Constitutive DNase I Hypersensitivity of p53-Regulated Promoters*

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The ability of p53 to alter, at the transcriptional level, the gene expression of downstream targets is critical for its role as a tumor suppressor. Most models of p53 activation postulate the stepwise recruitment by p53 of co-activators, histone acetyltransferases, and/or chromatin remodeling factors to a promoter region to facilitate the subsequent access of the general transcriptional machinery required for transcriptional induction. We demonstrate here, however, that the promoter regions for the p53 target genes, p21, 14-3-3σ, and KARP-1, exist in a constitutively open conformation that is readily accessible to DNase I. This conformation was not altered by DNA damage or by whether p53 was present or absent in the cell. In contrast, p53 response elements, which reside outside the immediate promoter regions, existed within DNase I-resistant chromatin domains. Thus, p53 activation of downstream target genes occurs without p53 inducing chromatin alterations detectable by DNase I accessibility at either the promoter or the response element. As such, these data support models of p53 activation that do not require extensive chromatin alterations to support cognate gene expression.

The differential chromatin structure and nuclease accessibility at a promoter region has long been recognized as a key distinguishing feature between active and inactive genes (reviewed in Ref. 1). Almost invariably, the promoter regions of active genes are marked experimentally by hypersensitivity to restriction endonucleases (2, 3) or, more commonly, DNase I (4). Although this hypersensitivity can be caused by torsional or topological stress in the DNA and distortions in the chromatin structure resulting from transcription factor binding, it is generally caused by the absence of nucleosomes or their remodeling (1, 4). The paucity of nucleosomes, in turn, is a direct consequence of the repressive effect that nucleosomes have on the transcriptional machinery and the requirement to alleviate that effect for productive transcription to occur (5–7). Thus, inactive genes usually have promoters that are nucleosomal, are insensitive to DNase I, and are regarded as being in a closed confirmation whereas active genes usually have core promoters that are nucleosome-free, hypersensitive to DNase I, and in an open configuration. Consequently, one of the hallmarks of gene induction mechanisms is the remodeling of the nucleosomal architecture of a promoter as it is activated (5, 6, 8).

Enormous strides have been made in the last decade in understanding transcriptional activation at the chromatin level. In particular, the activation of expression of many, although not all (9–11), inducible eukaryotic genes is consistent with what can collectively be called recruitment models of gene activation (3, 7, 8) (reviewed in Ref. 12). These models usually require, in response to some extracellular cue, the induction of a specific transcription factor and the subsequent interaction of that factor with its cognate response element, which is invariably a cis-acting sequence located within the minimal promoter region of the relevant target genes. These transcription factors then recruit either chromatin remodeling factors (13, 14) or histone transacetylases (HATs)1; reviewed in Ref. 15 or both, which catalyze the opening of the chromatin at the promoter. The subsequent (7) recruitment of additional coactivators, general transcription factors, and RNA polymerase II then facilitates gene expression. Implicit in all of these models is the assumption that chromatin alterations at the promoter will accompany and/or are required for gene induction (16, 17).

Although the signal transduction pathways responsible for the cellular response(s) to genotoxic stress are complex (reviewed in Refs. 18 and 19), it has become apparent that the p53 tumor suppressor lies at the heart of the matter. In particular, p53 suppresses tumorigenic growth by transcriptionally inducible genes that facilitate either survival or death of an injured cell (reviewed in Refs. 20–22). Active p53 binds, albeit with varying affinities (23), to consensus response elements (p53 REs) within genetic regulatory loci. p53 REs consist of tandem palindromic decamers of 5′-PuPuPuPuC(A/T)(A/T)GPyPyPyPy-3′ (where Pu represents purine and Py represents pyrimidine) (24). Binding of p53 to p53 REs alters the expression of a host of genes (reviewed in Ref. 25), which fall into five main categories and include genes 1) that act to arrest the cell cycle at G1/S and G2, 2) that are involved in the induction of the G0/M cell cycle checkpoint, 3) that are involved in DNA repair, 4) that play roles in the induction or suppression (26) of apoptosis, and 5) that are involved in autoregulation (20–22).

p53 is normally expressed at very low steady-state levels, because it is rapidly turned over via proteosome-mediated degradation. This degradation requires the specific MDM2-mediated ubiquitination of p53 (27, 28). The disruption of the inhibitory interaction between MDM2 and p53, which can occur by phosphorylation of either MDM2 (29, 30) or p53 (reviewed in Refs. 21 and 31), permits the opportunistic access of HAT complexes to the N terminus of p53. Binding of HATs to p53 results in acetylation of the C-terminal regulatory domain of p53, which strongly activates the latent specific DNA binding activity of p53 in vitro (32). The biological consequences of p53

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¶ The abbreviations used are: HAT, histone transacetylase; RE, response element; RT, reverse transcriptase; IR, ionizing radiation; Gy, gray; DHS, DNase I hypersensitive site; ChIP, chromatin immunoprecipitation; Ab, antibody; EKLF, erythroid Krueppel-like factor.
Acetylation in vivo, however, are less apparent, and it has been argued that at least one important aspect of p53-HAT interactions may rather be the ability of p30 to facilitate the delivery of the HATs to the adjacent p53-inducible promoters (33, 34) (reviewed in Ref. 35). Indeed, the association of p53 with HAT’s suggests that modulation of the promoter chromatin structure may be essential for the activation of p53-responsive genes (33, 34). Reconstitution in vitro in many models of p53 activation is the assumption that alteration or remodeling of the nucleosomal architecture of a promoter is likely to be a critical feature of the mechanism of gene activation.

We have experimentally begun to address the question of how p53 influences the chromatin structure of promoters and p53 REs of p53 target genes. Here we demonstrate that the chromatin at three separate target promoter elements was constituently open and accessible to DNase I, regardless of whether gene expression was induced and/or whether p53 was present. In contrast, the chromatin domains of p53 REs were closed and were not altered following genotoxic stress or by p53 binding. These experiments demonstrate that p53 activation of gene expression does not require extensive chromatin alterations at either the RE or the promoter of the target gene.

**EXPERIMENTAL PROCEDURES**

**Cells**—A set of matched p53+/− and p53−/− HCT116 cell lines (36, 37) were generously provided to us by Dr. Bert Vogelstein (Johns Hopkins University). All cells were cultured in McCoy’s 5A medium supplemented with 100 μg/ml penicillin and streptomycin, and 2 mM l-glutamine at 37 °C with 5% CO2.

**RT-PCR**—RT-PCR was performed essentially as described (38). Total RNA was isolated from p53+/− and p53+/− HCT116 cells at 0, 2, 4, and 6 h post-IR (10 Gy) treatment via the TRIZol (Invitrogen) protocol. 5 μg of total RNA was further purified with the DNA-Free RNA kit (Zymo Research; Orange, CA), assayed for the presence of contaminating genomic DNA, and then used in downstream protocols. cDNA synthesis reactions were performed via random hexamer priming with SuperScript™ (Invitrogen) reverse transcriptase (for p21, 14-3-3, and β-actin) or via gene-specific priming with ThermoScript™ (Invitrogen) thermostable reverse transcriptase at 55 °C (for KARP-1, 5′-CTTAT-TCCGGACAGCACCATGGTCGCGGTA-3′). The cDNA from each sample was then subjected to one round of PCR amplification consisting of an initial melting step (94 °C for 5 min) followed by a number, optimized for each amplicon, of repetitive cycles consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 30 s using primers (see below) specific to different exons within p21, 14-3-3, KARP-1, and β-actin (the latter to control for DNA dilution from cDNA pools). 26 PCR cycles were utilized for p21 and 14-3-3, 30 for KARP-1, and 24 for the β-actin control.

**Chromatin Immunoprecipitations**—Modifications of the published method (39) included harvesting in phosphate-buffered saline solution, pH 7.6, and cross-linking with 2% formaldehyde. Cross-linking was quenched by addition of 150 mM glycine for 5 min followed by two ice-cold washes with phosphate-buffered saline. Cells were permeabilized in lysis buffer (50 mM HEPES-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate/protease inhibitors) and kept on ice. Chromatin was sheared by sonication until the DNA was an average length of 600–1000 bp as assessed by agarose gel electrophoresis. Cellular debris was removed by centrifugation for 5 min at 13,000 rpm and then the supernatant was centrifuged again for 15 min. A 1/50 aliquot of the cleared extract was reserved as an input control for PCR reactions, and the remaining extract was incubated overnight with antibodies at 4 °C on a rotator. 50 μl of a protein A-Sepharose slurry was added and incubated for 1.5 h at 4 °C while rocking. The Sepharose beads were then washed twice (200 μl of elution buffer (50 mM Tris, 10 mM EDTA, 0.5 mM NaCl, 0.05% Nonidet P-40) followed by addition of precipitated protein-DNA complexes were eluted from the antibodies/beads by incubation at 65 °C for 30 min. The resulting supernatants, along with input aliquots, were subjected to cross-link reversal by heating to 65 °C overnight followed by treatment with 100 μg of proteinase K for 2 h at 37 °C. DNA was purified by phenol-chloroform extraction followed by addition of 2 μg of glycogen carrier for RNA precipitation with a 1/10 volume of 3 μl sodium acetate. Precipitated DNA was resuspended in 100 μl of water, and 20% of the immunoprecipitates or 100,000 of the input were used as templates in PCR reactions with locus-specific primers.

**DNase I Hypersensitivity Assays**—The basic protocol has been described (40), and all preparatory steps were carried out at 4 °C. Briefly, 1.5 to 2 × 106 triplyxened cells were centrifuged at 2000 rpm for 10 min. The cells were then resuspended in freshly made dyeing buffer (20 mM Tris-HCl, pH 7.4, 5 mM CaCl2, 2 mM MgCl2, 0.3% Nonidet P-40) and transferred to a Dounce homogenizer. Following incubation on ice for 10 min, a 1/100 dilution of an aliquot was used to quantitate cellular breakage, cells were broken apart with 100 μl of a hard strokes and checked by visual observation under a microscope. Following quantitative breakage, nuclei were isolated by centrifugation at 900 rpm for 7 min. Nuclei were then resuspended in 1 ml of resuspension buffer, and a 1/25 μl aliquot was diluted into 475 μl of 1% SDS for an A500 measurement. The nuclear stock was diluted with resuspension buffer until the A500 of the 1:20 dilution equaled 0.5. A quenched nuclei sample was removed and added directly to cell lysis solution (Gentra Systems, Minneapolis, MN). The purified, diluted nuclei were divided into untreated and treated aliquots and treated with 37 °C for 5 min and added to cell lysis solution and mixed well. Genomic DNA was isolated from each sample as described in the DNA isolation kit (Gentra Systems, Minneapolis, MN) protocol (scaled up based on cell number) and then resuspended in DNA hydration solution (10 mM Tris- HCl, pH 8.0) at 0.5 μg/μl. Genomic DNA was then analyzed by Southern blotting to detect hypersensitive sites at particular loci.

**Southern Blots**—All blots were performed with 10 μg of genomic DNA digested to completion with 0.6 to 2.0% agarose gels following appropriate restriction enzyme(s) as shown in each figure legend. Double digestions were performed simultaneously. Following electrophoresis, agarose gels were transferred to nitrocellulose membranes as described (41). Hybridizations were performed according to the PerfectHy Plus protocol (Sigma) with a 5 min prehybridization at 64 °C with sheared salmon sperm DNA followed by the addition of 32P radiolabeled probe prepared via the random hexamer Klenow synthesis Prime-It II protocol (Stratagene) and subsequently purified over a G-50 Sephadex column. Following overnight hybridizations, the blots were washed twice at room temperature with 2× SSC for 5 min, twice (once at room temperature, once at 64 °C) with 1× SSC for 10 to 20 min, and two times with 0.1× SSC at 64 °C with 0.1× SSC. The exposure of the blots to Eastman Kodak Co. autoradiographic film at ~ 80 °C in the presence of an intensifying screen varied from 1 h to 3 days.

**Antibodies, Primer Sequences, and Southern and Northern Blot Probes**—Antibodies used in chromatin immunoprecipitation analysis included phosphoserine 15-p30 (Cell Signaling Technology, Beverly, MA) and Ab421 and p21/WAF1 (Calbiochem) antibodies. The PCR primers used for the chromatin immunoprecipitation assays were as follows. For the KARP-1-responsive element: KP-ChIP-9, 5′-AAGATT-GAGGAAGAGTGGGGG-3′ and KP-ChIP-10, 5′-TGTAGTCAGTGGATGAGTAGT-3′; for the p21/WAF1-responsive element: WAF-ChIP-1, 5′-TCCACCTTTCACCATTCCC-3′ and WAF-ChIP-2, 5′-ATAATCTCTATAAGCCACACAC-3′; for the 14-3-3 responsive element: 1433-ChIP-1, 5′-AAATACCTACTCTCCTACTCCT-3′ and 1433-ChIP-2, 5′-TCTCCTGCTTATGCCTCCCAC-3′. The KARP-1 primers generate a 200-bp fragment located 380 bp distal from the RE. The p21 and the 14-3-3 primers generate 586- and 368-bp fragments that span their respective REs.

DNA was purified by phenol-chloroform extraction followed by addition of 2 μg of glycogen carrier for RNA precipitation with a 1/10 volume of 3 μl sodium acetate. Precipitated DNA was resuspended in 100 μl of water, and 20% of the immunoprecipitates or 100,000 of the input were used as templates in PCR reactions with locus-specific primers.
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RESULTS

IR-induced Up-regulation of p21, 14-3-3, and KARP-1 Gene Expression in Wild-type, but Not p53-Null, HCT116 Cells—

HCT116 is an immortalized human colonic cancer cell line that is diploid, contains wild-type p53 and p21 genes, and responds normally to DNA damaging agents with respect to the induction of p53 and cell cycle arrest (36, 37, 42). RT-PCR analysis was used to characterize the induction of mRNA for three p53-responsive genes in HCT116 cells exposed to 10 Gy of IR. The genes investigated were p21 and 14-3-3 and another p53-responsive gene, KARP-1. The promoter regions of p21, 14-3-3, and KARP-1 were already hypersensitive to DNase I indicating a hypersensitive site (DHS) detected resided immediately adjacent to the transcription start site (+1; see Fig. 3). Thinner vertical arrows represent the weaker DHSs, with the thickness of the arrow approximating the intensity of the DHS. The promoter regions are embedded within CpG islands, which are designated by rightward-hatched rectangles below the dark line. The areas utilized as probes for the DHS assays are shown as leftward-hatched rectangles under the dark line. Relevant restriction enzyme sites are shown as dashed vertical lines, and the distances between these sites is shown with the thin lines. Exons are represented as shaded rectangles. The p53 REs are shown as small ovals. Shaded ovals (responsive) correspond to REs that are required for transactivation and/or have been shown to bind p53. In the case of p21, two REs have been identified, and these are designated as distal and proximal based upon their proximity to the start of transcription. Blackened ovals (non-responsive) correspond to REs that are a good match for the p53 consensus sequence but that do not appear to be involved in either transactivation or p53 binding. The p53 REs are also characterized by their homology to the 10-bp consensus sequence; e.g. 10/10/0/0/10 indicates an RE that consists of 10 nucleotides that are a perfect match separated by 0 nucleotides from 10 nucleotides in which only 8 matches exist.

HCT116 cells that had been mock treated or X-irradiated (10 Gy), were harvested and mixed with DNase I for 0.5, 2, or 5 min. Genomic DNA was then purified, subject to the indicated restriction enzyme cleavage(s), and analyzed by Southern blotting. Probes specific to each locus (Fig. 2) were used to detect changes in the restriction enzyme cleavage patterns of DNA isolated from irradiated versus non-irradiated cells at the varying durations of endonuclease treatment. In the absence of DNA damage, the promoter regions of p21, 14-3-3, and KARP-1 were already hypersensitive to DNase I indicating a relatively open (i.e. non-nucleosomal) chromatin structure (see Figs. 2 and 3, Non-IR). In every case, the major DNase I hypersensitive site (DHS) detected resided immediately adjacent to the transcriptional start site (+1; see Fig. 2). For p21, a second strong DHS was also detected 2 kb downstream of the transcriptional start site within intron 1 (Fig. 3, EcoRI + EcoRV digest; see also Fig. 5), but this site was not characterized further. A distinct lack of DHSs was observed at all of the p53 REs save for a few weak DHSs flanking the p53 RE in the 14-3-3 locus (Figs. 2 and 3, Non-IR) (data not shown). To see whether the chromatin structure of these genes was altered upon DNA damage, cells were exposed to IR (10 Gy), nuclei were isolated, and DNase I hypersensitivity assays were performed again. Although the intensities of the DHSs varied somewhat from experiment to experiment, the only consistent...
salient difference detected between the irradiated and non-irradiated samples was that the irradiated samples were unexpectedly more resistant to nuclease digestion (Fig. 3, compare 2' of DNase I Treatment lanes for IR with Non-IR). This trait, which is poorly understood (see "Discussion") was not characterized further. Most importantly, no extra IR-inducible DHSs were ever observed. From these experiments we concluded that the promoter regions for p21, 14-3-3, and KARP-1 were constitutively open to DNase I. In addition, we concluded that none of the p53 REs were DNase I hypersensitive and that this attribute did not change immediately following X-irradiation.

The DNase I Hypersensitivity Profiles of p53 Target Gene Promoters Is Not Altered Following X-irradiation—To investigate whether alteration of the chromatin structure of the p53 target gene promoters required a lag or maturation phase, HCT116 cells were X-irradiated (10 Gy) and then DHS assays were carried out on cells isolated at varying times post-irradiation. The DNase I hypersensitivity profiles at 15 min, 30 min, and 1 h, or 1.5 h post-irradiation were indistinguishable from cells that were analyzed immediately following X-irradiation (Fig. 4) with the exception that once again IR-treated samples were invariably somewhat more resistant to DNase I digestion than non-IR samples (compare 0' lanes with 15'; see Fig. 4). Importantly, however, even as transcription for these genes rose over 5-fold (Fig. 1), there was no corresponding detectable alteration of the chromatin structure of the promoters or the p53 REs.

The Absence of p53 Does Not Alter the DNase I Hypersensitivity of the p21, 14-3-3, or KARP-1 Promoters—In the absence of p53, non-detectable levels of p53 target genes were expressed in cells exposed to IR (Fig. 1). Thus, to extend these studies, the chromatin structure of p53-inducible promoters was investigated in HCT116 p53-null cells, where the p53 alleles have been inactivated by two rounds of gene targeting (36, 37). The DNase I hypersensitivity patterns of p21, 14-3-3, and KARP-1 loci in p53−/− cells were indistinguishable from those observed in wild-type cells with the exception of the two weak DHSs at the 14-3-3 locus, which could not reproducibly be detected in p53-null cells (compare Fig. 5 with Fig. 3). Moreover, like in wild-type cells, the hypersensitivity pattern was not altered upon X-irradiation (Fig. 5, compare Non-IR with IR). Thus, despite a complete lack of detectable basal transcription of these genes in p53-null cells, the discrete hypersensitive regions of the respective promoters were present and indistinguishable from that of wild-type cells.

p53 Binding to Its REs Following X-irradiation Is Detectable by ChIP—The absence of chromatin alterations at p53 REs as detectable by DNase I accessibility compelled us to assess p53 interactions with its cognate binding sites by other techniques. Thus, chromatin immunoprecipitation (ChIP; reviewed in Ref. 50) was utilized to analyze the in vivo occupancy of p53 REs. Preparations of nuclei from either mock or IR-treated wild-type or p53-null HCT116 cells were cross-linked with 2% formaldehyde and subjected to sonication to disrupt chromatin into ~600- to 1,000-bp pieces. Subsequently, p53-DNA complexes were immunoprecipitated, the cross-links were reversed, and the DNA was purified and then subjected to PCR analysis for the enrichment of a particular genomic locus. To ascertain whether a particular subset of modified p53 was associated with p53 REs we carried out the ChIP experiments using monoclonal antibodies specific for phosphoserine 15 p53 (23, 51). The amount of phosphoserine 15 p53 associated with these p53 REs was initially low in untreated cells, and it increased ~4- to 10-fold in every case following IR exposure (Fig. 6). Similarly, the monoclonal Ab421 detected an increase in p53 occupancy at these sites following X-irradiation (Fig. 6), consistent with what has been reported (23, 51). In all cases, no or very low levels of p53 were detected in p53-null cells or when an irrelevant antibody (p21) was utilized (Fig. 6). From these experiments we concluded that p53 was indeed binding to its REs in a DNA damage-inducible manner.
even though this event was not accompanied by DNase I hypersensitivity.

**DISCUSSION**

In this study we have established that the chromatin structures of the promoters for three p53-inducible genes were accessible to DNase I in human HCT116 cells growing under normal conditions and regardless of whether the cells were damaged or p53 was present. We have demonstrated that the binding of p53 to its cognate REs in response to DNA damage occurs in the absence of detectable chromatin alterations at the RE. These data confirm and significantly extend the previous observations that the Mdm2 (52) and GADD45 (10) promoters, which are both dependent upon p53 for DNA damage-inducible gene expression, are constitutively nucleosome-free and DNase I hypersensitive. This study has important implications for the mechanism of p53 transactivation.

**Significance of DHSs at p53-regulated Promoters under Non-inducing Conditions**—Empirically, it has long been recognized that the promoters of genes that are being actively transcribed are hypersensitive to DNase I, usually because of the absence of nucleosomes (reviewed in Refs. 1 and 4). For example, the hypoxanthine phosphoribosyltransferase gene is X-linked. On the active X chromosome, where the gene is transcribed, the promoter is nucleosome-free (6) and is hypersensitive to DNase I (6, 53). In contrast, the hypoxanthine phosphoribosyltransferase gene on the inactive X chromosome is not transcribed, and its promoter is occupied by nucleosomes, and it is not DNase I hypersensitive. Reactivation of the inactive allele by azadecoxycytidine is accompanied by the appearance of hypoxanthine phosphoribosyltransferase mRNA and the DHS (54). Similarly, β-globin transcription is dependent upon the erythroid Kruppel-like factor (EKLF) transcription factor. Cells lacking EKLF do not produce globin transcripts and do not exhibit a DHS at the proximal β-globin promoter (55, 56). Restoration of EKLF to EKLF-null cells results in the reappearance of the DHS and of globin transcription (8). These and similar studies, coupled with the observation that the degree of DNase I hypersensitivity often correlates with nucleosome remodeling (57), demonstrate that the appearance of a DHS at a promoter usually implies that the core promoter is nucleosome-free and that the gene is being transcribed. Because the promoters for p21, 14-3-3σ, and KARP-1 contain constitutive DHSs even before the genes are induced (Fig. 3) a logical interpretation of this observation is that these promoters are nucleosome-free and that the genes are being expressed in the absence of DNA damage (Fig. 7B). Whether the p21, 14-3-3σ, and KARP-1 gene promoters are truly nucleosome-free and where the nucleosome-free boundaries reside will require additional MNase footprinting studies, although these data are completely consistent with the prior demonstration that the Mdm2 (52) and GADD45 (10) promoters are constitutively hypersensitive to DNase I and that positioned nucleosomes flank the GADD45 promoter, which itself is nucleosome-free (10). Taken together, these studies suggest that the vast majority of, if not all, p53-dependent DNA damage-inducible promoters reside in open chromatin. Moreover, the openness of these promoters is consistent with the genes being transcribed at low, basal levels in non-damaged wild-type cells (Fig. 1) (data not shown). It should be noted, however, that the basal expression of p21, 14-3-3σ, and KARP-1 in p53-null cells was greatly reduced over that observed in wild-type cells such that the transcripts could no longer be detected by Northern blotting (data not shown). It is unlikely that expression is reduced to zero, however, as the expression of p21, albeit at significantly reduced rates, has been reported in a p53-null cell line (33). Together, these data imply that although p53 is not essential for the basal transcription of these genes, its presence enhances constitutive expression. This conclusion is consistent with a study in which a temperature-sensitive p53 protein was shown to induce transcription of the Mdm2 gene in the absence of DNA damage only under permissive conditions (52). Whether p53 enhances basal transcription via the same mechanism that it uses to augment transcription following DNA damage (see below) is a subject that deserves more study.

**Importance of DHSs at p53-regulated Promoters under Inducing Conditions**—In higher eukaryotes, chromatin is the physiological template for transcription. The functional effect of nucleosomes on transcription initiation is generally repressive, and the ability to overcome these inhibitory effects is invariably required for transcription to occur (reviewed in Refs. 1, 4, and 12). More often than not, transcription factors accomplish this task not through intrinsic enzymatic activities but...
through association with coactivators, HATs, and chromatin remodeling complexes (1). This mechanism of transcriptional activation is often referred to as "recruitment," and a vast amount of data has demonstrated that recruitment is actively utilized at a number of well-characterized yeast promoters and eukaryotic viral promoters (16, 17) (reviewed in Refs. 12, 58, and 59). The documented association of p53 with HATs has led to the hypothesis that p53 similarly activates transcription by recruitment of factors that will facilitate chromatin opening at promoters (33, 34) (Fig. 7A).

In this study, however, we have demonstrated that although p53 is clearly essential for the induction of these genes following IR exposure (Fig. 1) (reviewed in Refs. 20–22), it does so without grossly altering the chromatin structure of the promoters that it transactivates (Fig. 3). These observations are virtually identical to those reported for the GADD45 promoter following IR exposure (10). Although our data cannot rule out subtle chromatin alterations at p21 and related p53-regulated promoters, they clearly do not support the contention that significant chromatin alteration is needed for p53-mediated gene activation. Indeed, the only reproducible alteration observed following X-irradiation was an increased resistance to DNase I immediately after irradiation (see Figs. 3 and 4). The mechanistic basis for this increased resistance is unknown. One possibility is that the exposure of cells to IR and general radioresistance have been correlated with the phosphorylation of the histones H2AX (60) and H3 (61), respectively. Histone phosphorylation, in turn, has long been associated with chromatin condensation, which would be consistent with the observed reduction in nuclease accessibility. Importantly, however, no other significant alterations in the DHS profile at the promoter were observed following X-irradiation (see Figs. 3 and 4) even over a time interval when p21, 14-3-3σ, and KARP-1 transcription was known to be induced manyfold (Fig. 1).

If the association of HATs with p53 is not required for promoter opening, then HATs are presumably assisting transactivation through another mechanism. One possibility would be through their ability to act as factor acetyltransferases (reviewed in Ref. 15) and activate either p53 (62–64) or associated cofactors. Thus, our data are most consistent with a basic activation model in which, in their uninduced state, p53 target gene promoters are open, and the genes are being transcribed at a low level (Fig. 7B). Following DNA damage, p53 is activated and induces transcription either through the recruitment or stimulation of coactivators (33), including HATs, general transcription factors, or RNA polymerase II without, however, concomitant chromatin alterations (Fig. 7B).

Importance of DHSs at p53-regulated Promoters in p53-null Cells—The open chromatin structure of p53 target genes was remarkably independent of p53 expression (Fig. 5). Although unexpected, there is precedent for the lack of a requirement for a transactivator in determining the chromatin structure of the target promoters. For example, major histocompatibility complex class II genes can be induced in a retinoblastoma tumor suppressor protein-dependent fashion (65, 66). In retinoblastoma−/− cells these genes are not induced although the promoter region is constitutively open as defined by DNase I hypersensitivity (67). Similarly, heat shock genes are highly induced following exposure to elevated temperatures. The promoters for these genes, however, are constitutively open and accessible to DNase I even in the absence of the transactivator, heat shock factor (reviewed in Ref. 68). In both of these cases the open architecture of the relevant promoters is established by a second transcription factor, whose expression precedes the expression of the transactivator. Thus, regulatory factor X is responsible for establishing the DHS at the HLA-DRA promoter (69) whereas GAGA factor performs the same role at heat shock gene promoters (70, 71). A logical extension of these observations is that there may be a transcription factor that binds to p53-regulated promoters and establishes the open chromatin confirmation prior to p53 activation. The identity of this putative transcription factor is unknown. The p21 promoter has been analyzed exhaustively, and a plethora of transcription factors have been implicated in its expression (reviewed in Ref. 44). Unfortunately, much less is known about the promoters for 14-3-3σ and KARP-1 (41), and so a direct comparison of potential common cis-acting binding sequences is not possible at this time. It is interesting to note, however, that all three of these promoters coincide within CpG islands (Fig. 2). CpG islands are frequently rich in Sp1 or Sp1-like binding sequences (e.g. GGGCGG). Moreover, Sp1 family members are known to interact with the HATs, p300, and cAMP-response element-binding protein-binding protein (44), and it is tempting to speculate that these HATs cooperate with Sp1 to establish the constitutive open chromatin structures observed at p53-regulated promoters.

The constitutive open chromatin structure of p53-regulated genes also suggests an alternative mechanism of transactivation. In the case of heat shock genes (9, 72) (reviewed in Ref. 68), c-fos (73), and c-myc (74, 75) activation is regulated by promoter proximal pausing. For these genes, the chromatin structure of the promoters is constitutively open and contains an engaged RNA polymerase II, which is paused during elongation just downstream of the initiation site. The subsequent
binding of the relevant transactivator then promotes rapid reinitiation of the stalled polymerase (Fig. 7C) (11). Clearly, the chromatin structure of the p21, 14-3-3-σ, and KARP-1 promoters is strikingly similar to the chromatin structure of the heat shock, c-fos, and c-myc genes. Moreover, the genes regulated by promoter proximal pausing and p53 are genes that need to be induced in a rapid fashion in response to a variety of cellular stresses and conditions. Given that, at least in vitro, the assembly of the core transcriptional machinery takes several min, whereas the transition from initiation to elongation occurs within seconds (76), it is logical that these stress-related genes might be regulated similarly. There are, however, some subtle differences between p53-regulated genes and the genes regulated by promoter proximal pausing. In the case of the promoter pausing, even though a large nucleosome-free region exists over the proximal promoter region, the subsequent initiation of transcription results in downstream nucleosomal remodeling, which can be detected as additional nucleosome hypersensitivity (11) (reviewed in Ref. 68). In the case of p53-regulated genes, no additional DHSs were detected (Fig. 3) even when transcription had increased manyfold (Fig. 1). Moreover, a strong argument has been made that the open chromatin region of genes regulated by promoter proximal pausing has even when transcription had increased manyfold (Fig. 1). More-
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49. Wu, C. (1989) Methods Enzymol. 170, 289–289
50. Hecht, A., Strahl-Bolsinger, S., and Grunstein, M. (1999) Methods Mol. Biol. 119, 469–479
51. Takenaka, I., Morin, F., Seizinger, B. R., and Kley, N. (1995) J. Biol. Chem. 270, 5405–5411
52. Xiao, G., White, D., and Bargenetti, J. (1998) Oncogene 16, 1171–1181
53. Liu, D., and Chinnault, A. C. (1988) Somatic Cell Mol. Genet. 15, 261–265
54. Litt, M. D., Hansen, R. S., Hornstra, I. K., Gartler, S. M., and Yang, T. P. (1997) J. Biol. Chem. 272, 14921–14926
55. Nuez, B., Michalovich, D., Bygrave, A., Ploemacher, R., and Grosveld, F. (1995) Nature 375, 316–318
56. Wijgerde, M., Gribnau, J., Trimborn, T., Nuez, B., Philipsen, S., Grosveld, F., and Fraser, P. (1996) Genes Dev. 10, 2894–2902
57. Pazin, M. J., Bhargava, P., Geiduschek, E. P., and Kadonaga, J. T. (1997) Science 276, 809–812
58. Berk, A. J. (1999) Curr. Opin. Cell Biol. 11, 330–335
59. Roberts, S. G. (2000) Cell. Mol. Life Sci. 57, 1149–1160
60. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998) J. Biol. Chem. 273, 5858–5868
61. Liu, Y., Ussery, G. D., Muncaster, M. M., Gallie, B. L., and Blanck, G. (2001) Oncogene 20, 726–738
62. Sakaguchi, K., Herrera, J. E., Saito, S., Bustin, M., Vassilev, A., Anderson, C. W., and Appella, E. (1998) Genes Dev. 12, 2831–2841
63. Liu, L., Scollnick, D. M., Triveel, R. C., Zhang, H. B., Marmorstein, R., Halasanetsis, T. D., and Berger, S. L. (1999) Mol. Cell. Biol. 19, 1202–1209
64. Ito, A., Lai, C. H., Zhao, X., Saito, S., Hamilton, M. H., Appella, E., and Yao, T. P. (2001) EMBO J. 20, 1331–1340
65. Lu, Y., Ussery, G. D., Muncaster, M. M., Gallie, B. L., and Blanck, G. (1994) Oncogene 9, 1015–1019
66. Zhou, X., Pattenden, S., and Bremner, R. (1999) Oncogene 18, 4940–4947
67. Osborne, A., Zhang, H., Yang, W. M., Seto, E., and Blanck, G. (2001) Mol. Cell. Biol. 21, 6495–6506
68. Lis, J., and Wu, C. (1993) Cell 74, 1–4
69. Genexy, P., Reith, W., Barras, E., Lisevska-Gospipierre, B., Griscelli, C., Hadam, M. R., and Mach, B. (1989) Mol. Cell. Biol. 9, 296–302
70. Lu, Q., Wallrath, L. L., Allan, B. D., Glaser, R. L., Lis, J. T., and Elgin, S. C. (1992) J. Mol. Biol. 225, 985–998
71. Lu, Q., Wallrath, L. L., Granok, H., and Elgin, S. C. (1993) Mol. Cell. Biol. 13, 2802–2814
72. Brown, S. A., Imbalzano, A. N., and Kingston, R. E. (1996) Genes Dev. 10, 1479–1490
73. Piet, A., Eick, D., and Blanchard, J. M. (1995) Oncogene 10, 319–328
74. Krumm, A., Meulia, T., Brunvand, M., and Groudine, M. (1992) Genes Dev. 6, 2201–2213
75. strebl, L. J., and Eick, D. (1992) EMBO J. 11, 3307–3314
76. Kadenaga, J. T. (1990) J. Biol. Chem. 265, 2624–2631
77. Pina, B., Bruggemeier, U., and Beato, M. (1999) Cell 90, 719–731
78. Taylor, I. C., Workman, J. L., Schuetz, T. J., and Kingston, R. E. (1991) Genes Dev. 5, 1285–1298
79. Becker, P. B. (1994) Bioessays 16, 541–547
80. Owen-Hughes, T., and Workman, J. L. (1994) Crit. Rev. Eukaryotic Gene Expression 4, 403–441
81. McPherson, C. E., Shum, E. Y., Friedman, D. S., and Zaret, K. S. (1993) Cell 75, 387–398
82. Tsukiyama, T., Becker, P. B., and Wu, C. (1994) Nature 367, 525–532
83. Owen-Hughes, T., and Workman, J. L. (1996) EMBO J. 15, 4702–4712
84. Adams, C. C., and Workman, J. L. (1995) Mol. Cell. Biol. 15, 1405–1421
85. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. (1994) Science 265, 346–355
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