Multivalent Binding of p53 to the STAGA Complex Mediates Coactivator Recruitment after UV Damage†

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The recruitment of transcriptional coactivators, including histone modifying enzymes, is an important step in transcription regulation. A typical activator is thought to interact with several cofactors, presumably in a sequential manner. The common use of several cofactors raises the question of how activators achieve both cofactor selectivity and diversity. Human STAGA is a multiprotein complex with the acetyltransferase GCN5L as the catalytic subunit. Here, we first show, through RNA interference-mediated knock-down and chromatin immunoprecipitation assays, that GCN5 plays a role in p53-dependent gene activation. We then employ p53 mutagenesis, in vitro binding, protein-protein cross-linking, and chromatin immunoprecipitation assays to establish a novel role for the second p53 activation subdomain (AD2) in STAGA recruitment and, further, to demonstrate that optimal binding of STAGA to p53 involves interactions of STAGA subunits TAF9, GCN5, and ADA2b, respectively, with AD1, AD2, and carboxy-terminal domains of p53. These results provide concrete evidence for mediation of transcription factor binding to coactivator complexes through multiple interactions. Based on our data, we propose a cooperative and modular binding mode for the recruitment of coactivator complexes to promoters.

The tumor suppressor p53, in large part through its action as a gene-specific transcriptional activator, mediates cell cycle arrest or apoptosis in mammalian cells in response to a variety of cellular stress conditions that include DNA damage, aberrant growth signals, and exposure to certain drugs. The p53 gene is the most frequent target of genetic alterations in cancer, and the majority of the observed p53 mutations map in its sequence-specific DNA-binding domain. The induction of p53 mutagenesis, in vitro binding, protein-protein cross-linking, and chromatin immunoprecipitation assays to establish a novel role for the second p53 activation subdomain (AD2) in STAGA recruitment and, further, to demonstrate that optimal binding of STAGA to p53 involves interactions of STAGA subunits TAF9, GCN5, and ADA2b, respectively, with AD1, AD2, and carboxy-terminal domains of p53. These results provide concrete evidence for mediation of transcription factor binding to coactivator complexes through multiple interactions. Based on our data, we propose a cooperative and modular binding mode for the recruitment of coactivator complexes to promoters.

As a transcription factor, p53 has been shown to act through cofactors involved either in preinitiation complex formation (25) or covalent modification of chromosomal histones (reviewed in reference 24). In the latter case our laboratory and others have shown that, through direct interactions, p53 recruits a variety of histone modifying enzymes (including p300, PRMT1, and CARM1) to p53-dependent genes (1). While the importance of the histone acetyltransferase p300 or the related CBP for p53-dependent transcription in vivo (24) and in vitro (reviewed in reference 1) has long been established, there is mounting evidence that GCN5 and PCAF, two closely related acetyltransferases that are homologues of yeast GCN5 (yGCN5) (11, 71), also play roles in p53-dependent gene activation.

As first reported for yGCN5, which is found in the Saccharomyces cerevisiae SAGA complex (23), mammalian GCN5 and PCAF are found in large complexes. These include the GCN5-containing STAGA complex (50), the GCN5-containing TFTC complex (64), and the PCAF complex (52). The mammalian (human) complexes contain homologues of yeast SAGA subunits, as well as associated factors involved in DNA repair and RNA processing (8, 51). Furthermore, the diversity of the mammalian SAGA-like complexes is increased by the presence of not only two paralogues (GCN5 and PCAF) but also alternatively spliced forms of mammalian GCN5 (68) and two variant forms of the ADA2 subunit (4). Mice lacking PCAF develop normally and do not have a distinct phenotype, whereas GCN5 null embryos die during embryogenesis (67, 69).

Human STAGA has been shown to interact with the activation domains of VP16 (51) and c-Myc (46) and to affect Gal4-VP16-dependent transcription from a chromatinized template (51). This interaction appears to be conserved in yeast since yeast SAGA binds the Myc activation domain and since transactivation by a Myc-Pho4 fusion protein depends on the Gcn5, Ada2, and Ada3 components of yeast SAGA (21). A TFTC-type complex was reported to show ligand-dependent estrogen receptor interaction and recruitment of TRRAP and GCN5 subunits to the cathepsin D and c-fos promoters (70).

Recently, several proteins common both to STAGA and to other complexes have been functionally or physically linked to p53. Thus, TRRAP was found to act synergistically with p53 in vivo (2) and to be recruited to the p21 promoter after gamma irradiation (5). However, TRRAP is found in at least five different large multisubunit complexes implicated in chromatin modification. These include the STAGA (51), PCAF (61), TFTC (9), TIP60 (29), and p400 (22) complexes. In relation to these complexes, the TIP60 complex has been implicated in p53 transactivation (reviewed in reference 55), TRRAP and GCN5 have been shown to coimmunoprecipitate with p53 from nuclear extract (5), and coexpressed ADA3 has been shown to stabilize p53 (39).

The amino terminal activation domain (amino acids 1 to 80) of p53 consists of two subdomains, AD1 (residues 1 to 40) and...
AD2 (residues 41 to 80), that show independent functions in various assays (reviewed in reference 73). Interestingly, in an artificial yeast-based assay employing Gal4 fusion proteins, both the intact activation domain and the two subdomains were found to depend upon yeast Ad2 (yAd2), yAd3, and yGcn5 (12). Human p53 containing mutations in AD1 (L22Q/W22S) is transcriptionally dysfunctional (44), and the corresponding L25Q/W26S mutation in murine p53 is embryonic lethal (33). However, studies with a conditional p53 mutant knock-in mouse strain have revealed that although the mutant is impaired in the activation of several known p53-dependent genes, including the p21 and MDM2 genes, it is able to activate transcription of certain targets that include the proapoptotic BAX and APAF-1 genes (32, 33). AD1 has been shown to directly bind p300/CBP (27), the human TFIID TAF9 (hTAF9)/TAF631 subunit (48, 59), and Mediator subunit TRAP80/MED17 (30), all of which are potentially responsible for transcription impairment by AD1 mutations.

Although early studies showed that subdomain AD2 also plays a role in p53-dependent transcription both in yeast (12) and in human cells (62), the underlying mechanism remains to be explained. AD2 previously has been shown to interact with replication protein A subunit 1 (6, 28) and with the p62 subunit of the general transcription factor TFIIH (17, 66). However, since replication protein A and TFIIH are also involved in DNA repair, the cellular events relevant to these interactions of p53 are presently unclear.

The present study shows that both the AD1 and AD2 activation subdomains interact with the STAGA complex in vitro and that both are needed for efficient recruitment of the complex in vivo, providing a novel explanation for the role of the second p53 activation subdomain. We show that the STAGA complex, through GCN5, is specifically involved in p53-dependent gene activation, and we document the recruitment of STAGA to promoters upon UV damage. We find that p53 binds the intact STAGA complex both in vivo and in vitro. Localizing direct physical interactions of the activator to various subunits of the complex, we find that unlike what was proposed for yeast SAGA (10), STAGA interaction with the activator is mediated by several distinct contacts. Our findings are indicative of a cooperative and modular activator-coactivator binding mechanism.

MATERIALS AND METHODS

Cell culture and transfection. H1299 and U2OS cells were kept in Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum. HeLa cells in spinner cultures were kept in phosphate-buffered Dulbecco’s modified Eagle medium with 10% bovine serum for large-scale nuclear extract preparation. Transfections were conducted according to the manufacturer’s instruction using medium with 10% bovine serum for large-scale nuclear extract preparation. spinner cultures were kept in phosphate-buffered Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum. HeLa cells in

RNA was isolated from cells with Trizol (Invitrogen) and reverse transcribed with Superscript II RT (Invitrogen) according to the manufacturer’s protocol. In RNA interference experiments, agarose gel bands after RT-PCR were quantified using the ImageJ program, version 1.34s, provided by the NIH. For the PCR of GADD45 shown in Fig. 1B, the 5′ and 3′ primers were located in the third and fourth exons, respectively (1). Alternatively, real-time PCR was used to quantify RNA levels. The primers for the real-time PCR were located in the 3′ untranslated region for GADD45, in the first and second exons for p21, and in the second and third exons for PUMA. Results and standard deviations refer to the outcome of at least three experiments.

Cloning of STAGA subunits. STAF65y and human ADA2b (hADA2b) were obtained from expressed sequence tags (clones HK04750 and BU80474). STAF42a was cloned from a fetal spleen cDNA library.

Antibodies. Antibodies against human SPT3 (hSPT3), hTAF9/TAF631, and hTAF12/TAF620 were from previously reported laboratory stocks. Antibodies against hADA2b were raised in rabbits injected with bacterially purified His-tagged hADA2b. TRRAP and hGCN5 antibodies were from Santa Cruz. Antibodies against hADA3, TAF5L/PAF65, and hTAF10/TAF630 were kindly provided by Yoshishiro Nakatani (Dana-Farber Cancer Institute) and Laszlo Tora (Institut de Genétique et de Biologie Moléculaire et Cellulaire, Illkirch, France).

GST pull-down assays. Glutathione S-transferase (GST) fusion proteins were expressed in the Escherichia coli strain XA90 and purified as described previously (26). Proteins were labeled with [35S]methionine using TNT reticulocyte lysates, following the standard protocol provided by Promega. In vitro translated proteins were incubated with resin-bound proteins (10 μg) by rotating at 4°C for 3 hours in 4 h in BC buffer (10% glycerol, 20 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol) containing 0.05% NP-40, 40 mM KCl, and 0.1 μg/ml bovine serum albumin. After incubation, BC buffer containing 0.05% NP-40 and 200 mM KCl, the resin was either stripped with a mixture of 20 mM Tris-HCl, pH 7.9, 100 mM NaCl, and 0.2% N-lauroyl sarcosine or directly boiled in sodium dodecyl sulfate (SDS) loading buffer before being subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Gels were visualized in Amplify (Amersham Pharmacia) before drying and autoradiography steps to enhance the signal. The same resin preparation, washing, and reaction conditions and reactions were used for hADA2b nuclear extract and the purified STAGA complex when they were incubated with the resin-coupled GST-fusion proteins. Eluates were subjected to SDS-PAGE and blotted onto nitrocellulose membrane (Protran; Schleicher and Schuell) for immunoblotting.

Nuclear extract preparation and complex purification from stable cell lines. Nuclear extract was prepared as described previously (16). The STAGA complex was purified from the nuclear extract of a cell line stably expressing FLAG-hemagglutinin (HA)-tagged SPT3 (51) by immunopurification with M2 agarose resin (Sigma). After successive washes with BC buffer containing 300 mM KCl–0.05% Nonidet P-40 and BC buffer containing 0.05% NP-40 and 200 mM KCl, the resin was either stripped with a mixture of 20 mM Tris-HCl, pH 7.9, 100 mM NaCl, and 0.2% N-lauroyl sarcosine or directly boiled in sodium dodecyl sulfate (SDS) loading buffer before being subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Gels were visualized in Amplify (Amersham Pharmacia) before drying and autoradiography steps to enhance the signal. The same resin preparation, washing, and reaction conditions were used for hADA2b nuclear extract and the purified STAGA complex when they were incubated with the resin-coupled GST-fusion proteins. Eluates were subjected to SDS-PAGE and blotted onto nitrocellulose membrane (Protran; Schleicher and Schuell) for immunoblotting.

Cross-linking experiments. The STAGA complex was bound to GST fusion proteins on glutathione-Sepharose and washed as described for the GST pull-down experiments. After an additional two washes with PBS–0.05% NP-40 to eliminate traces of Tris, the resin was suspended in 600 μl of PBS–0.05% NP-40, and 55 μl of 25 mM dithiothreitol (DTT) (in dimethyl sulfoxide) was added. After 2 min the supernatant was removed, and the reaction was quenched with 50 mM Tris-HCl, pH 7.5, for 30 min at room temperature. The proteins on the beads were denatured with 8 M urea for 1 hour before being washed twice with 1 ml of BC buffer, 200 mM KCl, and 0.05% NP-40. Elution

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and reversal of cross-linking were effected by stripping with N-lauroyl-sarcosine in the presence of 0.1 M dithiothreitol at 37°C for 30 min or by boiling in reducing SDS buffer. The former elution method gave generally cleaner immunoblots.

RESULTS

GCN5 is needed for efficient activation of the p53-inducible GADD45 gene. To investigate whether GCN5 is involved in p53-dependent gene expression, we derived a stable cell line (H9) expressing a temperature-sensitive p53 mutant (p53V143A) from p53-negative H1299 lung carcinoma cells. This p53 mutant activates a variety of target genes as effectively as wild-type p53 at 32°C but not at 37°C (72). Consistent with a report that GADD45α, cyclin G, p21, and HDM2 are induced by p53V143A at the permissive temperature (3), a shift from 37°C to 30°C led to increases of p21 and GADD45 RNA levels in H9 cells but not in control H1299 cells (data not shown). Treatment of H9 cells with GCN5 siRNA led to a greater than 90% decrease in the level of GCN5 (Fig. 1A). GCN5 knockdown in these cells resulted in a concomitant reduction in the accumulation of GADD45 RNA in response to the shift from 37°C to 30°C (Fig. 1B, lanes 1 to 3), whereas a control nontargeting siRNA had no significant effect on p53-dependent activation (lane 4). In an extension of these results, real-time PCR was used to quantify the effect of GCN5 siRNA on PUMA, p21, GADD45, and GAPDH RNA levels. GCN5 siRNA, but not control siRNA, resulted in impaired induction of the p53-dependent gene products PUMA, p21, and GADD45. GCN5 is thus required for the activation of several p53-dependent genes.

UV-induced recruitment of hGCN5 to promoter sites containing p53 response elements. In yeast, GCN5-containing complexes are recruited by transcription factors to promoters of several target genes (40, 42). To determine whether the hGCN5L-containing STAGA complex, an acetyltransferase complex with preference for histone H3 (50), is directly involved in p53 activation, a ChIP assay was employed to assess p53 and GCN5 binding to GADD45 and p21 promoters. The GADD45α gene contains a single p53 binding site 1.6 kb downstream of the core promoter in the third intron (35). The human p21 promoter contains two p53 response elements that are located 1.4 and 2.3 kb upstream of the transcription initiation site (18) (Fig. 2C). Both sites are at least partially bound by p53 in vivo (19, 34), p53-positive U2OS osteosarcoma cells showed, as expected, UV-induced increases in mRNAs from p53 target genes GADD45 and p21 (Fig. 2A). As reported for W51 fibroblasts (43), UV irradiation of U2OS cells led to phosphorylation of p53 at serine 15 and to a slow, modest increase in the p53 protein level (Fig. 2B). The GCN5 protein level was not affected by UV irradiation.

As shown by ChIP analyses, UV irradiation resulted in recruitment of GCN5 to p53 response elements at both the GADD45α and p21 promoters in U2OS cells (Fig. 2C). A control site downstream of the p53 binding site in the GADD45 promoter was not bound by GCN5. GCN5 binding and histone H3 acetylation appeared to be concomitant with p53 binding to the p21 promoter (Fig. 2D), supporting the hypothesis that STAGA is recruited to the promoter by p53.

The acidic amino terminus (residues 1 to 73) of p53 encompasses a domain needed for transcription activation (20, 36, 53). This activation domain has been implicated in the recruitment of cofactors p300 and PRMT1 to the GADD45 promoter but is dispensable for CARM1 recruitment (1). To test whether GCN5 is recruited to the p21 promoter by p53 and, if so, whether the activation domain of p53 is needed for the recruitment, p53-negative H1299 lung fibroblasts were transiently transfected with either FLAG-tagged wild-type p53 or a FLAG-tagged mutant lacking the amino terminal 73 residues. An immunoblot showed that the wild-type (Fig. 2E, W) and the mutant protein (Fig. 2E, ΔN) were equally well expressed, and ChIP assays confirmed that both proteins bound to the p21 promoter (Fig. 2F). However, only wild-type p53 resulted in accumulation of GCN5, as well as acetylated H3, on the promoter, indicating that the amino-terminal activation domain of p53 is essential for the recruitment of GCN5 in vivo.
Binding of STAGA to p53 in vitro and in vivo. GCN5 is a subunit of the human complex STAGA. To see whether the STAGA complex binds to p53, a bead-immobilized GST-full-length p53 fusion protein (GSTp53) was incubated with HeLa nuclear extract and washed. An immunoblot of bound proteins revealed binding of STAGA subunits TRRAP, GCN5L, TAF5L (PAF65/H9252), TAF9 (TAFII31), and SPT3 to GSTp53 but not to GST alone (Fig. 3A). Similarly, an affinity-purified STAGA complex (51), derived from a cell line that expresses a FLAG-tagged SPT3, bound to GSTp53 but not to GST alone (Fig. 3B). Thus, STAGA binding to p53 is direct and does not require other cofactors for a strong interaction.

We next determined whether STAGA also interacts with p53 in vivo. To this end we first characterized the purified STAGA complex with respect to STAF65/H9253 and STAF42/H9251, two associated proteins that are related, respectively, to the yeast SAGA subunits Spt7 and Ada1 (51). Immunoblotting for representative STAGA subunits in anti-FLAG (M2 agarose) immunoprecipitates from stable HeLa cell lines expressing FLAG-tagged STAF65/H9253 and STAF42/H9251 confirmed that the two proteins are integral subunits of the STAGA complex (data not shown). Because their yeast counterparts are thought to play structural roles in the related SAGA complex (65), we chose these two subunits for in vivo interaction studies. Pull-down assays with in vitro translated proteins confirmed that STAF65γ and STAF42α do not directly interact with GSTp53 (see Fig. 5D below).

Because the p53 pathway in HeLa cells is compromised by the endogenous human papillomavirus E6 protein (37, 57), we chose not to use the stable cell lines for in vivo interaction studies with p53. Instead, FLAG-HA-tagged STAF65/H9253 and STAF42/H9251 were individually coexpressed with p53 in H1299 cells. The STAGA complex with bound proteins was immunoprecipitated with M2 agarose and subjected to immunoblotting. As shown in Fig. 3C, p53 was immunoprecipitated with FLAG-HA-tagged STAF65/H9253 or STAF42/H9251 but not with FLAG-HA-tagged GAPDH (lanes 3 to 5). We also performed the reverse immunoprecipitation of endogenous STAGA with tagged p53 (Fig. 3D). H1299 cells were transfected with a vector expressing FLAG-tagged p53. Whole-cell extracts from transfected and untransfected cells were prepared and incubated with M2 agarose. Bound material was washed and analyzed by immunoblotting for representative STAGA subunits. STAGA was found to coprecipitate with tagged p53, indicating that p53 also associates with the STAGA complex in vivo.
FIG. 3. STAGA interacts with p53 in vitro and in vivo. (A) STAGA subunits in HeLa nuclear extract bind to p53. GST alone or GSTp53 was bound to glutathione-Sepharose and incubated with HeLa nuclear extract. The beads were washed, and bound proteins were analyzed by immunoblotting. (B) The STAGA complex purified from a tagged tSPT3 stable cell line binds to p53. GST and GSTp53 beads were incubated with purified STAGA, and bound proteins were analyzed as described in panel A. (C) In vivo interaction of STAGA with p53. Tagged STAGA subunit STAF65y or STAF42α was coexpressed with p53 in H1299 cells. After in vivo cross-linking, whole-cell extracts were prepared and incubated with M2 agarose. Bound material was washed and analyzed by immunoblotting with antibodies against p53 and HA. Cells expressing tagged GAPDH, p53 only, or STAF42α without p53 served as negative controls. (D) Immunoprecipitation of endogenous STAGA with tagged p53. Whole-cell extracts from H1299 cells transfected with a vector expressing FLAG-tagged p53 were prepared and incubated with M2 agarose. Bound material was washed and analyzed by immunoblotting. STAGA subunits were found to coprecipitate with tagged p53. Untransfected cells served as negative controls. f, Flag tag; ha, HA tag; IN, input; IP, immunoprecipitation.

STAGA binds to distinct p53 domains that include the amino terminal activation domain of p53. The requirement of the p53 amino terminus for GCN5 recruitment to endogenous target genes suggests that the STAGA complex binds directly to the activation domain of p53. To test this hypothesis, GST fusion proteins with full-length p53 [p53(1–393)], the p53 amino-terminal activation domain [p53(1–73)], or a p53 mutant lacking the amino terminus [p53(74–393)] were incubated with HeLa nuclear extract. As shown in Fig. 4A, endogenous STAGA, monitored by GCN5 and SPT3 subunits, showed only moderate (but significant) binding to the p53 activation domain (lane 3) compared to the intact p53 (lane 2) and only a very weak binding to the carboxy-terminal fragment (lane 4). These results indicate that while the amino-terminal activation domain of p53 is sufficient for a significant interaction of p53 with STAGA, a region(s) outside this domain contributes strongly to p53 binding to the complex.

In a further analysis, we checked for interactions of isolated AD1 and AD2 domains with STAGA in nuclear extracts. As shown in Fig. 4B, the endogenous STAGA complex did not bind measurably to either of the two isolated subdomains of p53 (lanes 2 to 4) or to a p53 activation domain, p53(1–73), with the double point mutation L22Q W23S (m22, 23S; lanes 5) are known to negatively affect p53-dependent transcription and cellular function, with a quadruple mutation, L22Q W23S W53Q F54S (53Q, 54S; lanes 5), showing the most severe effect. f, Flag tag; ha, HA tag; wt, wild type; mut, mutant.

Point mutations in either of the two amino-terminal activation subdomains of p53 affect STAGA binding. As mentioned, p53 regions containing amino acids 1 to 40 (AD1) and 41 to 80 (AD2) were proposed to function as independent activation domains. Interestingly, the mutations in either AD1 (L22Q W23S) or AD2 (W53Q F54S) negatively affect the transcription of a variety of p53-dependent genes, yet the mutants retain growth suppression activity, while a quadruple mutant completely loses transactivation and growth suppression activity (62). The deletion of p53 amino acids 1 to 23 or 63 to 91 leads to mutants with decreased, but still significant, activation of genes such as the p21 gene, whereas deletion of amino acids 1 to 63 or mutation of residues 22, 23, 53, and 54 completely...
shown to involve p53 residues 9 to 25 (60). To verify and map
been challenged by more recent cross-linking data (38, 56).
subunit mediating interaction with acidic activators (10) has
regarding yeast SAGA, the proposed role of Tra1 as the sole
could be responsible for a lack of observed binding. Of note
amino terminus (lanes 4 to 6). Of note, we did not observe
length p53, indicating that ADA2b binds p53 outside the
well as GCN5L and TAF9, was found to interact with full-
linked in the absence of STAGA (Fig. 5A).
stimulated to cross-linking with DSP (15, 47) according to the
GSTp53(1–393) or GST-p53(1–73) fusion proteins and sub-
interact with p53, purified STAGA was bound to immobilized
in p53. Consistent with these results, and indicative of a con-
these residues leads to weaker binding of STAGA than intact
p53 but stronger binding than p53(74–393) (data not shown).
Identification of STAGA subunits that are direct targets of
p53. In order to assess human STAGA subunits that directly
with p53, purified STAGA was bound to immobilized
STAGA subunit interactions, we used ChIP assays to deter-
mine whether AD1 (L22Q W23S) and AD2 (W53Q F54S) weakened the binding of STAGA to p53, with
the mutation in the second activation subdomain having a
more pronounced effect (Fig. 4C, lanes 4 and 5 versus
lane 2). A quadruple mutation of the activation domain dimin-
ished the interaction most severely (lanes 3).
These results are in agreement with a binding mode involv-
ing interaction sites within both activation subdomains and at
least one other interaction surface outside the amino terminus
in p53. Consistent with these results, and indicative of a con-
tribution of the first 40 amino acids of p53, a mutant lacking
these residues leads to weaker binding of STAGA than intact
p53 but stronger binding than p53(74–393) (data not shown).

Identification of STAGA subunits that are direct targets of
p53. In order to assess human STAGA subunits that directly
interact with p53, purified STAGA was bound to immobilized
GSTp53(1–393) or GST-p53(1–73) fusion proteins and sub-
ject to cross-linking with DSP (15, 47) according to the
protocol shown in Fig. 5A. This reversible homobifunctional
cross-linker, which mainly targets lysines, precludes possible
interference with target binding and altered mobilities that
might complicate assays with other (e.g., covalently linked pho-
toactivated cross-linking) agents. The cross-linking kinetics
were chosen so as to restrict cross-linking to two adjacent
partners and thus to avoid artificial positive signals due to
ternary linkages. As a control, a GST fusion protein was cross-
linked in the absence of STAGA (Fig. 5A).
In the analysis of Fig. 5B, in which representative STAGA
subunits were scored by immunoblotting after cross-link rever-
sal and elution, only GCN5L and hTAF9 were consistently
found to directly interact with the isolated activation domain
(residues 1 to 73) of p53 (lanes 1 to 3). In contrast, ADA2b, as
well as GCN5L and TAF9, was found to interact with full-
length p53, indicating that ADA2b binds p53 outside the
amino terminus (lanes 4 to 6). Of note, we did not observe
cross-linking to TRRAP or ADA3, despite previous reports of
direct interactions of p53 with isolated ADA3 or a fragment of
TRRAP (2, 39) and direct interactions of acidic activators with
yeast SAGA through the TRRAP homologue Tra1 (10). In the
case of TRRAP, detection limits of our immunoblot analysis
could be responsible for a lack of observed binding. Of note
regarding yeast SAGA, the proposed role of Tra1 as the sole
subunit mediating interaction with acidic activators (10) has
been challenged by more recent cross-linking data (38, 56).
As mentioned, the binding of (isolated) hTAF9 to p53 was
shown to involve p53 residues 9 to 25 (60). To verify and map
the observed interactions of ADA2b and GCN5L with p53, we
tested the binding of in vitro translated ADA2b and GCN5L to
GST-fused p53 deletion mutants. In agreement with the cross-
linking data, ADA2b bound very efficiently to a carboxy ter-
minal fragment, p53(74–393), but not detectably to the amino-
terminal activation domain (Fig. 5D). Also in agreement with
our cross-linking data, GCN5 bound efficiently to the intact
AD of p53 and, importantly, almost equally well to AD2 (res-
ides 41 to 80) but not to AD1 (residues 1 to 40) (Fig. 5D).
Thus, GCN5 binds to a subdomain distinct from that reported
to bind hTAF9 (48, 60). A further analysis with in vitro trans-
laced GCN5 showed that both hGCN5L and a shorter splice
variant (68) not present in the STAGA complex (hGCN5S)
bound p53 efficiently in vitro, indicating that the nonconserved
amino terminus of hGCN5L is not needed for the interaction
(data not shown). As a further control, and consistent with the
cross-linking data (Fig. 5B and C), three other STAGA sub-
units (STAF42α, hADA3, and hSPT3) showed no detectable
binding to p53 under the same conditions (Fig. 5D).
The results of the cross-linking and binding assays, indicat-
ing interactions of distinct p53 domains with three direct bind-
ing partners in STAGA, are in strong agreement with the
observed affinities of STAGA for p53 mutants bearing various
truncation and point mutations. Altogether, these data dem-
strate, for the first time, that simultaneous interactions of
different subunits with distinct activator regions contribute in
a cooperative manner to activator-coactivator binding.

STAGA interactions with both p53 activation subdomains,
AD1 and AD2, are important for STAGA recruitment in vivo.
In order to test the in vivo relevance of the observed p53-
STAGA subunit interactions, we used ChIP assays to deter-
mine whether AD1 (L22Q W23S) and AD2 (W53Q F54S)
point mutations that impair in vitro binding of STAGA also
impair GCN5 recruitment to endogenous p53 target genes.
H1299 cells that express an inducible p53 were harvested 24 h
after tetracycline withdrawal. These cells contain similar levels
of overexpressed p53 (45) and showed comparable binding of
p53 to the p21 promoter in a ChIP assay, indicating that the
point mutations do not affect p53 binding to the regulatory
elements under the present conditions (Fig. 6A). In contrast,
the two sets of point mutations, alone and in combination,
especially eliminated GCN5 recruitment and drastically re-
duced H3 acetylation. This observation that an intact p53 ac-
tivation domain is needed for detectable GCN5 binding to the
p53 response element underscores the importance of the indi-
vidual interactions of the p53 subdomains AD1 and AD2 with
the STAGA complex, and these interactions appear responsi-
ble, at least in part, for the detrimental effects of the mutations
on expression of p53 target genes (Fig. 6B). Residual transcrip-
tion by the individual sets of mutants could be explained by
residual interactions of AD1 or AD2 mutants with STAGA
that are too weak to be detected by ChIP assay. It is therefore
very likely that the same cooperative interactions that govern
p53 binding to the complex in vitro are also involved in
STAGA recruitment in vivo.

DISCUSSION
This report presents a functional analysis of the human
STAGA complex, which contains the histone acetyltransferase
GCN5, in p53-dependent gene activation and a biochemical analysis of STAGA interactions with p53. It demonstrates that p53-dependent transcription of \textit{GADD45} requires GCN5, that p53 recruits GCN5 to the p53-dependent p21 and GADD45 promoters after UV damage, and that STAGA binding to p53 involves cooperative interactions of three distinct subunits of the complex with three distinct p53 domains. These results provide evidence that STAGA is directly involved in p53-dependent gene activation. They also provide evidence for a cooperative binding model of activators with multiprotein co-activator complexes, including clues on the possible regulation of cofactor recruitment and corresponding gene activation.

\textbf{STAGA, through GCN5, acts as a coactivator of p53.} Studies of p53 function in yeast and reports that p53 interacts directly with several isolated proteins that are subunits of STAGA, as well as other complexes (2, 39, 48), have led to the speculation that the human STAGA complex itself might interact with p53 and contribute to p53 function. Here, we show through RNA
interference-mediated knockdown and ChIP assays that GCN5 is important for p53-dependent gene activation. We further show that p53 can bind the STAGA complex specifically, both in vitro and in vivo, and that p53 effects GCN5 recruitment, concomitant with H3 acetylation, to endogenous p53-dependent promoters. These results are consistent with our previous demonstration (1) showing that, on the GADD45 gene, UV-induced recruitment of GCN5 correlates with enhanced acetylation of H3 (but not H4), whereas the UV-induced recruitment of p300 correlates with enhanced acetylation of H4 (but not H3). Similar to Drosophila GCN5, hGCN5 is found in the ADA2b-containing STAGA complex and also in a distinct complex containing ADA2a (A. M. Gamper, J. Kim, and R. G. Roeder, unpublished data). Our unpublished ChIP assays showing the recruitment of STAGA-specific ADA2b but not of ADA2a after UV irradiation to p53-dependent promoters strongly supports the idea that STAGA is recruited by p53. While STAGA is clearly involved in transcription of some p53 target genes, the extent to which it is generally required for transcription in mammalian cells remains to be determined.

Distinct domains of p53 interact with specific STAGA subunits. By using a bifunctional cross-linker to analyze interactions of p53 or the p53 amino-terminal activation domain with bound STAGA, we identified subunits TAF9, ADA2b, and GCN5L as direct interaction partners of p53 in the context of the intact STAGA complex. These interactions were confirmed by the demonstration of direct interactions of p53 with isolated TAF9 (48, 59) and with isolated GCN5 and ADA2b proteins (this study). Further studies showed that the amino terminal p53 activation domain, comprised of the AD1 (residues 1 to 40) and AD2 (residues 41 to 80) subdomains, interacts with TAF9 and GCN5L, whereas the ADA2b interaction is mediated by an undefined domain(s) in the remaining p53 carboxy-terminal region, p53(74–393).

In relation to AD1, it has been suggested that the reduced transcription caused by AD1 mutations affecting the TAF9-p53 interaction reflects reduced p53-TFIID interaction (48, 59). However, our demonstration that TAF9 is involved in STAGA interactions with p53 and that mutations (L22Q W23S) in AD1 diminish p53 STAGA interactions in vitro and in vivo suggests decreased transcription due to reduced p53-STAGA interactions. Furthermore, AD1 also has been implicated in p53 interactions with the Mediator (30) and with p300 (27). Hence, the AD1 domain may well facilitate the recruitment and function of several coactivators.

In relation to AD2, we have demonstrated, first, that mutations (W53Q and F54S) previously shown to reduce p53-dependent transcription dramatically decrease p53-STAGA interactions and, second, that GCN5L binds strongly to the isolated AD2 subdomain. These results provide a novel explanation for the well-established physiological role of AD2 in transcription of various p53 target genes (62, 73, 74).

Beyond the p53 AD1 and AD2 interactions with STAGA through TAF9 and GCN5, respectively, we demonstrate an
important role for a carboxy-terminal domain(s) in p53 interactions with STAGA through the ADA2b subunit. In an extension of the present work, our preliminary studies (data not shown) have shown ADA2b binding both to a central p53 fragment (residues 120 to 290) that contains the DNA binding domain and to a carboxy-terminal fragment (residues 290 to 393).

A cooperative and modular binding mode. Despite the ability of the p53(1–40), p53(41–80), and p53(74–393) fragments to bind TAF9, GCN5, and ADA2b relatively well, none of these domains alone suffices for detectable interaction with the complete STAGA complex in vitro. However, certain combinations of two domains significantly increase the binding, with p53(1–73) and p53(41–393) showing easily detectable binding of STAGA (data not shown). These results underscore the cooperativity in the overall binding mechanism.

Interaction of a single activator such as p53 with multiple subunits of a coactivator complex such as STAGA, Mediator, or TFIID would make sense from energetic, evolutionary, and regulatory points of view. Thus, a combinatorial set of binding partners provides a means both for fine-tuning the strength of the overall interaction and for a wider range of control. It also allows the modular “construction” of activators from interacting (sub)domains during evolution—similar to the combinatorial use of activator binding sites in promoters—and the assembly of diverse coactivator complexes using common subunits. Importantly, individual activation domain-coactivator subunit interactions need not be strong, thus allowing for greater (perhaps stepwise) reversibility and for a dynamic interplay and exchange, potentially in a temporal sequence of multiple coactivators with distinct functions.

In the case of p53, it will be important to extend the current study of interaction multivalency to other relevant cofactor complexes. These include Mediator, TFIID, and the HDAC1 complex with demonstrated p53 interactions that involve, minimally, p53 AD1-MEDI17/TRAP80 (30; also S. Yamamura and R. G. Roeder, unpublished results), p53 AD1-TAF9 (48, 59), and p53 AD1-PID/MTA2 (49) interactions, respectively.

Regulation of p53-STAGA interactions. p53 is subject to a number of constitutive and stress-induced posttranslational modifications in both the amino-terminal activation domain and the carboxy-terminal regulatory domain (reviewed in reference 7). The phosphorylation of specific p53 residues was shown to enhance p300/CPB interactions (41), and phosphorylation was also proposed to differentially regulate HDM2 and TAF9 binding to p53 (31), whereas mutations in a primary AD1 phosphorylation site were shown to reduce activation of these domains alone suffices for detectable interaction with the complete STAGA complex in vitro. However, certain combinations of two domains significantly increase the binding, with p53(1–73) and p53(41–393) showing easily detectable binding of STAGA (data not shown). These results underscore the cooperativity in the overall binding mechanism.

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Finally, another important question is whether requirements for each of these multivalent p53-STAGA interactions show any target gene specificity. In this regard, gene-specific variations in AD1 and AD2 requirements have been noted (14, 33, 73, 74).


Liu, G., T. Xia, and X. Chen. 2003. The activation domains, the proline-rich domain, and the C-terminal basic domain in p53 are necessary for acetylation of histones on the proximal 212 promoter and interaction with p300 CREB-binding protein. J. Biol. Chem. 278:17557–17565.


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