made accessible by p53-mediated recruitment of ATP-dependent chromatin remodeling enzymes, such as CHD8 (50) and SWI/SNF complexes (51–53).

The p53-RE interactions in nucleosomes could be facilitated by intrinsic flexibility of the DNA response element. Our analysis showed that the p53RE favors the same direction of bending in solution as observed in the reconstituted nucleosomes that show stronger binding with p53. These flexible sequence elements (e.g. CATG core motifs) could present a p53 binding site in a pre-bent conformation on the nucleosome, thus decreasing the energy required for p53 binding to the response element. The intrinsic bending of the DNA response element plays a significant role as demonstrated by a higher affinity of p53 to REs containing CATG tetramers in the two half-sites as well as a stronger p53 binding to a pre-bent RE embedded in a DNA minicircle (54). Similarly, wrapping of p53RE in the nucleosome in a proper orientation could present DNA in the bent configuration, poised for p53 binding.

The sequence-specific binding of p53 to DNA has been studied for several years by crystallography, NMR, cryoelectron microscopy, and other biophysical methods (55–57). These studies have provided a wealth of information on p53-DNA interactions and how mutations in p53 affect its binding to DNA. However, few studies have sought to map p53-DNA interactions in the context of natural chromatin, and it is not yet clear how chromatin architecture imposes constraints on p53 binding to DNA response elements. Our results show that positioning of nucleosomes over p53 response elements can have dramatic effects on the potential recognition of these elements by the protein. This work represents the first comprehensive effort to characterize the interaction of the p53 protein with well-positioned nucleosomes. We further propose that nucleosome positioning and dynamics could play an important role in the p53 recognition process. Therefore, a comprehensive evaluation of p53 binding affinity and the accessibility of its target sites in chromatin are necessary.

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REFERENCES

1. Toledo, F., and Wahl, G. M. (2006) Nat. Rev. Cancer 6, 909–923
2. Staples, O. D., Steele, R. J., and Lain, S. (2008) Surgeon. 6, 240–243
3. Ventura, A., Kirsch, D. G., McLaughlin, M. E., Tuveson, D. A., Grimm, J., Lintault, L., Newman, J., Reczek, E. E., Weissleder, R., and Jacks, T. (2007) Nature 445, 661–665
4. Laptenko, O., and Prives, C. (2006) Cell Death Differ. 13, 951–961
5. Murray-Znimjewski, F., Slew, E. A., and Lu, X. (2008) Nat. Rev. Mol. Cell Biol. 9, 702–712
6. Appella, E., and Anderson, C. W. (2001) Eur. J. Biochem. 268, 2764–2772
7. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., and Vogelstein, B. (1991) Science 252, 1708–1711
8. Speidel, D., Helmhold, H., and Deppert, W. (2006) Oncogene 25, 940–953
9. Friedman, P. N., Chen, X., Bargonetti, J., and Prives, C. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 3319–3323
10. Chen, X., Ko, L. J., Jayaraman, L., and Prives, C. (1996) Genes Dev. 10, 2438–2451
11. Hainaut, P., and Hollstein, M. (2000) Adv. Cancer Res. 77, 81–137
12. Kitayner, M., Rozenberg, H., Kessler, N., Rabinovich, D., Shaulov, L., Han, T. E., and Shakked, Z. (2006) Mol. Cell 22, 741–753
13. Zhao, K., Chai, X., Johnston, K., Clements, A., and Marmorstein, R. (2001) J. Biol. Chem. 276, 12120–12127
14. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. (1994) Science 265, 346–355
15. Jordan, J. J., Menendez, D., Inga, A., Nourredine, M., Bell, D. A., and Resnick, M. A. (2008) PLoS Genet. 4, e1000104
16. Balagurumoorthy, P., Sakamoto, H., Lewis, M. S., Zambrano, N., Cleore, G. M., Gronenborn, A. M., Appella, E., and Harrington, R. E. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 8591–8595
17. Nagaich, A. K., Appella, E., and Harrington, R. E. (1997) J. Biol. Chem. 272, 14842–14849
18. Durell, S. R., Jernigan, R. L., Appella, E., Nagaich, A. K., Harrington, R. E., and Zhurkin, V. B. (1998) in Structure, Motion, Interaction, and Expression of Biological Macromolecules: Proceedings of the Tenth Conversation (Sarma, R. H., and Sarma, M. H., eds) Vol. 2, pp. 277–296, Adenine Press, New York
19. Nagaich, A. K., Zhurkin, V. B., Durell, S. R., Jernigan, R. L., Appella, E., and Harrington, R. E. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 1875–1880
20. Ho, W. C., Fitzgerald, M. X., and Marmorstein, R. (2006) J. Biol. Chem. 281, 20494–20502
21. Nagaich, A. K., Zhurkin, V. B., Sakamoto, H., Gorin, A. A., Clore, G. M., Gronenborn, A. M., Appella, E., and Harrington, R. E. (1997) J. Biol. Chem. 272, 14830–14841
22. Hayes, J. J., and Lee, K. M. (1997) Methods 12, 2–9
23. Fitzgerald, D. J., Dryden, G. L., Bronson, E. C., Williams, J. S., and Anderson, J. N. (1994) J. Biol. Chem. 269, 21303–21314
24. Fitzgerald, D. J., and Anderson, J. N. (1998) Nucleic Acids Res. 26, 2526–2535
25. Drew, H. R., and Travers, A. A. (1985) *J. Mol. Biol.* 186, 773–790
26. Riley, T., Sontag, E., Chen, P., and Levine, A. (2008) *Nat. Rev. Mol. Cell Biol.* 9, 402–412
27. Hayes, J. I., Tullius, T. D., and Wolff, A. P. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87, 7405–7409
28. Brukner, I., Dlakic, M., Savic, A., Suic, S., Pongor, S., and Suck, D. (1993) *Nucleic Acids Res.* 21, 1025–1029
29. Studitsky, V. M. (1999) *Methods Mol. Biol.* 119, 17–26
30. Hayes, J. J., Tullius, T. D., and Wolffe, A. P. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87, 7405–7409
31. Davey, C. A., Sargent, D. F., Luger, K., Maeder, A. W., and Richmond, T. J. (2002) *J. Mol. Biol.* 319, 1097–1113
32. McLachlan, A. D. (1982) *Acta Crystallogr. Sect. A* 38, 871–873
33. Zhurkin, V. B., Ulyanov, N. B., Gorin, A. A., and Jernigan, R. L. (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88, 7046–7050
34. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., Swaminathan, S., and Karplus, M. (1983) *J. Comput. Chem.* 4, 187–217
35. Blomquist, P., Li, Q., and Wrange, O. (1996) *J. Biol. Chem.* 271, 153–159
36. Taylor, I. C., Workman, J. L., Schuetz, T. J., and Kingston, R. E. (1991) *Genes Dev.* 5, 1285–1298
37. Fryer, C. I., and Archer, T. K. (1998) *Nature* 393, 88–91
38. Tsukiyama, T., Becker, P. B., and Wu, C. (1994) *Nature* 367, 525–532
39. Nagaich, A. K., Walker, D. A., Woldford, R., and Hager, G. L. (2004) *Mol. Cell* 14, 163–174
40. Lee, D. Y., Hayes, J. I., Pruss, D., and Wolffe, A. P. (1993) *Cell* 72, 73–84
41. Vettese-Dadey, M., Grant, P. A., Hebbes, T. R., Crane-Robinson, C., Allis, C. D., and Workman, J. L. (1996) *EMBO J.* 15, 2508–2518
42. Perlmann, T., and Wrange, O. (1988) *EMBO J.* 7, 3073–3079
43. Cirillo, L. A., and Zaret, K. S. (1999) *Mol. Cell* 4, 961–969
44. Li, B., Adams, C. C., and Workman, J. L. (1994) *J. Biol. Chem.* 269, 7753–7763
45. Wechsler, D. S., Papoulas, O., Dang, C. V., and Kingston, R. E. (1994) *Mol. Cell. Biol.* 14, 4097–4107
46. Li, Q., and Wrange, O. (1995) *Mol. Cell. Biol.* 15, 4375–4384
47. Li, Q., and Wrange, O. (1993) *Genes Dev.* 7, 2471–2482
48. Espinosa, J. M., and Emerson, B. M. (2001) *Mol. Cell* 8, 57–69
49. Cui, F., Sirotin, M., and Zhurkin, V. B. (2006) in *Biomolecular Pathways and System Biology, Proceedings of the 2006 International Conference on Bioinformatics and Computational Biology (BIOCOMP ’06)* (Arabnia, H. and Valafar, H., eds) pp. 48–55, CSREA Press, Athens, GA
50. Nishiyama, M., Oshikawa, K., Tsukada, Y., Nakagawa, T., Iemura, S., Natsume, T., Fan, Y., Kikuchi, A., Skoultchi, A. I., and Nakayama, K. I. (2009) *Nat. Cell Biol.* 11, 172–182
51. Lee, D., Kim, J. W., Seo, T., Hwang, S. G., Choi, E. J., and Choe, I. (2002) *J. Biol. Chem.* 277, 22330–22337
52. Xu, Y., Zhang, J., and Chen, X. (2007) *J. Biol. Chem.* 282, 37429–37435
53. Oh, J., Sohn, D. H., Ko, M., Chung, H., Jeon, S. H., and Seong, R. H. (2008) *J. Biol. Chem.* 283, 11924–11934
54. McKinney, K., and Prives, C. (2002) *Mol. Cell. Biol.* 22, 6797–6808
55. Jeffrey, P. D., Gorina, S., and Pavletich, N. P. (1995) *Science* 267, 1498–1502
56. Rippin, T. M., Freund, S. M., Veprintsev, D. B., and Fersht, A. R. (2002) *J. Mol. Biol.* 319, 351–358
57. Joerger, A. C., Ang, H. C., and Fersht, A. R. (2006) *Proc. Natl. Acad. Sci. U.S.A.* 103, 15056–15061