

SAGA-associated Sgf73p facilitates formation of the preinitiation complex assembly at the promoters either in a HAT-dependent or independent manner *in vivo*

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ABSTRACT

Although Sgf73p, a yeast homologue of human Sca7p, has recently been implicated as a new component of Spt-Ada-Gcn5-acetyltransferase (SAGA), its association with SAGA and functional role in regulation of transcription remain unknown *in vivo*. Here, using a chromatin immunoprecipitation (ChIP) assay, we show *in vivo* that, like SAGA, Sgf73p is recruited to the upstream activating sequence (UAS) of a SAGA-dependent gene, *GAL1*, in an activator-dependent manner. Further, Sgf73p is required for recruitment of SAGA to the *GAL1* UAS, and facilitates formation of the preinitiation complex (PIC) assembly at the *GAL1* promoter. When PIC is not formed in Δ *sgf73*, histone H3 is not evicted from the *GAL1* promoter. Interestingly, PIC formation at *GAL1* is not regulated by histone H3 acetylation or histone acetyltransferase (HAT) activity of SAGA. Similarly, Sgf73p facilitates PIC formation at another SAGA-dependent gene, *ADH1*, independent of histone H3 acetylation or HAT. In contrast, Sgf73p stimulates PIC formation at *PHO84* (a SAGA-dependent gene), in a HAT-dependent-manner. Collectively, our data reveal that Sgf73p is required for SAGA recruitment, and stimulates PIC formation either in a HAT-dependent or -independent manner, providing significant information on how Sgf73p and possibly human Sca7p function physiologically.

INTRODUCTION

Spt-Ada-Gcn5-acetyltransferase (SAGA), a large multi-protein complex with Gcn5p-HAT (histone acetyltransferase) activity, is required for the normal transcription of ~10%

of yeast genes (1). The role of SAGA in transcriptional activation has been studied extensively at *GAL1* (2–8). At the *GAL1* promoter, the activator Gal4p first recruits SAGA to the upstream activating sequence (UAS), and then the UAS-bound SAGA facilitates recruitment of TATA-box binding protein (TBP) to the core promoter, thereby stimulating formation of preinitiation complex (PIC) and hence transcription (5,7,8).

Recently, Sgf73p has been biochemically identified as a new component of SAGA (9,10). Sgf73p is a yeast homologue of the human Spinocerebellar ataxia type 7 (*SCA7*) gene product, ataxin-7 or Sca7p. It is one of the eight known autosomal dominant neurodegenerative disorders which are caused by CAG (that encodes glutamine, Q) nucleotide repeat expansions from <35 (non-pathogenic) to an extreme of 300 (pathogenic) copies (11–15). However, it is not known how CAG nucleotide repeat expansion within ataxin-7 causes neurodegenerative diseases. Recent biochemical studies (16,17) have demonstrated that, like Sgf73p, wild-type ataxin-7 is a component of mammalian SPT3-TAF_{II}31-GCN5_L acetylase (STAGA) transcription coactivator complex (18,19), a human homologue of yeast SAGA complex, and it regulates HAT activity of STAGA and its interaction with activators. The poly(Q)-expanded ataxin-7 gets incorporated into STAGA as well as SAGA (10,16), but inhibits Gcn5p-HAT activities of both STAGA and SAGA (10,16), and thus alters gene expression, providing significant information on the molecular basis of poly(Q) disorders.

Although previous biochemical studies (10,16,17) have defined the functional roles of Sgf73p and ataxin-7 in maintaining the overall structural integrities of SAGA and STAGA, respectively, and their HAT activities, the molecular role of Sgf73p or ataxin-7 in transcriptional regulation remains mostly unknown *in vivo*. Here, using a formaldehyde-based *in vivo* crosslinking and chromatin immunoprecipitation (ChIP) assay, we have analyzed the role of Sgf73p in recruitment of SAGA and formation of the PIC assembly (and hence transcription) at the SAGA-dependent promoter

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in *Saccharomyces cerevisiae*. Further, we have determined the role of histone H3 acetylation or the HAT activity of SAGA in regulation of the PIC formation at the SAGA-dependent promoters *in vivo*. Our results reveal that Sgf73p is required for SAGA recruitment and stimulates formation of the PIC assembly at the SAGA-dependent promoters. Consistently, transcription of the SAGA-regulated genes is significantly impaired in Δ *sgf73*. Interestingly, PIC formation at the SAGA-regulated genes is differentially regulated by histone H3 acetylation or the HAT activity of SAGA *in vivo*.

MATERIALS AND METHODS

Plasmids

Plasmid SGP4 was generated by cloning a DNA fragment containing three Gal4p-binding sites into the low copy number plasmid pRS416 (5). The plasmid pFA6a-13Myc-KanMX6 (20) was used for genomic myc epitope-tagging of the proteins of interest. The Plasmid pRS416 (21) was used in the PCR-based gene disruption.

Yeast strains and media

Yeast strain harboring null mutation in *GCN5* (FY1370), and its isogenic wild-type equivalent FY1369 were obtained from Fred Winston (Harvard Medical School, Boston, MA) (2). Multiple myc-epitope tags were added at the original chromosomal locus of *SGF73* in W303a to generate ASY3 (Sgf73p-myc, *Kan*). The plasmid SGP4, carrying three Gal4p-binding sites, was transformed into ASY3 to generate ASY6. The endogenous *SGF73* gene of W303a was disrupted using the PCR-based gene knock-out method (22) to generate ASY9 (Δ *sgf73::URA3*). Multiple myc-epitope tags were added at the original chromosomal locus of *SPT20* in W303a and ASY9 to generate ASY10 (Spt20p-myc, *Kan*) and ASY13 (Spt20p-myc, *Kan*; Δ *sgf73*), respectively.

For the studies at the *GALI* promoter in Δ *sgf73* and Δ *gcn5* mutant strains, and their isogenic wild-type equivalents, cells were first grown in YPR (yeast extract containing peptone plus 2% raffinose) to an optical density at 600 nm (OD_{600}) of 0.9 and then transferred to YPG (yeast extract-peptone plus 2% galactose) for 90 min at 30°C prior to formaldehyde crosslinking. Minimal media containing either 2% galactose or raffinose were used for the strain (ASY6) with reporter plasmid, SGP4. The yeast strains were grown in YPD (yeast extract containing peptone plus 2% dextrose) to an OD_{600} of 1.0 at 30°C for the studies at the *ADHI*, *PHO84* and *RPS5* promoters.

ChIP assay

The ChIP assay was performed as described previously (23,24). Briefly, yeast cells were treated with 1% formaldehyde, collected and resuspended in lysis buffer. Following sonication (that generates the average size of ~400 bp of sheared DNA), cell lysates (400 μ l lysate from 50 ml of yeast culture) were precleared by centrifugation, and then 100 μ l lysate was used for each immunoprecipitation (IP). Immunoprecipitated (IP) protein-DNA complexes were treated with proteinase K, the crosslinks were reversed, and

the DNA was purified. IP DNA was dissolved in 20 μ l TE 8.0 [10 mM Tris-HCl (pH 8.0) and 1 mM EDTA], and 1 μ l of IP DNA was analyzed by PCR. The PCR (a total of 23 cycles) contained [α -³²P]dATP (2.5 μ Ci for each 25 μ l 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 μ l lysate without going through the IP step and suspended in 100 μ l TE 8.0. To compare the PCR signal arising from the IP DNA with the input DNA, 1 μ l of input DNA was used in PCR analysis.

For analysis of histone H3 levels at the promoters, we modified the above ChIP protocol as follows. Eight hundred microlitre lysate was prepared from 100 ml of yeast culture. Four hundred microlitre lysate was used for each IP (using 5 μ l of anti-histone H3 antibody from Abcam and 100 μ l of protein A/G plus agarose beads from Santa Cruz Biotechnology, Inc.), and IP DNA sample was dissolved in 10 μ l TE 8.0 of which 1 μ l was used in PCR analysis. In parallel, the PCR for 'input' DNA was performed using 1 μ l DNA that was prepared by dissolving purified DNA from 5 μ l lysate in 1000 μ l TE 8.0.

Serial dilutions of the input and IP DNAs were used to assess the linear range of PCR amplification as described previously (6). The PCR data presented in this paper are within the linear range of PCR analysis. All the ChIP experiments were repeated at least three times and consistent results were obtained. Primer-pairs used for PCR analysis were as follows: *GALI*(UAS): 5'-CGCTTAAGTCTCATTGCTATATTG-3', 5'-TTGTTTCGGAGCAGTGC GGCGC-3'; *GALI*(Core): 5'-ATAGGATGATAATGCGATTAGTTTTTTAGCCTT-3', 5'-GAAAATGTTGAAAGTATTAGTTAAAGTGGTTATGCA-3'; *ADHI*(Core): 5'-GGTATACGGCCTTCCTTCCAGT-TAC-3', 5'-GAACGAGAACAATGACGAGGAAACAA-AAG-3'; *PHO84*(Core): 5'-GATCCACTTACTATTGTG-GCTCGT-3', 5'-GTTTGTGTGTGCCCTGGTGATCT-3'; *RPS5*(Core): 5'-GGCCAACCTTCTACGCTCACGTTAG-3', 5'-CGGTGTCAGACATCTTTGGAATGGTC-3'; *GAL4* [open reading frame (ORF)]: 5'-CTTGTTC AATGCAGTC-CTAGTACCC-3', 5'-CACAAAGTCTGGATTTTAAAAGT-GGCC-3'.

Primers flanking Gal4p-binding sites in the plasmid SGP4 are 5'-GGTGGCGCCGCTCTAGA ACTAGT-3' and 5'-TTGACCGTAATGGGATAGGTCACG-3'.

Autoradiograms were scanned and quantitated by the National Institutes of Health image 1.62 program. The ratio of IP DNA over the input was presented as %IP. For mutant strains, DNA IP relative to wild-type (WT) was presented as %WT.

Primer extension analysis

Primer extension analysis was performed as described previously (5-7,23,25,26). Briefly, total RNA was prepared from 10 ml of yeast culture. Fifteen micrograms of total RNA was used in the primer extension analysis using ³²P-labeled primer and AMV reverse transcriptase (Promega). The primers used for analysis of *GALI*, *ADHI*, *PHO84* and *RPS5* mRNAs were as follows: *GALI*: 5'-CCTTGACGTTACCTTGACGTTAAAGTATAGAGG-3'; *ADHI*: 5'-TATCCTTGTGTTCCAATTTACCGTGG-3'; *PHO84*: 5'-GAAGACTTCTTTTCAGCAACATG-3'; *RPS5*: 5'-GACTGGGGTG AAT-TCTTCAACA ACTTC-3'.

RESULTS

Sgf73p is an integral component of SAGA *in vivo*

Yeast SAGA is a large multi-protein complex that contains Ada1p, Ada2p, Ada3p, Ada5p/Spt20p, Spt3p, Spt7p, Spt8p, Gcn5p, Tra1p, TAF5p, TAF6p, TAF9p, TAF10p, TAF12p, Ubp8p, Chd1 and Sgf11 (9,27–35). Recently, Sgf73p has been biochemically implicated as a new component of SAGA (10). To confirm whether Sgf73p is a new component of SAGA *in vivo*, we analyzed recruitment of Sgf73p to the UAS of a well-characterized SAGA-dependent gene, *GALI*, using a ChIP assay. We used either a c-myc mouse monoclonal antibody and yeast strains expressing a C-terminal myc epitope-tagged Sgf73p or polyclonal antibody to SAGA TAF (e.g. TAF12p). Two sets of promoter-specific primer-pairs were used that, as shown below in Figure 1A, could distinguish binding to the *GALI* UAS or core promoter. If Sgf73p is an integral component of the SAGA complex, it would be recruited to the *GALI* UAS, but not core promoter, since previous ChIP studies (5) have demonstrated recruitment of SAGA to the *GALI* UAS.

Figure 1A shows that under condition in which Gal4p activation domain was active (galactose-containing growth medium), the TAF12p subunit of SAGA as well as Sgf73p were present at the *GALI* UAS. Similarly, Gal4p was recruited to the *GALI* UAS (Figure 1A). Significantly, these same proteins were not associated with the *GALI* core promoter as well as an irrelevant DNA sequence (*GAL4* ORF) (Figure 1A). These observations supported the possibility that Sgf73p is a component of SAGA *in vivo*. However, because these experiments were performed with the intact *GALI* promoter, it remained possible that other transcription components, such as general transcription factors (GTFs) played a role in recruitment of Sgf73p to the UAS. To address this issue, we asked whether Sgf73p could be recruited by Gal4p to a plasmid bearing only Gal4p-binding sites, but not other promoter elements. Figure 1B shows that Sgf73p was recruited to a plasmid bearing Gal4p-binding sites in galactose-containing growth medium in which Gal4p activation domain was active. Significantly, Sgf73p was not associated with the Gal4p-binding sites when Gal4p activation domain was inactive (raffinose-containing growth medium) even though the level of Gal4p recruitment was almost equal to that in galactose-containing growth medium (Figure 1B). Further, our previous studies (5) have demonstrated that, like Sgf73p, other SAGA components, such as Spt3p and Spt20p, were also recruited to the minimal Gal4p-binding sites in the plasmid in galactose-containing growth medium. Significantly, the GTFs, such as TBP, TFIIB, Mediator and RNA polymerase II, were not recruited to the minimal Gal4p binding sites in the plasmid (5). Taken together, our data provide evidence that Sgf73p is a new component of the SAGA complex *in vivo*, consistent with previous biochemical data (9,10).

Sgf73p regulates the integrity of SAGA *in vivo*

To understand the role of the newly identified Sgf73p component of SAGA in transcriptional activation in greater detail, it is necessary to determine its contribution in maintaining the integrity of SAGA. Recently, McMahon

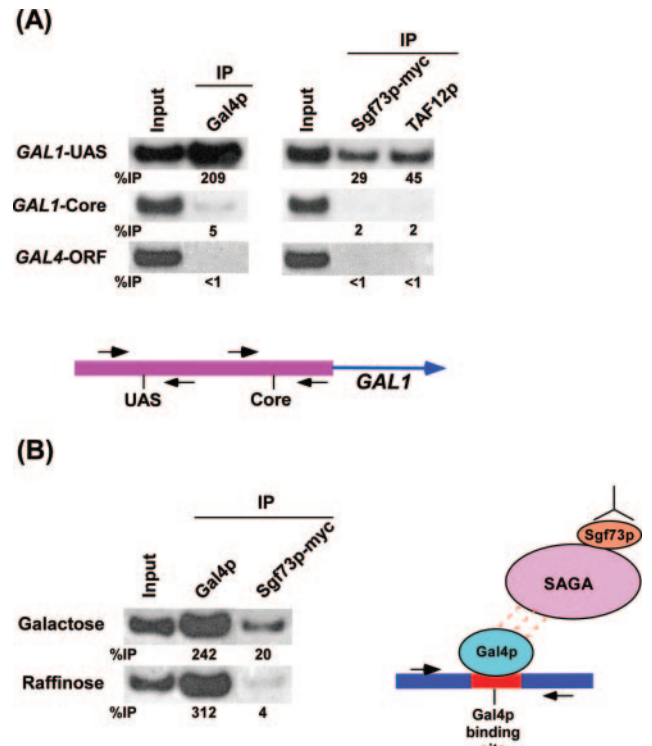


Figure 1. Recruitment of Sgf73p to the *GALI* UAS or minimal Gal4p binding site. (A) Recruitment of Sgf73p to the UAS, but not core promoter, of the *GALI* gene. Yeast strain expressing myc-epitope tagged Sgf73p was grown at 30°C in 1% yeast extract containing 2% peptone plus 2% galactose (YPG) up to an OD₆₀₀ of 1.0 prior to formaldehyde-based *in vivo* crosslinking. The chromatin (IP) was performed as previously described (23,24). Primer-pair located at the UAS or core promoter of the *GALI* gene (see Materials and Methods section) was used for PCR analysis of the (IP) DNA samples. A PCR fragment corresponding to the *GAL4* ORF was used as a control for background binding. (IP) was performed using a mouse monoclonal antibody against the c-myc epitope-tag (9E10; Santa Cruz Biotechnology, Inc.) or a polyclonal antibody against TAF12p (from Michael R. Green, University of Massachusetts Medical School). A mouse monoclonal antibody against the DNA binding domain of Gal4p (RK5C1; Santa Cruz Biotechnology, Inc.) was used. The primer-pairs are indicated on the left. The ratio of immunoprecipitate over the input is indicated below each band as %IP. (B) Recruitment of Sgf73p to the minimal Gal4p-binding sites. The plasmid containing minimal Gal4p-binding sites was transformed into the yeast strain expressing myc-epitope tagged Sgf73p. Yeast strain thus generated, was grown at 30°C in 1% yeast extract containing 2% peptone plus 2% galactose or raffinose. The ChIP analysis was performed as described in (A). The primers used for the PCR analysis are adjacent to the Gal4p-binding sites in the plasmid (see Materials and Methods section). The growth media are indicated on the left.

et al. (10) have demonstrated biochemically that Sgf73p is required to maintain SAGA integrity. Similarly, if Sgf73p maintains SAGA integrity *in vivo*, Spt20p and other SAGA components will not be recruited to the *GALI* UAS in the Δ *sgf73* strain. To test this idea, we analyzed recruitment of SAGA to the *GALI* UAS in the *SGF73* deletion mutant. Figure 2A shows that the deletion of *SGF73* significantly lowered recruitment of the Spt20p and TAF10p components of SAGA to the *GALI* UAS. Similarly, our previous studies (5,6) have also demonstrated that recruitment of SAGA was lost in the *SPT20* [that integrates SAGA (3)] deletion mutant strain. On the other hand, recruitment of the SAGA components such as TAF10p, TAF12p and Spt3p was not altered

in the deletion mutant of *GCN5* (5) that is dispensable for the integrity of SAGA (3). Since Spt20p maintains the integrity of SAGA (3,5) and its recruitment was significantly reduced in the absence of Sgf73p (Figure 2A), thus Sgf73p, like Spt20p, seems to regulate the structural integrity of the SAGA complex *in vivo*, consistent with previous biochemical data (10). However, our ChIP data also raised the possibility that Sgf73p might be required for recruitment of Spt20p (and hence SAGA) to the *GAL1* UAS by its interaction with the activator, Gal4p. In fact, our previous studies (7) have demonstrated that SAGA is recruited to the *GAL1* UAS by interaction of its Tra1 subunit with Gal4p *in vivo*. Thus, it is more likely that Sgf73p is required to maintain the integrity of SAGA *in vivo*, consistent with previous biochemical studies (10).

Sgf73p facilitates formation of the PIC assembly at the core promoters of the SAGA-dependent genes *in vivo*

To better understand the role of Sgf73p in formation of the PIC assembly and hence transcriptional activation, we analyzed its contribution in recruitment of TBP and RNA polymerase II (two important components of the PIC assembly) to the *GAL* core promoter. Figure 2B shows that recruitment of TBP and the largest subunit (Rpb1p) of RNA polymerase II to the *GAL1* core promoter was dramatically reduced in the *SGF73* deletion mutant strain. Consistently, transcription of *GAL1* was significantly impaired in Δ *sgf73* (Figure 2C). Thus, the Sgf73p component of SAGA regulates *GAL1* transcription by facilitating formation of PIC at the core promoter. However, formation of the PIC assembly at the *GAL1* core promoter was not completely impaired in Δ *sgf73*. On the other hand, our previous studies (5,6) have demonstrated that PIC formation at the *GAL1* core promoter was completely impaired in the absence of Spt20p which maintains SAGA integrity. Such a discrepancy in PIC formation in the absence of Sgf73p or Spt20p is due to the fact that the loss of Spt20p leads to complete impairment of SAGA recruitment to the *GAL1* UAS (5,6) while SAGA recruitment was not completely lost in Δ *sgf73* (Figure 2A). Consistently, SAGA has been shown biochemically to be completely disintegrated in the absence of Spt20p but not Sgf73p (3,10).

Since Sgf73p regulates recruitment of SAGA, the formation of the PIC assembly at the SAGA-dependent promoters in general would be altered in Δ *sgf73*. To test this idea, we analyzed recruitment of TBP and RNA polymerase II to the core promoters of two other SAGA-dependent genes, namely *ADH1* and *PHO84* (6). Figure 3A shows that recruitment of TBP and RNA polymerase II to the *ADH1* and *PHO84* core promoters was significantly reduced in Δ *sgf73*. Consistently, transcription of these genes was also altered in the *SGF73* deletion mutant strain (Figure 3B). As a negative control, we demonstrate that the formation of the PIC assembly (and hence transcription) at a SAGA-independent gene, *RPS5*, was not altered in Δ *sgf73* (Figure 3C). Thus, SAGA-associated Sgf73p regulates gene expression by stimulating formation of the PIC assembly *in vivo*. However, such a function of Sgf73p in PIC formation seems to be mediated by the regulatory role of Sgf73p in recruitment of SAGA or its integrity. In addition, Sgf73p may also contact with the component(s) of the PIC assembly to promote PIC formation.

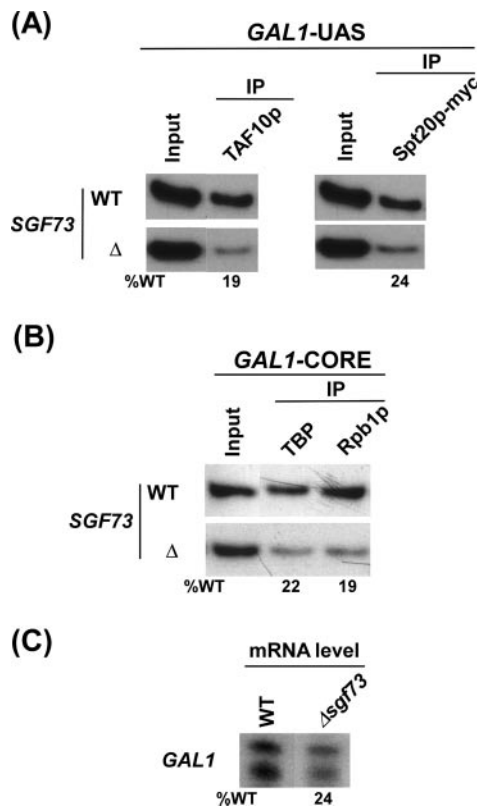


Figure 2. Requirement of Sgf73p for recruitment of SAGA and formation of the PIC assembly at *GAL1*. (A) Sgf73p is required for recruitment of SAGA to the *GAL1* UAS. The *SGF73* deletion mutant and its isogenic wild-type equivalent were first grown in raffinose-containing (YPR) and then shifted to galactose-containing (YPG) growth media 90 min before treatment with formaldehyde. (IPs) were performed as described in Figure 1A. A polyclonal antibody against the TAF10p (from Michael R. Green, University of Massachusetts Medical School) was used. Primer-pair located at the *GAL1* UAS was used for PCR analysis of the IP DNA samples. The percentage of DNA immunoprecipitated relative to wild-type (%WT) is indicated below each band of the mutant strains. (B) Sgf73p is required for formation of the PIC assembly at the *GAL1* core promoter. Yeast strains were grown, crosslinked and immunoprecipitated as in panel A. Primer-pair located at the *GAL1* core promoter was used for PCR analysis of the immunoprecipitated DNA samples. IPs were performed using polyclonal antibodies against TBP (obtained from Michael R. Green, University of Massachusetts Medical School) and the mouse monoclonal antibody 8WG16 (Covance) against the carboxy terminal domain of the RNA polymerase II large subunit (Rpb1p). (C) Transcription. Total cellular RNA was prepared from the wild-type or Δ *sgf73*, and mRNA level from the *GAL1* gene was quantitated by primer-extension. The percentage transcription relative to wild-type is indicated below.

Formation of the PIC assembly is differentially regulated by HAT or histone H3 acetylation *in vivo*

SAGA-associated Gcn5p acetylates primarily histone H3 at K9 (lysine 9) and K14. Histone H3 acetylation or the HAT activity of SAGA has been previously implicated in regulation of transcriptional initiation (3,36–41). However, how histone H3 acetylation or HAT regulates PIC formation and hence transcriptional initiation is not clearly known *in vivo*. Thus, we asked whether formation of Sgf73p-mediated PIC assembly at the core promoters of the aforementioned SAGA-dependent genes is dependent on histone H3 acetylation or HAT. To address this question, we analyzed recruitment of TBP and RNA polymerase II to the core promoters

of the *GAL1*, *PHO84* and *ADH1* genes in the *GCN5* deletion mutant and its isogenic wild-type equivalent. Figure 4A shows that the *GAL1*, *PHO84* and *ADH1* core promoters were acetylated at histone H3. Histone H3 acetylation at these core promoters were significantly reduced in $\Delta gcn5$. Interestingly, such a reduction in the level of histone H3 acetylation did not alter recruitment of RNA polymerase II and TBP to the *GAL1* and *ADH1* core promoters (Figure 4B). Thus, at these two promoters, Sgf73p facilitates formation of the PIC assembly in a HAT-independent-manner. In contrast, recruitment of RNA polymerase II and TBP to the *PHO84* core promoter was dependent on histone H3 acetylation or Gcn5p-HAT (Figure 4A and B), demonstrating the

requirement of histone H3 acetylation or HAT for formation of the PIC assembly. Thus, our data demonstrate that histone H3 acetylation or the HAT activity of SAGA is differentially required for formation of the PIC assembly *in vivo*, revealing a complex regulation of the PIC formation.

Role of Sgf73p in regulation of histone H3 acetylation or histone H3 eviction at the SAGA-dependent promoters

We next asked whether Gcn5p-HAT activity of SAGA is regulated by Sgf73p *in vivo*. Previous biochemical studies (36) have demonstrated that Gcn5p-HAT activity on nucleosomal histone H3 is dependent on the Ada2p and Ada3p components of SAGA. Similarly, deletion of *SGF73* has been shown to biochemically impair the HAT activity of SAGA (10). However, it is not known whether Sgf73p regulates histone H3 acetylation at the promoters of the SAGA-dependent genes *in vivo*. To address this question, we analyzed the levels of histone H3 acetylation at the *GAL1* core promoter in the *SGF73* wild-type and deletion mutant strains. Since Sgf73p is required for recruitment of Gcn5p as well as SAGA, histone H3 acetylation at the *GAL1* core promoter is expected to be significantly impaired in $\Delta sgf73$. Interestingly, our ChIP data demonstrate the elevated level of histone H3 acetylation at the *GAL1* core promoter in $\Delta sgf73$ when compared with wild-type equivalent (Figure 5A). Likewise, the elevated levels of histone H3 acetylation were also observed at the *ADH1* and *PHO84* core promoters in $\Delta sgf73$ (Figure 5A). In contrast, histone H3 acetylation level at the core promoter of a SAGA-independent *RPS5* gene was not altered in the absence of Sgf73p (Figure 5A). Since H3-K9/14 acetylation antibody also recognizes unacetylated histone H3 (Upstate biotechnology, Inc.; Figure 4A), the increase in the levels of histone H3 acetylation at the core promoters of *GAL1*, *ADH1* and *PHO84* in $\Delta sgf73$ may be

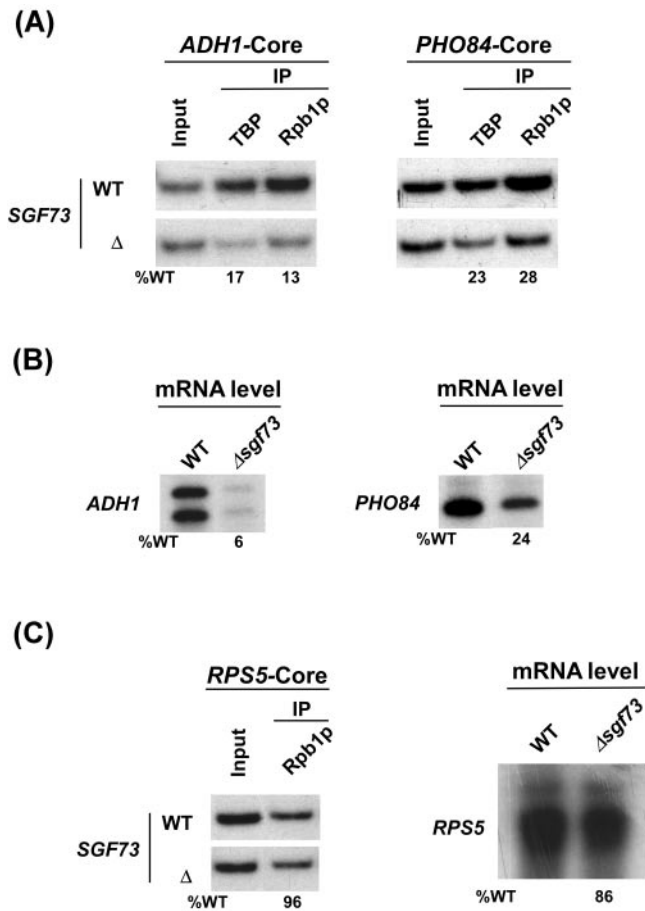


Figure 3. Sgf73p is required for PIC formation (and hence transcriptional activation) at the *ADH1* and *PHO84* promoters. (A) The analysis of the PIC assembly at the *ADH1* and *PHO84* core promoters in $\Delta sgf73$. The yeast strains were grown in YPD at 30°C up to an OD₆₀₀ of 1.0 prior to crosslinking. IPs were performed as described in Figure 2B. Primer-pairs located at the *ADH1* and *PHO84* core promoters were used for PCR analysis of the immunoprecipitated DNA samples. (B) Transcription. Total cellular RNA was prepared from the wild-type or $\Delta sgf73$, and mRNA levels from the *ADH1* and *PHO84* genes were quantitated by primer-extension. (C) Sgf73p is not required for PIC formation (and hence transcription) at the *RPS5* promoter. Left panel: Analysis of recruitment of RNA polymerase II at the *RPS5* core promoter in the wild-type and *SGF73* deletion mutant strains. Both the wild-type and deletion mutant strains were grown, crosslinked and immunoprecipitated as in panel A. Primer-pair located at the *RPS5* core promoter was used for PCR analysis of the IP DNA samples. Right panel: Transcription. Total cellular RNA was prepared from the wild-type or $\Delta sgf73$, and mRNA level from the *RPS5* gene was quantitated by primer-extension.

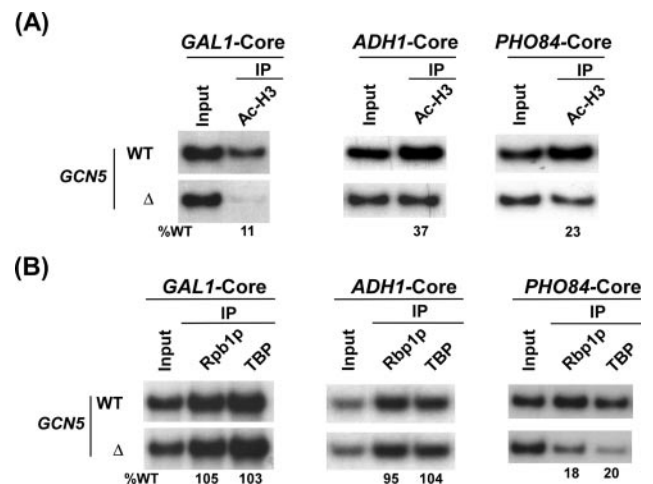


Figure 4. Histone H3 acetylation differentially regulates formation of the PIC assembly at the core promoters of the SAGA-dependent genes. (A) The levels of histone H3 acetylation at the *GAL1*, *ADH1* and *PHO84* promoters are significantly reduced in the *GCN5* deletion mutant. The wild-type and *GCN5* deletion mutant strains were grown and crosslinked as described in Materials and Methods section. IP was performed using a rabbit polyclonal antibody against Lys-9/14 di-acetylated H3 (06-599, Upstate Biotechnology, Inc.). (B) Gcn5p is required for formation of the PIC assembly at the *PHO84* core promoter, but not *GAL1* and *ADH1* core promoters. Yeast strains were grown and crosslinked as in (A). IPs were performed as in Figure 2B.

caused by the elevated levels of histone H3 at the core promoters of these genes, possibly because of reduction of histone H3 eviction in the absence of the PIC formation or active transcription (37). To test this possibility, we analyzed the levels of histone H3 at the *GALI*, *ADH1* and *PHO84* core promoters in the *SGF73* deletion mutant strain and its isogenic wild-type equivalent. Figure 5B shows that the levels of histone H3 at the core promoters of *GALI*, *ADH1* and *PHO84*, but not *RPS5*, were elevated in Δ *sgf73*. Interestingly, the increase in the level of histone H3 acetylation is remarkably similar to that of histone H3 at each of the SAGA-dependent promoters in Δ *sgf73*. Thus, Sgf73p does not seem to elevate the relative level of histone H3 acetylation or the HAT activity of Gcn5p *in vivo*. Nonetheless, our data reveal that the loss of Sgf73p lowers histone H3 eviction from the SAGA-dependent promoters *in vivo*.

DISCUSSION

Although Sgf73p has been shown to be a new component of SAGA on the basis of the biochemical studies (9,10), it is not known whether Sgf73p is a SAGA component *in vivo*. If so, it would be recruited along with other SAGA components to the *GALI* UAS but not to its core promoter, since SAGA is specifically recruited to the *GALI* UAS by the activator Gal4p (5,7). Here, we show that, like SAGA, Sgf73p is recruited to the *GALI* UAS, but not core promoter, or even to the minimal Gal4p-binding sites in a plasmid, implicating Sgf73p as a SAGA component *in vivo*. Furthermore, we show that the deletion of *SGF73* significantly impairs recruitment of the SAGA complex, consistent with biochemical requirement of Sgf73p for SAGA integrity (10). Thus, our results support the fact *in vivo* that Sgf73p is a SAGA component and is required for the structural integrity of SAGA, consistent with recent biochemical data (9,10).

Since Sgf73p is essential for recruitment of SAGA, the PIC would thus not be formed at the SAGA-dependent promoters in Δ *sgf73*. Indeed, our study demonstrates that recruitment of the PIC components such as TBP and RNA polymerase II is significantly impaired at the core promoters of the three representative SAGA-dependent genes, *GALI*, *ADH1* and *PHO84*, in the absence of Sgf73p. Consistently, transcription of these genes is altered in Δ *sgf73*. However, whether Sgf73p plays a dual role in assembling PIC by interacting with the component(s) of PIC as well as maintaining structural integrity of SAGA to regulate gene expression remains to be elucidated.

Previous studies (3,38–41) have demonstrated the functional role of histone H3 acetylation at the promoters in regulation of transcriptional initiation. Here, we demonstrate that all three SAGA-dependent promoters are acetylated at histone H3. Acetylation of histone H3 at these promoters is significantly lost in Δ *gcn5*. However, formation of the PIC assembly at the *GALI* and *ADH1* promoters is not altered in Δ *gcn5*. Thus, our study reveals that histone H3 acetylation status does not regulate PIC formation at *GALI* and *ADH1*. In contrast, PIC formation is significantly reduced at the *PHO84* promoter in Δ *gcn5*, demonstrating the role of histone H3 acetylation in regulation of the PIC formation, consistent with previous studies (3,38–41). Thus, our study reveals

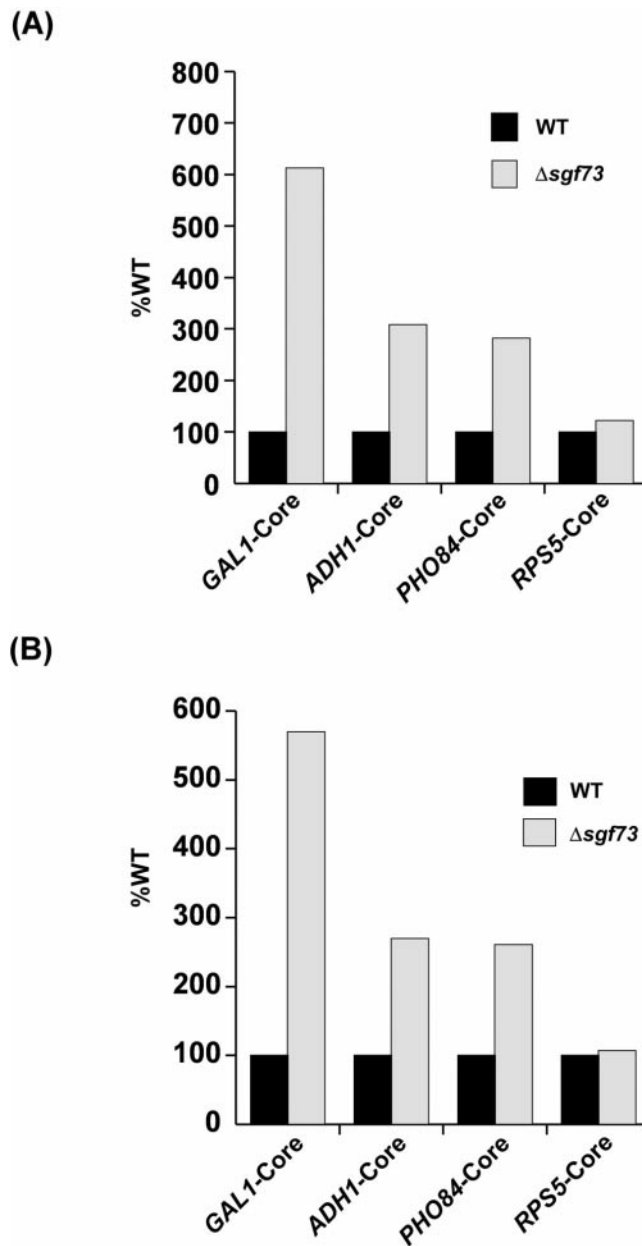


Figure 5. Elevation of the levels of histone H3 as well as histone H3 acetylation at the core promoters of *GALI*, *ADH1* and *PHO84* in Δ *sgf73*. (A) Analysis of the levels of histone H3 acetylation at the promoters of *GALI*, *ADH1*, *PHO84* and *RPS5* in the *SGF73* deletion mutant strain. The wild-type and *SGF73* deletion mutant strains were grown, crosslinked and immunoprecipitated as in Figure 4A. The percentages of DNA immunoprecipitated in Δ *sgf73* relative to wild-type (%WT) at the core promoters of *GALI*, *ADH1*, *PHO84* and *RPS5* are plotted in the form of a histogram. (B) Analysis of the levels of histone H3 at the promoters of *GALI*, *ADH1*, *PHO84* and *RPS5* in the *SGF73* deletion mutant strain. The wild-type and *SGF73* deletion mutant strains were grown and crosslinked as in panel A. IP was performed using an anti-histone H3 antibody (Ab1791; Abcam) and a modified ChIP protocol as described in Materials and Methods section.

that histone H3 acetylation differentially regulates formation of the PIC assembly. However, the molecular basis for such differential roles of histone H3 acetylation in regulation of the PIC formation remains unknown. According to the ‘histone-code’ hypothesis (42), it is likely that histone H3

acetylation in combination with other histone modifications at the *GAL1* and *ADHI* promoters may contribute to the role of chromatin structure in PIC formation and hence transcriptional activation.

Histones are evicted during active transcription *in vivo* (37). Since Sgf73p is required for formation of the PIC assembly (and hence transcriptional activation), histone eviction from the SAGA-dependent promoters would thus be significantly reduced in Δ *sgf73*. Indeed, our study demonstrates the elevated levels of histone H3 at the SAGA-dependent promoters in the absence of Sgf73p. Thus, impairment of PIC formation in Δ *sgf73* decreases histone H3 eviction and hence alters chromatin structure at the SAGA-dependent promoters.

In summary, we provide evidence that Sgf73 is a component of the SAGA complex *in vivo*, consistent with previous biochemical data (9,10). Sgf73p regulates SAGA recruitment, and is required for the assembly of PIC at the core promoters of the SAGA-dependent genes. However, formation of the PIC assembly at the core promoters is differentially regulated by histone H3 acetylation or Gcn5p-HAT activity of SAGA. Further, impairment of PIC formation in Δ *sgf73* lowers histone H3 eviction from the SAGA-dependent promoters, and thus alters chromatin structure. These results provide valuable information on the functional role of yeast Sgf73p (and possibly human ataxin-7 or Sca7p) in regulation of gene expression *in vivo*.

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Conflict of interest statement. None declared.

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