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

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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 Δsgf73, 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-TAFII31-GCN5 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...
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 Δsgf73. 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 (Δsgf73::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; Δsgf73), respectively.

For the studies at the *GAL1* promoter in Δsgf73 and Δgcen5 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–peptone–dextrose) plus 2% galactose for 90 min at 30°C. Total RNA was prepared from 10 ml of yeast culture. Fifteen micrograms of total RNA were used in the primer extension analysis using 32P-labeled primer and AMV reverse transcriptase (Promega). The primers used for analysis of *GAL1*, *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 μl lysate from 50 ml of yeast culture) were precleared by centrifugation, and then 100 μl 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 μl TE 8.0 [10 mM Tris–HCl (pH 8.0) and 1 mM EDTA], and 1 μl of IP DNA was analyzed by PCR. The PCR (a total of 23 cycles) contained [α-32P]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 lysate without going through the IP step and suspended in 100 μl TE 8.0. To compare the PCR signal arising from the IP DNA with the input DNA, 1 μl 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 μl of anti-histone H3 antibody from Abcam and 100 μl of protein A/G plus agarose beads from Santa Cruz Biotechnology, Inc.), and IP DNA sample was dissolved in 10 μl TE 8.0 of which 1 μl was used in PCR analysis. In parallel, the PCR for ‘input’ DNA was performed using 1 μl DNA that was prepared by dissolving purified DNA from 5 μl lysate in 1000 μl 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: GAL1 (UAS): 5'-CGCTTAAACTGCTCATTTGCTATATTG-3', 5'-TTTCTCGGAGCTTGCGG-3'; GAL1 (Core): 5'-ATAGAGTAGTAAATGCGATTAGTTAAAGTGGTTATGCA-3'; ADH1 (Core): 5'-GGTTATACGGCCTTCCTCCTCAGT-TAC-3', 5'-GAAACAGAAACAATGACAGGGAAACAA-AAG-3'; PHO84 (Core): 5'-GACTCACTTACTATTGTTG-GGCCTG-3', 5'-GTTTGTGTTGGTGTCCCTGTTGATCT-3'; RPS5 (Core): 5'-GGCCAAACTTCTACGTCAGCTAGTAG-3', 5'-CGGTGTCAGAATCCTTGGGAAATGTC-3'; GAL4 (open reading frame (ORF)): 5'-CTTTGTCTAATGCAGTCGTCATGTACC-3', 5'-CACAAGTCTGGATTTTAAAGTGGCC-3'. Primer pairs flanking Gal4p-binding sites in the plasmid SGP4 are 5'-GTTGGCGGCGCGCTCTAGAAACTATG-3' and 5'-TGGACCTAATGGGATAGTTCAGC-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 *GAL1*, *ADH1*, *PHO84* and *RPS5* mRNAs were as follows: GAL1: 5'-CTTGTGACGCTTCCAGCTTACGTAATGGGATAGG-3'; ADH1: 5'-CTATTCTTGTTCCAATTTACCAGG-3'; PHO84: 5'-GAAAGACTTCTTTACGCAAAATG-3'; RPS5: 5'-GACTGGGGTGAAATCCTTCAACAACACTTC-3'.
RESULTS

**Sgf73p is an integral component of SAGA in vivo**

Yeast SAGA is a large multi-protein complex that contains Adalp, 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 vitro, we analyzed recruitment of Sgf73p to the UAS of a well-characterized SAGA-dependent gene, **GAL1**, 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 **GAL1** UAS or core promoter. If Sgf73p is an integral component of the SAGA complex, it would be recruited to the **GAL1** UAS, but not core promoter, since previous ChIP studies (5) have demonstrated recruitment of SAGA to the **GAL1** 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 **GAL1** UAS. Similarly, Gal4p was recruited to the **GAL1** UAS (Figure 1A). Significantly, these same proteins were not associated with the **GAL1** 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 **GAL1** 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 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 **GAL1** UAS in the Δsgf73 strain. To test this idea, we analyzed recruitment of SAGA to the **GAL1** 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 **GAL1** 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 Δsgf73 (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 Δsgf73. 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 Δsgf73 (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 Δsgf73. 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 Δsgf73. 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 Δsgf73 (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.

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 \(\Delta\)gc5. 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), our ChIP data demonstrate the elevated level 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 OD_{600} 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 GAL1, 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 GAL1, ADH1 and PHO84, but not RPS5, were elevated in Δsgf73. 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 Δsgf73. 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 GAL1 UAS but not to its core promoter, since SAGA is specifically recruited to the GAL1 UAS by the activator Gal4p (5,7). Here, we show that, like SAGA, Sgf73p is recruited to the GAL1 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 Δsgf73. 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, GAL1, ADH1 and PHO84, in the absence of Sgf73p. Consistently, transcription of these genes is altered in Δsgf73. 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 Δgen5. However, formation of the PIC assembly at the GAL1 and ADH1 promoters is not altered in Δgen5. Thus, our study reveals that histone H3 acetylation status does not regulate PIC formation at GAL1 and ADH1. In contrast, PIC formation is significantly reduced at the PHO84 promoter in Δgen5, demonstrating the role of histone H3 acetylation in regulation of the PIC formation, consistent with previous studies (3,38–41). Thus, our study reveals 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 differentially regulates formation of the PIC assembly.

Figure 5. Elevation of the levels of histone H3 as well as histone H3 acetylation at the core promoters of GAL1, ADH1 and PHO84 in Δsgf73. (A) Analysis of the levels of histone H3 acetylation at the promoters of GAL1, 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 Δsgf73 relative to wild-type (%WT) at the core promoters of GAL1, ADH1, PHO84 and RPS5 are plotted in the form of a histogram. (B) Analysis of the levels of histone H3 at the promoters of GAL1, 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.
acetylation in combination with other histone modifications at the GALI and ADH1 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 Δsgf73. 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 Δsgf73 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 Δsgf73 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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