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Sgf29p Facilitates the Recruitment of TATA Box Binding Protein but Does Not Alter SAGA's Global Structural Integrity in Vivo

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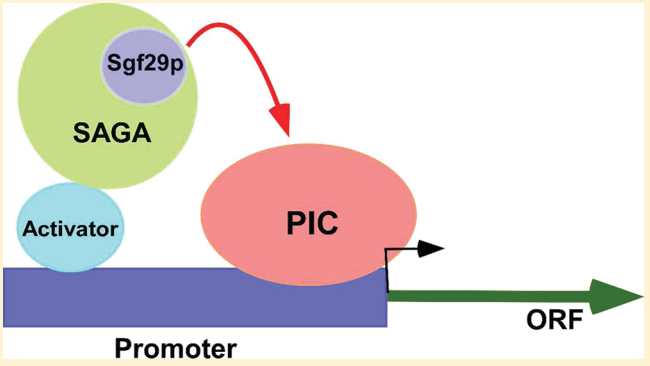
ABSTRACT: Although Sgf29p has been biochemically implicated as a component of SAGA (Spt-Ada-Gcn5 acetyltransferase), its precise mechanism of action in transcription is not clearly understood in vivo. Here, using a formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation (ChIP) assay in conjunction with transcriptional and mutational analyses, we show that Sgf29p along with other SAGA components is recruited to the upstream activating sequence (UAS) of a SAGA-regulated gene, *GAL1*, in an activation domain-dependent manner. However, Sgf29p does not alter the recruitment of Spt20p that maintains the overall structural and functional integrity of SAGA. The recruitment of other SAGA components such as TAF10p, TAF12p, and Ubp8p to the *GAL1* UAS is also not altered in the absence of Sgf29p. Interestingly, we find that the recruitment of TBP (TATA box binding protein that nucleates the assembly of general transcription factors to form the preinitiation complex for transcriptional initiation) to the core promoter of *GAL1* is weakened in Δ*sgf29*. Likewise, Sgf29p also enhances the recruitment of TBP to other SAGA-regulated promoters. Such weakening of recruitment of TBP to these promoters subsequently decreases the level of transcription. Taken together, these results support the idea that SAGA-associated Sgf29p facilitates the recruitment of TBP (and hence transcription) without altering the global structural integrity of SAGA in vivo.



SAGA is a large multiprotein complex with histone acetyltransferase (HAT) and deubiquitinase activities.[1,2] In yeast, SAGA has at least 14 nonessential and 6 essential components and is required for transcription of approximately 10% of genes.[3] The nonessential components include Gcn5p-HAT, Ubp8p-histone deubiquitinase, Ada1p, Ada2p, Ada3p, Spt3p, Spt7p, Spt8p, Spt20p, Sgf11p, Sgf29p, Sgf73p, Sus1p, and Chd1p. The essential components of SAGA include the ATM/PI-3-kinase-related protein Tra1p and a set of TBP-associated factors (TAFs). SAGA's global structural integrity is maintained by Ada1p, Spt7p, and Spt20p,[1,2,4,5] and it is targeted to the promoter via interaction of its Tra1p component with transcriptional activators.[6−9] For several yeast SAGA subunits, conserved homologues are found in mammals. For example, TRRAP (transformation/transcription domain-associated protein) has been identified as a mammalian homologue of yeast Tra1p. Like yeast Tra1p, TRRAP has been reported to be the target of several activators.[10] Moreover, there is a TRRAP-containing mammalian complex, STAGA (SPT3-TAF_{II}31-$GCN5_L$ acetylase), which is a homologue of the yeast SAGA complex.[11] Although many conserved homologues of yeast SAGA components are found in humans, homologues of yeast Spt8p and Chd1p have not yet been identified in human SAGA or STAGA.[12] Like in yeast and humans, the SAGA complex is also present in *Drosophila*.[12] However, the homologues of yeast Spt8p, Spt20p, TAF6p, Sgf73p, and Chd1p have not yet been identified in *Drosophila*.[12] Similar to the yeast and human SAGA complexes, *Drosophila* SAGA is also targeted by acidic activators.[12,13] Additionally, histone covalent modifications are also involved in recruiting SAGA to the active gene. For example, SAGA interacts with acetylated histone H3, and such an interaction is mediated by the bromodomain of its Gcn5p component.[1,14−21] Similarly, the chromodomain of the Chd1p component of yeast SAGA interacts with di- and trimethylated K4 (lysine 4) of histone H3[22−24] and plays an important role in the recruitment of SAGA onto chromatin. Collectively, these studies demonstrated that activators and histone covalent modifications play crucial roles in recruiting and stabilizing SAGA on the promoter. Interestingly, a very recent structural and biochemical study[25] revealed that yeast SAGA interacts with methylated K4 of histone H3 via the tudor domain of its Sgf29p component. Consistently, this study further demonstrated that the absence of histone H3K4 methyltransferase weakens the recruitment of SAGA.[25] Likewise, another recent study in human cell lines also revealed the interaction of Sgf29p with methylated K4 of histone H3.[26] Thus, the recognition of histone H3 K4 methylation by SAGA via its Sgf29p component appears to be evolutionarily conserved among eukaryotes.

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How is the recruitment of SAGA correlated with the association of TBP [which nucleates the assembly of general transcription factors for formation of the preinitiation complex (PIC) to initiate transcription] with the promoter? Previous biochemical and genetic studies[5,27,28] identified the interaction of SAGA with TBP via its Spt3p and Spt8p components. Consistent with these studies, the chromatin immunoprecipitation (ChIP) experiments demonstrated the requirement of Spt3p and Spt8p for recruitment of TBP at several SAGA-regulated promoters such as *ADH1*, *PHO84*, *BDF2*, and *VTC3*.[4] However, Spt3p and Spt8p have also been implicated in inhibiting TBP recruitment at several other promoters such as *HIS3*, *TRP3*, and *HO*.[29,30] Intriguingly, the ChIP experiments further revealed that Spt3p, but not Spt8p, is required for TBP recruitment at the promoter of a well-characterized SAGA-dependent gene, *GAL1*.[4] Together, these results support the idea that Spt3p and Spt8p perform distinct functions in recruiting TBP. Further, Qiu et al.[31] demonstrated that SAGA can promote the recruitment of RNA polymerase II in a manner that is independent of TBP. In addition to its role in the recruitment of TBP and RNA polymerase II, SAGA also regulates transcriptional initiation via its HAT[1,4,32−36] and histone H2B deubiquitinase[37−42] activities. Together, these studies reveal a complex regulation of transcriptional initiation by SAGA. Further, recent studies revealed SAGA's participation in transcriptional repression at the telomere, transcriptional elongation, mRNA export, active gene translocation, and genome repair,[1,2,43−49] thus implicating it as an important regulator of gene expression.

Previous biochemical studies[50] identified Sgf29 as a component of SAGA that is conserved from yeast to humans. A recent study in yeast demonstrated the role of Sgf29p in transcriptional stimulation.[25] Further, another study in rats implicated Sgf29p in the regulation of the expression of genes involved in tumorogenesis.[51] While the function of Sgf29p in transcription has been documented in yeast and mammalian systems, its precise mechanism of action in transcriptional regulation is not clearly understood in living cells. Recently, Bian et al.[25] implicated Sgf29p in the recruitment of SAGA via its interaction with methylated K4 on histone H3 in yeast. Similarly, another recent study in human cell lines[26] also showed the requirement of Sgf29p in the recruitment of SAGA via the interaction of Sgf29p with methylated K4 of histone H3. Although these studies[25,26] demonstrated an important role for Sgf29p in the recruitment of SAGA (and hence SAGA's function in transcription) via histone H3 K4 methylation, it is not known whether Sgf29p can also play additional function(s) in transcription by regulating SAGA's structural integrity, TBP recruitment, or histone H3 acetylation in vivo. A recent MudPIT (multidimensional protein identification technology)-based biochemical study[52] revealed that the absence of Sgf29p does not alter the global structural integrity of SAGA. Similarly, Bian et al.[25] also demonstrated biochemically the dispensability of Sgf29p in maintaining SAGA's overall structural integrity. However, in striking contrast to their in vitro results, Bian et al.[25] implied that Sgf29p is required for SAGA's structural integrity as well as histone H3 acetylation in vivo. Here, using the ChIP assay in conjunction with transcriptional and mutational analyses, we show that Sgf29p is not required for the recruitment of SAGA components such as Spt20p, TAF10p, TAF12p, and Ubp8p, supporting the dispensability of Sgf29p in maintaining SAGA's global structural integrity in vivo. Further, our results reveal that Sgf29p facilitates the recruitment of TBP (and hence transcription) in vivo. Such a role for Sgf29p does not appear to be mediated via histone H3 acetylation at the SAGA-dependent but Gcn5p-independent gene.

MATERIALS AND METHODS

Plasmids. Plasmid SGP4[53] was generated by cloning a DNA fragment containing three Gal4p-binding sites into low-copy number plasmid pRS416. Plasmid pFA6a-13Myc-KanMX6[54] was used for genomic myc epitope tagging of the proteins of interest. Plasmid pRS416[55] was used in polymerase chain reaction (PCR)-based gene disruption.

Yeast Strains. Multiple myc epitope tags were added at the original chromosomal locus of *SGF29* in W303a to generate ASY2 (Sgf29p-myc, *Kan*). Plasmid SGP4, carrying three Gal4p-binding sites, was transformed into ASY2 to generate ASY5. The endogenous *SGF29* gene (encompassing the whole protein-encoding sequence) of W303a was disrupted using the PCR-based gene knockout method[56] to generate ASY8 (Δ*sgf29*::*URA3*). Multiple myc epitope tags were added at the original chromosomal locus of *SPT20* in W303a and ASY8 to generate ASY10 (Spt20p-myc, *Kan*) and ASY12 (Spt20p-myc, *Kan*; Δ*sgf29*::*URA3*), respectively. Strains ASY4 (Ubp8p-myc, *Kan*) and SLY18 (Ubp8p-myc, *Kan*; Δ*sgf29*::*URA3*) were generated by inserting multiple myc epitope tags at the original chromosomal locus of *UBP8* in strains W303a and ASY8, respectively.

Growth Media. For the studies at the *GAL1* promoter in Δ*sgf29* and its isogenic wild-type equivalent, cells were first grown in YPR (yeast extract containing peptone and 2% raffinose) to an optical density at 600 nm (OD_{600}) of 0.9 and then transferred to YPG (yeast extract containing peptone and 2% galactose) for 90 min at 30 °C prior to formaldehyde-based in vivo cross-linking. Minimal media containing either 2% galactose or raffinose were used for the strain (ASY5) with reporter plasmid SGP4. The yeast strains were grown in YPD (yeast extract containing peptone and 2% dextrose) to an OD_{600} of 1.0 at 30 °C for the studies at the *ADH1*, *PHO84*, and *RPS5* promoters.

ChIP Assay. The ChIP assay was performed as described previously.[57,58] Briefly, yeast cells were treated with 1% formaldehyde, collected, and resuspended in lysis buffer. Following sonication, cell lysates (400 μL of lysate from 50 mL of yeast culture) were precleared by centrifugation, and then 100 μL of lysate was used for each immunoprecipitation. Immunoprecipitated protein−DNA complexes were treated with proteinase K; the cross-links were reversed, and then DNA was purified. Immunoprecipitated DNA was dissolved in 20 μL of TE 8.0 [10 mM Tris-HCl (pH 8.0) and 1 mM EDTA], and 1 μL of immunoprecipitated DNA was analyzed by PCR. PCR mixtures contained [α-^{32}P]dATP (2.5 μCi for each 25 μL reaction), and the PCR products were detected by autoradiography after separation on a 6% polyacrylamide gel. As a control, "input" DNA was isolated from 5 μL of lysate without going through the immunoprecipitation step and suspended in 100 μL of TE 8.0. To compare the PCR signal arising from the immunoprecipitated DNA with the input DNA, 1 μL of input DNA was used in the PCR analysis. The ChIP assay for histone H3 was conducted following the protocol described previously.[37,59] Primer pairs used for PCR analysis were as follows: *GAL1*(UAS), 5′-CGCTTAACTGCTCATTGCTATATTG-3′ and 5′-TTGTTCGGAGCAGTGCGGCGC-3′; *GAL1*(Core), 5′-ATAGGATGATAATGCGATTAGTTTTTTAGCCTT-3′

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and 5′-GAAAATGTTGAAAGTATTAGTTAAAGTGGTTATGCA-3′; *ADH1*(Core), 5′-GGTATACGGCCTTCCTTCCAGTTAC-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′; *PHO84*(Core), 5′-GATCCACTTACTATTGTGGCTCGT-3′ and 5′-GTTTGTTGTGTGCCCTGGTGATCT-3′; *RPS5*(Core), 5′-GGCCAACTTCTACGCTCACGTTAG-3′ and 5′-CGGTGTCAGACATCTTTGGAATGGTC-3′. Primers flanking Gal4p-binding sites in plasmid SGP4 are 5′-GGTGGCGGCCGCTCTAGAACTAGT-3′ and 5′-TTGACCGTAATGGGATAGGTCACG-3′.

Autoradiograms were scanned and quantitated with National Institutes of Health image version 1.62. Immunoprecipitated DNAs were quantitated as the ratio of immunoprecipitate to input in the autoradiogram.

Total mRNA Preparation. Total mRNA was prepared from a yeast cell culture following the standard protocol. Briefly, 10 mL of a yeast culture of a total OD_{600} of 1.0 was harvested and suspended in 100 μL of RNA preparation buffer (500 mM NaCl, 200 mM Tris-HCl, 100 mM Na_2EDTA, and 1% SDS) along with 100 μL of a phenol/chloroform/isoamyl alcohol mixture and 100 μL (equivalent volume) of glass beads (acid-washed; Sigma). Subsequently, the yeast cell suspension was vortexed with a maximal speed (10 in the VWR mini-vortexer, catalog no. 58816-121) five times (30 s each). The cell suspension was put in ice for 30 s between pulses. After the sample had been vortexed, 150 μL of RNA preparation buffer and 150 μL of a phenol/chloroform/isoamyl alcohol mixture were added to the yeast cell suspension followed by vortexing for 15 s with a maximal speed on the VWR mini-vortexer. The aqueous phase was collected following a 5 min centrifugation at a maximal speed in a microcentrifuge machine. The total mRNA was isolated from the aqueous phase by ethanol precipitation.

Primer Extension Analysis. Primer extension analysis was performed as described previously.[7] The primers used for analysis of *GAL1*, *ADH1*, *PHO84*, and *RPS5* mRNAs were as follows: *GAL1*, 5′-CCTTGACGTTACCTTGACGTTAAAGTATAGAGG-3′; *ADH1*, 5′-TATCCTTGTGTTCCAATTTACCGTGG-3′; *PHO84*, 5′-GAAGACTTCTTTCAGCAACATG-3′; *RPS5*, 5′-GACTGGGGTGAATTCTTCAACAACTTC-3′.

RESULTS

Sgf29p Is an Integral Component of SAGA in Vivo. Sanders et al.[50] identified biochemically Sgf29p as a component of SAGA. To determine whether Sgf29p exists in the same form as that defined by its biochemical copurification with SAGA at the promoter in vivo, we analyzed the recruitment of Sgf29p to the UAS of a well-characterized SAGA-dependent gene, *GAL1*, using a ChIP assay. If Sgf29p is an integral component of SAGA in vivo, it will be recruited to the *GAL1* UAS but not to the core promoter, because we have previously shown that SAGA components including TAFs are predominantly recruited to the *GAL1* UAS but not the core promoter.[53] Panels A and B of Figure 1 show that the TAF12p subunit of SAGA as well as Sgf29p was present at the *GAL1* UAS but not the core promoter following seven cycles (each cycle with 10 s at a power output of 8 in a Misonix sonicator) of sonication in the ChIP assay. Similarly, Gal4p was recruited to the *GAL1* UAS (Figure 1B). These observations suggest that Sgf29p is a component of SAGA in vivo. However, it remained possible that other transcription components might have played a

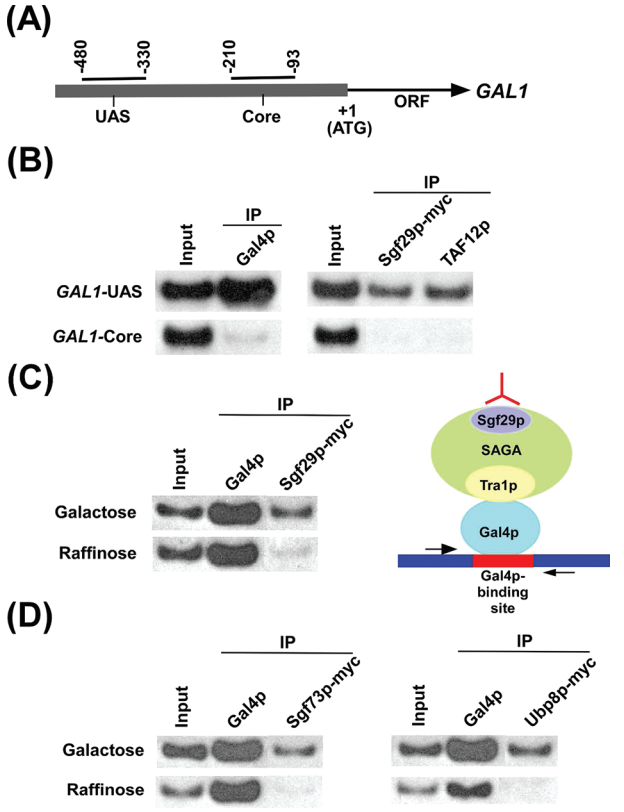

Figure 1. Recruitment of Sgf29p to the *GAL1* UAS or minimal Gal4p-binding site. (A) Schematic diagram showing the PCR amplification regions at the UAS and core promoter of the *GAL1* gene. The numbers are presented with respect to the position of the first nucleotide of the initiation codon (+1). (B) Recruitment of Sgf29p to the UAS, but not core promoter, of the *GAL1* gene. The yeast strain expressing myc epitope-tagged Sgf29p was grown at 30 °C in 1% yeast extract containing 2% peptone and 2% galactose (YPG) to an OD_{600} of 1.0 prior to formaldehyde-based in vivo cross-linking. The ChIP assay was performed as described previously.[37,57,58] Immunoprecipitation was performed using a mouse monoclonal antibody against the c-myc epitope tag (9E10, Santa Cruz Biotechnology, Inc.) or a polyclonal antibody against TAF12p. A mouse monoclonal antibody against the DNA binding domain of Gal4p (RK5C1, Santa Cruz Biotechnology, Inc.) was used. A primer pair targeted to the UAS or core promoter of the *GAL1* gene was used for PCR analysis of the immunoprecipitated DNA samples. (C) Recruitment of Sgf29p to the minimal Gal4p-binding sites. The plasmid containing minimal Gal4p-binding sites was transformed into the yeast strain expressing myc epitope-tagged Sgf29p. The yeast strain thus generated was grown at 30 °C in 1% yeast extract containing 2% peptone and 2% raffinose (YPR) or YPG. ChIP analysis was performed as described for panel B. The primers used for the PCR analysis are adjacent to the Gal4p-binding sites in the plasmid. The growth media are indicated at the left. (D) Recruitment of Sgf73p and Ubp8p to the minimal Gal4p-binding sites. The plasmid containing minimal Gal4p-binding sites was transformed into the yeast strain expressing myc epitope-tagged Sgf73p or Ubp8p. Yeast strains thus generated were grown at 30 °C in YPG or YPR. ChIP analysis was performed as described for panel B.

crucial role in the recruitment of Sgf29p to the *GAL1* UAS, because the experiments described above were performed on the intact *GAL1*. To address this issue, we next analyzed whether Sgf29p is recruited by Gal4p to a plasmid bearing only Gal4p-binding sites but not other promoter elements. Figure 1C shows that Sgf29p was recruited to the Gal4p-binding sites in the plasmid when the Gal4p activation domain was active in

galactose-containing growth medium. On the other hand, Sgf29p was not associated with the Gal4p-binding sites when the Gal4p activation domain was inactive in raffinose-containing growth medium. However, the levels of recruitment of Gal4p to the Gal4p-binding sites in both galactose- and raffinose-containing growth media were the same (Figure 1C). Similarly, the Ubp8p and Sgf73p components of SAGA were also recruited to the Gal4p-binding site by the Gal4p activation domain (Figure 1D[37,58]). Further, our previous studies[53] demonstrated that other SAGA components such as Spt3p and Spt20p were also recruited to the minimal Gal4p-binding sites in the plasmid when the Gal4 activation domain was active in galactose-containing growth medium. Significantly, the general transcription factors were not recruited to the minimal Gal4p-binding sites.[53] Together, these results support the idea that Sgf29p exists in the same form at the promoter in vivo as observed biochemically,[50] and thus, it is recruited to the *GAL1* UAS or Gal4p activation domain along with other SAGA components.

Sgf29p Does Not Regulate the Global Structural Integrity of SAGA in Vivo. To determine the role of Sgf29p in transcriptional regulation, we next analyzed its contribution in SAGA recruitment or integrity. If Sgf29p maintains SAGA's integrity, the SAGA components will not be recruited to the *GAL1* UAS in the Δ*sgf29* strain. Figure 2A shows that deletion of *SGF29* did not alter the recruitment of the SAGA components such as Spt20p, Ubp8p, TAF10p, and TAF12p. Similarly, the recruitment of the activator Gal4p to the *GAL1* UAS was not altered in the Δ*sgf29* strain (Figure 2B). Likewise, our previous studies have demonstrated that the recruitment of the SAGA components was not changed in the deletion mutant of *GCN5* that is dispensable for the integrity of SAGA.[4,5,53] On the other hand, recruitment of SAGA was almost lost in the *SPT20* (that integrates SAGA[5]) deletion mutant strain.[4,53] Because Spt20p maintains the global structural integrity of SAGA and its recruitment (along with other SAGA components such as TAF10p, TAF12p, and Ubp8p) is not altered in the absence of Sgf29p, thus, like Gcn5p, Sgf29p does not appear to regulate the global structural integrity of SAGA in vivo.

Sgf29p Facilitates the Recruitment of TBP and Hence Transcription in Vivo. So far, we find that the absence of Sgf29p did not alter the global structural integrity of SAGA in vivo. Similarly, previous studies revealed that the Spt3p component of SAGA is also not required for SAGA's global structural integrity.[5,53,60,61] Rather, it promotes transcription by facilitating the recruitment of TBP at the promoter.[53,60,61] Thus, Spt3p is essential for SAGA's functional integrity, even though it is dispensable for maintaining the overall structural integrity of SAGA. On the basis of these studies, we hypothesize that like Spt3p, Sgf29p might be regulating transcription by modulating the recruitment of TBP at the promoter for nucleation of the PIC assembly to initiate transcription. To test this hypothesis, we next analyzed Sgf29p's contribution in the recruitment of TBP to the *GAL1* core promoter. Figure 3A shows that the recruitment of TBP to the *GAL1* core promoter was decreased in the *SGF29* deletion mutant strain. Such a decrease in the level of recruitment of TBP to the *GAL1* core promoter in Δ*sgf29* would reduce the level of transcription. Indeed, we find that the level of transcription of *GAL1* was also decreased in Δ*sgf29* (Figure 3B). As a control, we show that transcription of a SAGA-independent gene, *RPS5*,[53] was not altered in Δ*sgf29* (Figure

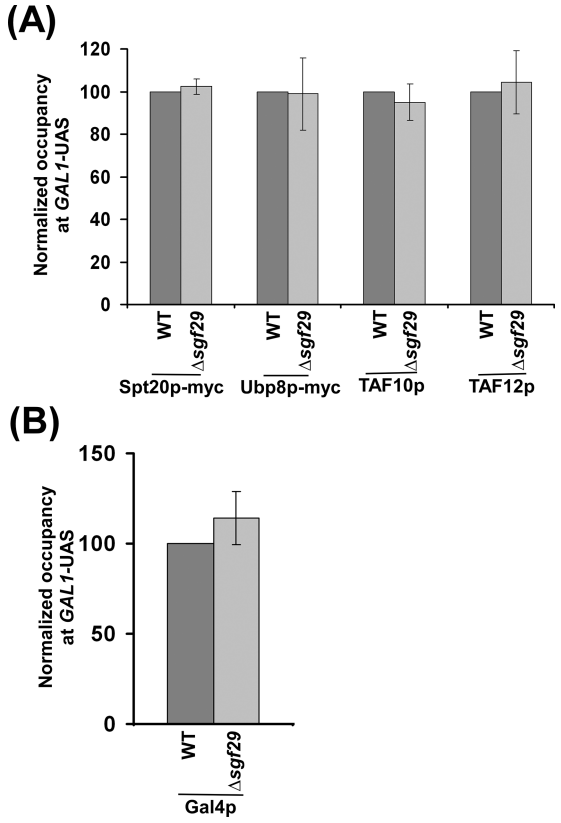

Figure 2. Sgf29p is dispensable for recruitment of SAGA (A) and Gal4p (B) to the *GAL1* UAS. The *SGF29* deletion mutant and its isogenic wild-type (WT) equivalent were first grown in YPR, and then shifted to YPG for 90 min before being treated with formaldehyde. Immunoprecipitations were performed as described in the legend of Figure 1B. A polyclonal antibody against TAF10p was used. The primer pair targeted to the *GAL1* UAS was used for PCR analysis of the immunoprecipitated DNA samples. The ratio of immunoprecipitate to the input in the autoradiogram was measured and termed the ChIP signal. The ChIP signal of the wild-type strain was set to 100, and the ChIP signal of the Δ*sgf29* strain was normalized with respect to 100. The normalized ChIP signal (represented as normalized occupancy) is plotted in the form of a histogram.

3B). Consistent with the *RPS5* transcription data, we further show that the recruitment of TBP to the *RPS5* core promoter was not altered in the absence of Sgf29p (Figure 3C). Together, these results demonstrate that the Sgf29p component of SAGA promotes *GAL1* transcription by facilitating the recruitment of TBP to the core promoter.

To determine whether Sgf29p also facilitates TBP recruitment (and hence transcription) at other genes, we analyzed the association of TBP with the core promoters of two other SAGA-dependent genes, namely, *ADH1* and *PHO84*,[4] in the *SGF29* deletion mutant and its isogenic wild-type equivalent. Panels A and B of Figure 4 show that the recruitment of TBP to the *ADH1* and *PHO84* core promoters was weakened in Δ*sgf29*. Consistently, the level of transcription of these genes was also decreased in the *SGF29* deletion mutant strain (Figure 4C). Thus, SAGA-associated Sgf29p enhances transcription by facilitating the recruitment of TBP in vivo.

Role of Sgf29p in the Regulation of Histone H3 Acetylation in Vivo. We next asked whether the Gcn5p-HAT activity of SAGA is regulated by Sgf29p in vivo. Previous studies have indicated that Gcn5p-HAT activity on nucleoso-

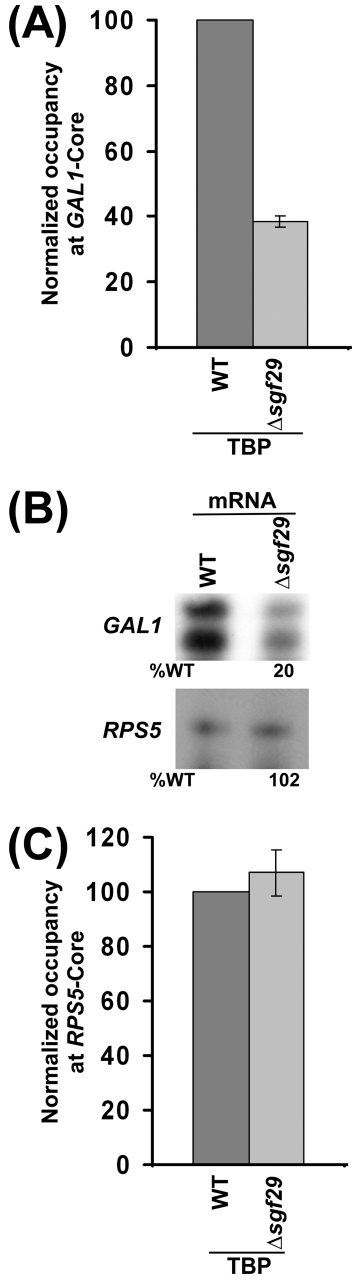

Figure 3. Requirement of Sgf29p for the recruitment of TBP to the *GAL1* core promoter. (A) Sgf29p is required for TBP recruitment at the *GAL1* core promoter. Yeast strains were grown, cross-linked, and immunoprecipitated as described in the legend of Figure 2. Immunoprecipitation was performed using a polyclonal antibody against TBP. The primer pair targeted to the *GAL1* core promoter was used for PCR analysis of the immunoprecipitated DNA samples. (B) Transcription. Total cellular RNA was prepared from the wild-type and Δ*sgf29* strains, and mRNA levels from the *GAL1* and *RPS5* genes were quantitated by primer extension analysis. The percentage mRNA level relative to that of the wild-type strain (%WT) is indicated below. (C) Analysis of the recruitment of TBP to the *RPS5* core promoter in the Δ*sgf29* and wild-type strains.

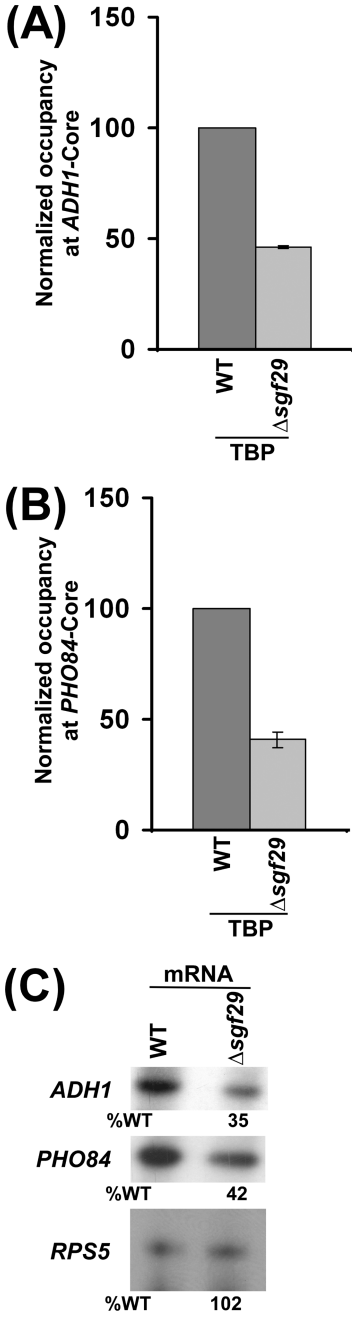

Figure 4. Sgf29p is required for TBP recruitment (and hence transcription) to the *ADH1* and *PHO84* promoters. (A and B) Analysis of the recruitment of TBP to the *ADH1* and *PHO84* core promoters in Δ*sgf29*. Yeast strains were grown in YPD (yeast extract containing peptone and 2% dextrose) at 30 °C up to an OD_{600} of 1.0 prior to cross-linking. Immunoprecipitation was performed as described in the legend of Figure 3A. Primer pairs targeted to the *ADH1* and *PHO84* core promoters were used for PCR analysis of the immunoprecipitated DNA samples. (C) Transcription. Total cellular RNA was prepared from the wild-type and Δ*sgf29* strains, and mRNA levels from the *ADH1*, *PHO84*, and *RPS5* genes were quantitated by primer extension analysis.

mal histone H3 is dependent on the Ada2p and Ada3p components of SAGA.[4,58,62−65] Further, a recent MudPIT-based study[52] implied that Sgf29p along with Ada2p, Ada3p, and Gcn5p forms a HAT module within SAGA. Thus, like Ada2p and Ada3p,[4,58,64,65] Sgf29p may regulate the HAT activity and hence histone H3 acetylation at the SAGA-regulated promoters. To test this, we analyzed the levels of histone H3 acetylation and histone H3 at the core promoters of the SAGA-dependent *ADH1* and *PHO84* genes in the Δ*sgf29* mutant and wild-type strains. Interestingly, our ChIP data revealed that the levels of histone H3 at the *ADH1* and *PHO84*

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core promoters were increased in Δ*sgf29* when compared with the wild-type equivalent (Figure 5). This is as expected, because

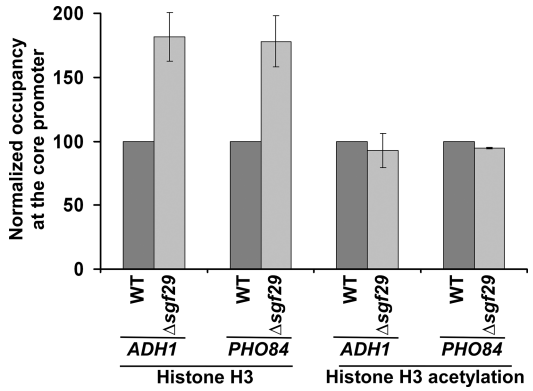

Figure 5. Analysis of the levels of histone H3 and its acetylation at K9 and K14 at the core promoters of *ADH1* and *PHO84* in the *SGF29* deletion mutant and wild-type strains. The wild-type and *SGF29* deletion mutant strains were grown and cross-linked as described in the legend of Figure 4. Immunoprecipitations were performed using anti-histone H3 (Ab1791, Abcam) and anti-K9/14-diacetylated histone H3 (06-599, Upstate Biotechnology, Inc.) antibodies.

previous studies[58,66] revealed an inverse correlation between TBP recruitment (or PIC formation and/or transcription) and nucleosomal disassembly. As the level of recruitment of TBP at the *ADH1* and *PHO84* core promoters was decreased in Δ*sgf29* (Figure 4A,B), an increased level of histone H3 was observed at these core promoters in the absence of Sgf29p (Figure 5). A similar increase in the level of histone H3 acetylation would be observed in the ChIP assay in Δ*sgf29*, if Sgf29p did not regulate histone H3 acetylation at these promoters. Intriguingly, we find that the levels of histone H3 acetylation at these promoters did not increase in the same proportions as that of histone H3 in Δ*sgf29* (Figure 5). These results support the idea that the level of histone H3 acetylation is decreased in the absence of Sgf29p in vivo.

DISCUSSION

Here, we show that, like other SAGA components, Sgf29p is recruited to the UAS but not the core promoter of a well-characterized SAGA-dependent gene, *GAL1* (Figure 1A,B). Further, we demonstrate that the Gal4p activation domain targets the recruitment of Sgf29p to the minimal Gal4p-binding site as a SAGA component in vivo (Figure 1C). These results suggest that Sgf29p exists in vivo in the same form as that defined by its biochemical copurification with SAGA. Further, we find that the deletion of *SGF29* does not impair the recruitment of SAGA components such as TAF10p, TAF12p, Spt20p, and Ubp8p (Figure 2A). These observations support the idea that Sgf29p is not essential for the maintenance of the global structural integrity of SAGA in vivo.

Consistent with our in vivo results, a recent study[25] also demonstrated that Sgf29p is not required for SAGA's overall integrity in vitro. Further, another MudPIT-based recent biochemical study[52] revealed the dispensability of Sgf29p in maintaining SAGA's global structural integrity. However, in striking contrast to these biochemical data[25,52] and our in vivo results, Bian et al.[25] implicated the requirement of Sgf29p in maintaining SAGA's structural integrity in vivo. Using the ChIP assay, they demonstrated the decrease in the level of recruitment of the Ada2p component of SAGA in Δ*sgf29*. Ada2p is not essential for maintaining SAGA's global structural integrity. It is also an integral component of the ADA complex. Bian et al.[25] used Ada2p as a representative component of SAGA to evaluate the effect of Sgf29p in the recruitment of SAGA and found that the absence of Sgf29p decreased the level of recruitment of Ada2p by ∼2-fold at the *GAL1* UAS. A recent study[52] implicated Sgf29p in the HAT module (containing Sgf29p, Ada2p, Ada3p, and Gcn5p) within SAGA, and thus, the absence of Sgf29 may alter the association of Ada2p with SAGA in vivo. Indeed, a decrease in the level of recruitment of Ada2p in Δ*sgf29* was observed in the previous studies.[25] Further, Lee et al.[52] demonstrated Sgf29p as a common component of the SAGA and ADA complexes. Thus, the decrease in the level of recruitment of Ada2p in Δ*sgf29* could also occur via the ADA complex if it associates with *GAL1* UAS. Therefore, it is important to look at several components of the SAGA complex to evaluate the role of Sgf29p in maintaining SAGA's structural integrity in vivo. In this study, we analyzed the recruitment of several SAGA components (TAF10p, TAF12p, Spt20p, and Ubp8p) to the *GAL1* UAS. These SAGA components represent different structural modules of the SAGA complex. Recently, MudPIT-based biochemical studies[52] identified four structural modules (TAF, SPT, DUB, and HAT) within SAGA. Our ChIP data demonstrate that the recruitment of TAF10p (TAF module), TAF12p (TAF module), Spt20p (SPT module), and Ubp8p (DUB module) is not altered in the absence of Sgf29p (Figure 2A). These results support the idea that SAGA's overall integrity is not altered in the absence of Sgf29p in vivo. Further, our results suggest that Sgf29p could be a peripheral component of SAGA. Indeed, a recent biochemical study[52] identified Sgf29p as a peripheral SAGA component. This is further substantiated by the fact that Sgf29p is accessible to interact with methylated K4 of histone H3 to enhance the recruitment of SAGA.[25,26]

Because Sgf29p is dispensable for SAGA's overall structural integrity, it may not have any effect on TBP recruitment and hence initiation of transcription. Alternatively, it is quite possible that it can promote TBP recruitment (and hence transcription) without altering the recruitment of SAGA, similar to the functional role of Spt3p.[5,53,60,61] Indeed, our data support this possibility. We find that the level of recruitment of TBP to the promoters of several SAGA-dependent genes such as *GAL1*, *ADH1*, and *PHO84* is decreased in the absence of Sgf29p (Figures 3A and 4A,B). Such a decrease in the level of TBP recruitment subsequently reduces the level of transcription of these genes (Figures 3B and 4C). Consistent with our results, Lee et al.[52] demonstrated the growth defect of the Δ*sgf29* cells in galactose-containing as well as ethanol- and glycerol-containing growth media. Yeast cells require the expression of the *GAL1* and *ADH1* genes for growth in these media.[52] Further, Bian et al.[25] also demonstrated the transcriptional defect of *GAL1* in the absence of Sgf29p. Taken together, Sgf29p promotes transcription by facilitating the recruitment of TBP without altering SAGA's global structural integrity.

Previous studies[58] demonstrated histone H3 acetylation at the promoters of the three genes mentioned above. Such an acetylation is significantly lost in the absence of Gcn5p.[58] However, the recruitment of TBP to the core promoters of *GAL1* and *ADH1* is not altered in the deletion mutant of *GCN5*,[4,58] implying that histone H3 acetylation is dispensable for TBP recruitment at these promoters. Therefore, these

studies support the idea that the decrease in the level of recruitment of TBP to the *GAL1* and *ADH1* core promoters in the absence of Sgf29p is not mediated via histone H3 acetylation. On the other hand, Gcn5p or histone H3 acetylation promotes the recruitment of TBP to the *PHO84* promoter.[4,58] Thus, it may be likely that the decrease in the level of recruitment of TBP to the *PHO84* promoter in Δ*sgf29* is due to an impaired recruitment of Gcn5p (or associated HAT activity). We rule out this possibility as the deletion of *SGF29* does not alter the overall recruitment of SAGA (Figure 2A). In addition, Lee et al.[52] recently demonstrated biochemically that Gcn5p remains associated with SAGA in the absence of Sgf29p. Similarly, Bian et al.[25] also showed that Sgf29p does not alter SAGA's global structural integrity in vitro. If Sgf29p would have regulated the recruitment of TBP via the modulation of histone H3 acetylation, a decreased level of recruitment of TBP to the core promoter of *GAL1* or *ADH1* in Δ*sgf29* would not have been observed (as the recruitment of TBP to the *GAL1* and *ADH1* promoters is not altered in the absence of Gcn5p or HAT activity[4,58]). However, our data (Figures 3A and 4A) have demonstrated a decreased level of recruitment of TBP to the *ADH1* and *GAL1* promoters in vivo. Thus, the role of Sgf29p in promoting the recruitment of TBP to these three promoters does not appear to be mediated via histone H3 acetylation. This is further substantiated by a recent observation that the deletion of *SGF29* does not alter the HAT activity of SAGA in vitro.[25]

As mentioned above, a recent MudPIT-based study implicated the presence of Sgf29p in SAGA as well as the ADA HAT complex.[52] Further, the deletion of *SGF29* has been shown to reduce the level of global acetylation of histone H3 in Western blot analysis.[25] Such a decrease in the level of global histone H3 acetylation may be mediated via ADA, SAGA, or both. Because a recent MudPIT-based study[52] implicated the presence of Sgf29p in the HAT module of SAGA, Sgf29p may likely regulate the HAT activity of SAGA (and hence histone H3 acetylation), similar to the roles of Ada2p and Ada3p (that are present in the HAT module) in the regulation of SAGA's HAT activity.[4,58,64,65] However, previous biochemical studies[25,52] demonstrated that the structural integrity of SAGA is not altered in the absence of Sgf29p, and such a SAGA complex (without Sgf29p) does not alter histone H3 acetylation in vitro,[25] and hence SAGA's HAT activity. Thus, the global decrease in the level of histone H3 acetylation in the absence of Sgf29p may occur through the Sgf29p-associated ADA HAT complex.

To determine the role of Sgf29p in histone H3 acetylation at the promoter in vivo, we analyzed histone H3 acetylation at the promoters of the SAGA-regulated *ADH1* and *PHO84* genes in the presence and absence of Sgf29p. We find that Sgf29p moderately enhances histone H3 acetylation at these SAGA-regulated promoters (Figure 5). However, Bian et al.[25] demonstrated a dramatic decrease in the level of histone H3 acetylation at the *GAL1* promoter in Δ*sgf29*, even though their in vitro results show no effect of Sgf29p on histone H3 acetylation. One possible reason for this discrepancy could be the absence of histone H3 control in their ChIP experiments.[25] Therefore, it is not clear how much histone H3 acetylation signal was altered in proportion to histone H3 in Δ*sgf29*. Further, they have used the K9 monoacetylated histone H3 antibody in their study.[25] On the other hand, the K9/14-diacetylated histone H3 antibody was used in this study. Although our results demonstrate that Sgf29p moderately enhances histone H3 acetylation, such acetylation does not appear to enhance TBP recruitment at the *GAL1* and *ADH1* promoters as described above. However, such a moderate regulation of histone H3 acetylation by Sgf29p may have a positive effect on TBP recruitment at the Gcn5p-regulated genes, as previous studies[36,58] showed a correlation between histone H3 acetylation and TBP recruitment.

Previous studies have identified the enhancement of SAGA recruitment via the interaction of the Sgf29p's tudor domain with K4-methylated histone H3.[25,26] Therefore, the deletion of *SET1* (that is involved in histone H3 K4 mono, di and trimethylation) would impair the recruitment of TBP (and hence transcription) at the SAGA-regulated genes. However, previous studies have demonstrated that the absence of Set1p does not alter TBP recruitment at the core promoters of several SAGA-dependent genes such as *GAL1*, *ADH1*, and *PHO84*.[37,67] Consistently, the transcription of these genes is also not altered in the *SET1* deletion mutant when compared with the wild-type equivalent.[37,67] Although previous studies[25,26] have identified the interaction of Sgf29p with methylated K4 of histone H3, the loss of such interaction does not appear to have a dramatic effect on transcription. Similarly, Gcn5p has been shown to enhance the recruitment of SAGA via the interaction of its bromodomain with acetylated histone H3.[1,14−21,63,68,69] However, the deletion of *GCN5* does not alter the global recruitment of SAGA or its integrity.[4,5] Further, Gcn5p is not required for transcription of all SAGA-dependent genes.[3,58]

The role of Sgf29p in promoting the recruitment of TBP does not appear to be primarily mediated via histone H3 acetylation, nor is the decrease in the level of recruitment of TBP due to disintegration of the SAGA complex in the absence of Sgf29 (Figure 2A[25,52]). Although we find that the absence of Sgf29p lowers the level of recruitment of TBP, its effect on TBP recruitment is not as dramatic as that observed in other SAGA mutants such as Δ*spt20*, Δ*ada1*, Δ*spt7*, and Δ*sgf73*.[4,37,58] Even though the effect of Sgf29p on TBP recruitment is relatively weak, it may have important roles during differentiation and development in higher eukaryotes. Indeed, the absence of Sgf29p in rats has been implicated in oncogenic transformation via modest transcriptional alteration.[51] Further, the loss of the Sgf29p homologue in plants delays flowering[70] and has a moderate effect on transcription.[70]

In summary, we show here that Sgf29p exists in vivo in the same form as that defined by its biochemical copurification with SAGA. Sgf29p is not required for the recruitment of SAGA components such as Spt20p, TAF10p, TAF12p, and Ubp8p (or its overall structural integrity) but facilitates the association of TBP (and hence transcription) in vivo. These results provide significant insight into how yeast Sgf29p (and possibly its mammalian homologue) functions physiologically to regulate transcription.

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