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The mRNA Export Factor Sus1 Is Involved in Spt/Ada/Gcn5 Acetyltransferase-mediated H2B Deubiquitinylation through Its Interaction with Ubp8 and Sgf11[D]

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Sus1 acts in nuclear mRNA export via its association with the nuclear pore-associated Sac3–Thp1–Cdc31 complex. In addition, Sus1 plays a role in transcription through its interaction with the Spt/Ada/Gcn5 acetyltransferase (SAGA) complex. Here, we have analyzed function and interaction of Sus1 within the SAGA complex. We demonstrate that Sus1 is involved in the SAGA-dependent histone H2B deubiquitinylation and maintenance of normal H3 methylation levels. By deletion analyses, we show that binding of Sus1 to SAGA depends on the deubiquitinylating enzyme Ubp8 and Sgf11. Moreover, a stable subcomplex between Sus1, Sgf11, and Ubp8 could be dissociated from SAGA under high salt conditions. In vivo recruitment of Sus1 to the activated *GAL1* promoter depends on Ubp8 and vice versa. In addition, histones coenrich during SAGA purification in a Sus1–Sgf11–Ubp8-dependent way. Interestingly, *sgf11* deletion enhances the mRNA export defect observed in *sus1*Δ cells. Thus, the Sus1–Sgf11–Ubp8 module could work at the junction between SAGA-dependent transcription and nuclear mRNA export.

INTRODUCTION

Gene expression in eukaryotes depends on several multiprotein complexes that regulate transcription, pre-mRNA processing, and the export of mature mRNA through the nuclear pore complexes (NPCs). These cellular machines are organized into an intricate coupled network (Reed, 2003; Rodríguez *et al*., 2004; Aguilera, 2005). Gaining access to chromatin is a prerequisite for transcription and requires a conversion from inactive to active chromatin states. Histone-modifying enzymes impose a pattern of posttranslational modifications that can either activate or repress transcription, depending on the type and location of the modification on the histone octamer (Jenuwein and Allis, 2001; Cosgrove *et al*., 2004; Mellor, 2005). The 1.8-MDa Spt/Ada/Gcn5 acetyltransferase (SAGA) complex is one of the best studied examples of a histone modifier, with acetylating and deubiquitinylating activities, that regulates RNA polymerase II (Grant *et al*., 1997; Henry *et al*., 2003).

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Abbreviations used: NPC, nuclear pore complex; SAGA, Spt/Ada/Gcn5 acetyltransferase.

The SAGA complex contains a small protein Sus1, which was shown to be present also in the Sac3–Thp1–Cdc31 mRNA export complex (Fischer *et al*., 2004; Rodríguez-Navarro *et al*., 2004). The Sac3–Thp1–Cdc31–Sus1 complex binds to nucleoporins at the nuclear basket, and in concert with the export receptor Mex67-Mtr2, it mediates nuclear export of messenger ribonucleoprotein particles (Fischer *et al*., 2004). Consequently, Sus1 has functional roles in both mRNA export and transcriptional regulation. This suggests that Sus1 could be a physical bridging factor between a transcriptional coactivator and nuclear pore-associated mRNA export factors (Rodríguez *et al*., 2004). Recently, Sus1 was shown to be involved in the repositioning and subsequent confinement of dynamic motility of the *GAL1* locus to the nuclear periphery upon transcriptional activation (Cabal *et al*., 2006).

SAGA is organized into distinct domains that have been extensively studied by biochemical, genetic, and structural methods. Electron-microscopic (EM) analyses of human TATA-binding protein (TBP)-associated factor-containing complex and yeast SAGA complexes together with immuno-EM labeling of subunits have revealed specialized structural and functional modules. These modules include components for histone acetylation and deubiquitinylation, activator interactions, TBP regulation, and components that constitute the SAGA structural backbone (Brand *et al*., 1999; Wu *et al*., 2004).

SAGA-dependent modification of histones plays an important role in the regulation of gene expression. The SAGA acetyltransferase Gcn5 has been implicated in acetylation of histones H3 and H2B, which is necessary for full transcriptional activation (Candau *et al*., 1997; Grant *et al*., 1999;

Berger, 2002; Carrozza *et al*., 2003). Ubp8, the SAGA-deubiquitylating enzyme, removes ubiquitin moieties from histone H2B (Holstege *et al*., 1998; Sanders *et al*., 2002; Henry *et al*., 2003; Daniel *et al*., 2004). Different studies have demonstrated that Lys-123 at the carboxy terminus of H2B is ubiquitinylated by the E2/E3 enzymes Rad6/Bre1 (Robzyk *et al*., 2000; Hwang *et al*., 2003; Wood *et al*., 2003). H2B monoubiquitinylation induces a *trans*-tail methylation of histone H3 at Lys-4 and Lys-79 mediated by Set1 and Dot1, respectively (Briggs *et al*., 2002; Sun and Allis, 2002). H2B ubiquitinylation rises early during gene activation and declines shortly afterward. Both the addition and subsequent removal of ubiquitin seem to be required for optimal gene activation (Henry *et al*., 2003).

Recently, Sgf11, a SAGA subunit (Lee *et al*., 2004; Powell *et al*., 2004), has been shown to be important for Ubp8 association with SAGA. Several lines of evidence suggest that Sgf11 and Ubp8 form a distinct functional module inside of SAGA required for deubiquitinylation of H2B (Ingvarsdottir *et al*., 2005; Lee *et al*., 2005). Moreover, a recent study has shown that Ubp8 differentially regulates Lys-4 methylation of histone H3 in vivo (Shukla *et al*., 2006).

In this study, we show that Sus1 binds to the SAGA complex through Ubp8 and Sgf11 and thereby extend the concept of a deubiquitinylating module into a heterotrimeric subcomplex. Moreover, Sus1 is required for Ubp8 binding to the *GAL1* promoter under conditions of transcription activation. In vivo, Sus1 is required for H2B deubiquitinylation and global H3 methylation. Furthermore, deletion of *SGF11* from cells aggravates the growth and mRNA export defects that are detected in *sus1*Δ cells. Together, the data suggest that Sus1, Ubp8, and Sgf11 are organized in a functional module of the SAGA complex that could couple transcription with mRNA export.

MATERIALS AND METHODS

Yeast Strains, DNA Recombinant Work, and Microbiological Techniques

Yeast strains used in this study are listed in Table 1. Microbiological techniques, yeast plasmid transformation, mating, sporulation of diploids, and tetrad analysis were done essentially as described previously (Santos-Rosa *et al*., 1998). Chromosomal integration of green fluorescent protein (GFP) (HIS3/KANMX6 marker), TAP (TRP1 marker), MYC (HIS3 marker), and hemagglutinin (HA) (HIS3 marker) as C-terminal tags was performed as described previously (Longtine *et al*., 1998; Gavin *et al*., 2002). TAP-tagged strains with deletions were obtained by polymerase chain reaction (PCR)-targeted disruption of the gene of interest or by mating individual TAP-tagged strains and deletion strains. Gene deletion strains that were not obtained from EUROSCARF were made by transformation with a PCR disruption cassette derived from pFA6a-KanMX6 (Longtine *et al*., 1998) and from pRS425-LEU2 (Brachmann *et al*., 1998) plasmids. Strains were grown under standard conditions. For growth analysis, yeast cells were diluted to 0.5 OD_{600}, and serial dilutions (1:10) were spotted onto YPD and incubated at various temperatures.

TAP Purifications, Immunoprecipitations, and Western Blot Analysis

Purification of Sus1-TAP and Ada2-TAP in wild-type and mutant strains was performed as described previously (Rodríguez-Navarro *et al*., 2004). TAP-purified complexes were analyzed by SDS-PAGE by using Novex 4–12% gradient gels (Invitrogen, Carlsbad, CA) and visualized by staining with PageBlue protein-staining Coomassie (MBI Fermentas, Hanover, MD). For isolation of a Sus1–Sgf11–Ubp8 subcomplex, IgG-bound Sus1-TAP was incubated with a buffer containing 1 M $MgCl_2$, 50 mM Tris-HCl, pH 6.0, 0.5 mM dithiothreitol for 1 h at 4°C. Resin was then washed with 50 bed volumes of the same buffer followed by 5 volumes of TAP buffer. All other steps were performed essentially as published previously (Gavin *et al*., 2002). The protein bands were excised, digested with trypsin, and analyzed by matrix-assisted laser desorption ionization/time of flight (TOF)/TOF mass spectrometry (Voyager 4700; Applied Biosystems, Foster City, CA).

Total amounts of H3 and H4 were analyzed using anti-TAP (Open Biosystems, Huntsville, AL), anti-H3, and anti-H4 (Abcam, Cambridge, United Kingdom) antibodies following the manufacturer's protocol.

Global levels of ubH2B were assessed by using YZ6276, YZ6276*sus1*Δ, YZ6276*ubp8*Δ, and YZ6276*sgf11*Δ strains expressing FLAG-tagged H2B (Robzyk *et al*., 2000). Whole-cell extracts were prepared in 20% trichloroacetic acid to precipitate histones, and the resuspended precipitates were either used directly for Western blot analysis or used for immunoprecipitation with anti-FLAG M2 agarose (Sigma-Aldrich, St. Louis, MO). The immunoprecipitates were washed twice in immunoprecipitation (IP) buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.5% NP-40, 0.5% bovine serum albumin, protease inhibitors) and three times in IP buffer without bovine serum albumin. Elution was performed with 300 ng of FLAG peptide (generous gift from Dr. Saus, Centro de Investigación Príncipe Felipe), and input and eluate samples were subjected to SDS-PAGE and Western blotting with anti-H2B (Abcam) and anti-FLAG antibodies (Sigma-Aldrich), respectively. Global levels of H3 methylation were tested with antibodies against K4 monomethylated H3 and K79 trimethylated H3 (Abcam) following the manufacturer's protocol.

Chromatin Immunoprecipitation (ChIP)

PCR assays were performed as described previously (De Nadal *et al*., 2004). In all ChIP experiments, yeast cultures were grown to early log phase (0.6 OD_{600}) before cells were transferred to YPG for 5 h. The oligonucleotides used to amplify the *GAL1* chromosomal region were located from −537 to −216 base pairs with respect to ATG. TEL corresponds to a 177-base pair region located 500 base pairs before the right end of chromosome VI. Quantification was performed using Quantity One software (Bio-Rad, Hercules, CA).

Miscellaneous

Sus1-GFP localization was performed with exponentially growing cells using a Leica DM6000B fluorescence microscope (Leica, Wetzlar, Germany) with a 63× PL APO objective. Localization of poly(A)$^+$ RNA by in situ hybridization was performed using a Cy3 end-labeled oligo$(dT)_{50}$ as described in Santos-Rosa *et al*. (1998).

RESULTS

Association of Sus1 with SAGA Requires Ubp8 and Sgf11

Understanding the molecular basis of Sus1 function requires a better knowledge of its association with the SAGA complex. As shown previously, TAP-tagged Sus1 coenriched all the SAGA subunits, and vice versa TAP-tagged SAGA subunits (e.g., Ada2-TAP or TBP-associated factor [Taf]6-TAP) coprecipitated Sus1 during tandem affinity purification (Rodríguez-Navarro *et al*., 2004). Notably, when Ada2-TAP (Figure 1A) or Taf6-TAP (our unpublished data) were affinity purified from a yeast strain lacking chromosomal *SUS1* (*sus1*Δ), most of the SAGA subunits still coprecipitated, but a band at ~53 kDa, which we identified as Ubp8, was specifically absent (our unpublished data). To demonstrate that binding of Ubp8 to SAGA depends on Sus1, we purified Ada2-TAP from wild-type and *sus1*Δ cells that express myc-tagged Ubp8. Mass spectrometry of the corresponding band in the wild-type strain and Western blot analysis revealed that Ubp8 does not copurify with SAGA when Sus1 is absent (Figure 1A). These data suggested that Sus1 could be required for the association of Ubp8 with SAGA.

To further substantiate this finding, we affinity purified Ada2-TAP from a yeast strain with disrupted chromosomal *UBP8*. Western blot analysis revealed that Sus1 (which was myc tagged to facilitate its detection) was absent when Ada2-TAP was purified from the *ubp8*Δ strain, but it was readily detectable when isolated from an isogenic *UBP8*$^+$ wild-type strain (Figure 1A). Together, these analyses revealed that both Sus1 and Ubp8 are required for their binding to the SAGA complex.

The observation that Ubp8 is necessary for binding of Sus1 to SAGA prompted us to test whether Sus1 is still associated with its second complex, the Sac3–Thp1–Cdc31 complex, when Ubp8 is absent. It was suggested that Sus1 via Cdc31 binds to this mRNA export complex that is located at the nuclear site of the NPC (Fischer *et al*., 2004). Sus1 together with Cdc31 binds to the Ct-interacting domain motif, which is present in the Sac3 carboxy-terminal domain. Sus1-TAP affinity purified from the *ubp8*Δ strain was still

Table 1. Strains used in this study

Yeast strain	Genotype	Reference
ADA2-TAP	*Mat a, ade2, arg4, leu2-3,112, ura3-52, trp1-289, ADA2-TAP::K.l. URA3*	Rodríguez-Navarro *et al*. (2004)
ADA2-TAP *sus1*Δ	*Mat a, ade2, arg4, leu2-3,112, ura3-52, trp1-289, ADA2-TAP::K.l. URA3, sus1::KanMX4*	This study
ADA2-TAP SUS1-MYC	*Mat a, ade2, arg4, leu2-3,112, ura3--52, trp1-289, ADA2-TAP::K.l. URA3, SUS1-MYC::KanMX4*	Rodríguez-Navarro *et al*. (2004)
ADA2-TAP SUS1-MYC *ubp8*Δ	*Mat a, leu2, trp1,his3, ura3, ADA2-TAP::K.l. URA3, SUS1-MYC::KanMX4, ubp8::KanMX4*	This study
ADA2-TAP UBP8-MYC *sus1*Δ	*Mat a, leu2, trp1,his3, ura3, ADA2-TAP::K.l. URA3, UBP8-MYC::HIS3, sus1::KanMX4*	This study
ADA2-TAP UBP8-MYC	*Mat a, leu2, trp1,his3, ura3, ADA2-TAP::K.l. URA3, UBP8-MYC::HIS3*	This study
SUS1-TAP	*Mat* α, *ade2, his3, leu2, trp1, ura3, SUS1-TAP::TRP1*	Rodríguez-Navarro *et al*. (2004)
SUS1-TAP *ubp8*Δ	*Mat* α, *ade2, his3, leu2, trp1, ura3, SUS1-TAP::TRP1, ubp8::KanMX4*	This study
SUS1-TAP *ubp8-C46/49A*	*Mat* α, *ade2, his3, leu2, trp1, ura3, SUS1-TAP::TRP1, ubp8-C46/49A-3HA:his*	This study
SUS1-TAP *ubp8-H77A*	*Mat* α, *ade2, his3, leu2, trp1, ura3, SUS1-TAP::TRP1, ubp8-H77A-3HA:his*	This study
SUS1-TAP *sgf11*Δ	*Mat* α, *ade2, his3, leu2, trp1, ura3, SUS1-TAP::TRP1, sgf11::KanMX4*	This study
UBP8-TAP	*Mat a, his3*Δ*1, leu2*Δ*0, met15*Δ*0, ura3*Δ*0:UBP8-TAP::HIS3 MX6*	Lee *et al*. (2005)
SUS1-MYC	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, SUS1-MYC::HIS3*	This study
SUS1-MYC *ubp8*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, SUS1-MYC::HIS3, ubp8::KanMX4*	This study
UBP8-HA	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, UBP8-HA::HIS3*	This study
UBP8-HA *sus1*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, UBP8-HA::HIS3, sus1::KanMX4*	This study
SUS1-MYC *spt20*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, SUS1-MYC::HIS3, spt20::KanMX4*	This study
SPT20-HA	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, SPT20-HA::HIS3*	YEN239
SPT20-HA *sus1*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, SPT20-HA::HIS3, sus1::KanMX4*	This study
ADA2-MYC	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, ADA2-MYC::HIS3*	YMZ44
ADA2-MYC *sus1*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, ADA2-MYC::HIS3, sus1::KanMX4*	This study
SUS1-GFP	*Mat* α, *ade2, ade3, his3, leu2, trp1, ura3, SUS1-GFP::KanMX4*	Rodríguez-Navarro *et al*. (2004)
SUS1-GFP *ubp8*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0 , SUS1-GFP::HIS3, ubp8::KanMX4*	This study
SUS1-GFP *sgf11*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0 , SUS1-GFP::HIS3, sgf11::KanMX4*	This study
SUS1-GFP *ada2*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0 , SUS1-GFP::HIS3, ada2::KanMX4*	This study
YZS276	*Mat a, hta1-htb1*Δ*, hta2-htb2*Δ*::LEU2 ura3-1, trp1-1, his3, ade2-1 + pRS413-HTA1-Flag-HTB1*	Robzyk *et al*. (2000)
YZS276 *sus1*Δ	*Mat a, hta1-htb1*Δ*, hta2-htb2*Δ*::LEU2 ura3-1, trp1-1, his3, ade2-1, sus1::KanMX4 + pRS413-HTA1-Flag-HTB1*	This study
YZS276 *ubp8*Δ	*Mat a, hta1-htb1*Δ*, hta2-htb2*Δ*::LEU2 ura3-1, trp1-1, his3, ade2-1, ubp8::KanMX4 + pRS413-HTA1-Flag-HTB1*	This study
YZS276 *sgf11*Δ	*Mat a, hta1-htb1*Δ*, hta2-htb2*Δ*::LEU2 ura3-1, trp1-1, his3, ade2-1, sgf11::KanMX4 + pRS413-HTA1-Flag-HTB1*	This study
*ubp8*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, ubp8::KanMX4*	EUROSCARF
*sgf11*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, sgf11::KanMX4*	EUROSCARF
*sgf11*Δ *sus1*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, sgf11::KanMX4, , sus1::LEU2*	This study
*ubp8*Δ*sus1*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, ubp8::natNT2 sus1::KanMX4*	This study
BY4741	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0*	EUROSCARF
*sus1*Δ	*Mat a, leu2-*Δ*0, his3-*Δ*1, met15-*Δ*0, ura3-*Δ*0, sus1::KanMX4*	This study

associated with the subunits of the mRNA export complex (Sac3, Thp1, and Cdc31), but remarkably, members of the SAGA complex were absent (Figure 1B). However, a protein of ~11 kDa, which was identified as the SAGA component Sgf11 by mass spectrometry, was still coenriched with Sus1-TAP isolated from the *ubp8*Δ strain. Ubp8 association with SAGA requires a putative zinc (Zn) finger domain in the N-terminal region. To determine whether this domain was also required for the Sus1 interaction with SAGA, we TAP tagged Sus1 in two Ubp8 mutants with substitutions in its Zn finger domain (Ingvarsdottir *et al*., 2005). As shown for the complete deletion of Ubp8, these Zn finger mutants also cause the loss of Sus1 from SAGA (Figure 1B). Together, these results suggest that wild-type Ubp8 is required for interaction of Sus1 with the SAGA complex.

To test whether Sgf11 is also required to recruit Sus1 to the SAGA complex, we affinity purified Sus1-TAP from an *sgf11*Δ strain. Similar to the *ubp8*Δ strain, Sus1-TAP no longer coprecipitated the SAGA complex when isolated from the *sgf11*Δ strain, but interaction with the Sac3 complex was not impaired (Figure 1B). Notably, Ubp8 does not co-purify with Sus1 in the absence of Sgf11, whereas Sgf11 is still present in Sus1-TAP when Ubp8 is deleted or mutated (see above). These data demonstrate that the interaction of Sus1 with SAGA requires Ubp8 and Sgf11 and suggest that Sgf11 could be the direct binding partner of Sus1.

A Salt-Stable Sus1–Sgf11–Ubp8 Subcomplex Can Be Dissociated from SAGA

To further investigate whether Sus1, Sgf11, and Ubp8 could constitute a stable subcomplex of SAGA, we affinity purified Sus1-TAP from yeast lysates, but before eluting the immobilized Sus1 from IgG-Sepharose with tobacco etch virus (TEV) protease, the beads were treated with increasing concentrations of $MgCl_2$. Interestingly, most of the SAGA subunits (e.g., Tra1 and Spt7) were released from beads bound with Sus1 at 1 M $MgCl_2$, whereas Ubp8, Sgf11, Cdc31, Thp1, and Sac3 were specifically retained along with Sus1 (Figure

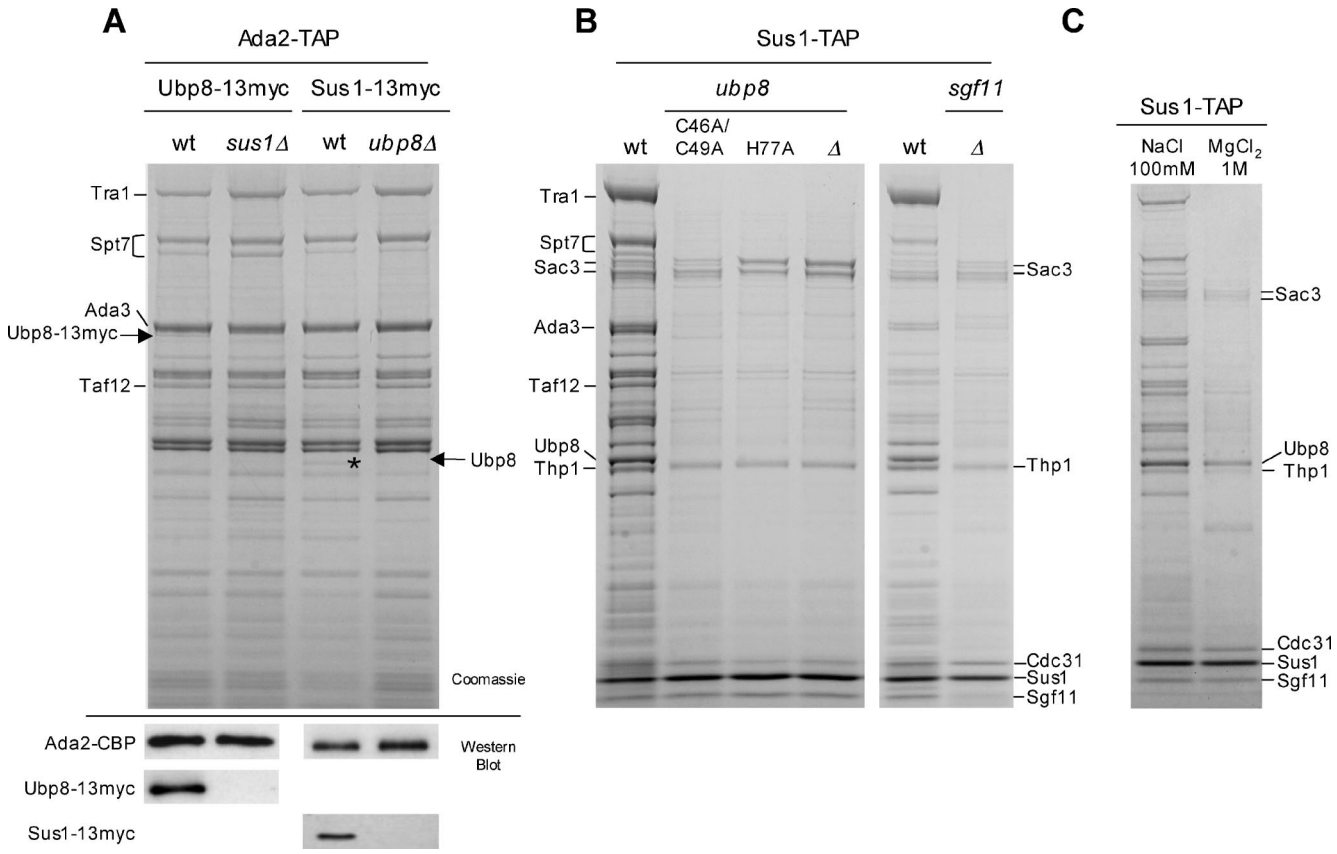

Figure 1. Association of Sus1 with SAGA requires Ubp8 and Sgf11. (A) Ada2-TAP was purified from a strain expressing Ubp8-13myc in a wild-type (wt) or *sus1*Δ background by the TAP method. Reciprocally, an Ada2-TAP strain expressing Sus1-13myc was compared with a strain where *ubp8* had been deleted. Purifications were analyzed on a SDS 4–12% gradient polyacrylamide gel, and proteins were stained with Coomassie. Ubp8 (star) and Ubp8-myc (arrow) were identified by mass spectrometry; known SAGA components are indicated as landmarks. Note that Ubp8-13myc is present in the preparation from wild-type but is absent from *sus1*Δ cells (top). Equal loading was ensured by detecting the bait (Ada2-calmodulin-binding protein [CBP]) with an anti-CBP antibody. Loss of Ubp8-myc and Sus1-myc in the respective deletion strains is demonstrated by Western blotting by using anti-myc antibodies (bottom). (B) Sus1-TAP was affinity purified from wild-type (wt), *ubp8*Δ, *ubp8-C46/49A*, *ubp8-H77A*, and *sgf11*Δ strains, showing that Sus1 is unable to associate with the SAGA complex in these mutant conditions. Ubp8, Thp1, and Sgf11 bands were verified by mass spectrometry. (C) Sus1-TAP was affinity purified from wild-type cells by IgG-Sepharose. IgG-bound Sus1-TAP was treated with 1 M $MgCl_2$ before TEV elution and subsequent purification via the CBP tag (right lane). For comparison, Sus1-TAP was also affinity purified under standard conditions (i.e., 100 mM NaCl; left lane). All indicated copurifying proteins were identified by mass spectrometry.

1C). Thus, high $MgCl_2$ concentrations could dissociate most SAGA subunits from Sus1, but Ubp8 and Sgf11 stayed bound. Moreover, Sus1 remained associated with its second complex (Sac3, Thp1, and Cdc31) under conditions of high $MgCl_2$. Notably, when immobilized Sus1-TAP was incubated with 1.2 M $MgCl_2$, Ubp8 but not Sgf11 was dissociated (our unpublished data), supporting the idea that Sgf11 could be more tightly bound to Sus1 than Ubp8. Together, the biochemical data suggest that Sus1, Sgf11, and Ubp8 form a structural entity within the SAGA holoenzyme.

Sus1 and Ubp8 Are Codependent for Their Recruitment to the GAL1 Promoter

Previously, we showed that Sus1 is recruited to the *GAL1* promoter after transcriptional activation with galactose (Rodríguez-Navarro *et al.*, 2004). That Sus1 does not coprecipitate with SAGA in the absence of Ubp8 suggested that Ubp8 might be required for Sus1 recruitment to chromatin. To test this possibility, we carried out ChIP assays of Sus1 to the *GAL1* promoter after induction with galactose in wild-type and *ubp8*Δ cells. After galactose induction, Sus1 immunopurified from wild-type cells specifically purified a DNA fragment corresponding to the *GAL1* promoter, indicating that Sus1 is bound to the chromatin. Interestingly, in *ubp8*Δ cells no specific purification of the DNA fragment containing the *GAL1* promoter region compared with the control fragment (*TEL*) was observed. This analysis revealed that Sus1 recruitment to the *GAL1* promoter depends on Ubp8 (Figure 2A).

Sus1 is required for Ubp8 association with SAGA (Figure 1A). Hence, it is conceivable that Sus1 somehow mediates the recruitment of Ubp8-SAGA to its regulated promoters. To test this idea, we used ChIP to analyze the recruitment of Ubp8 to the *GAL1* promoter. After induction with galactose, Ubp8 is recruited to the promoter of the SAGA-regulated gene *GAL1* (Figure 2B). However, in the absence of Sus1, Ubp8 is not recruited to the chromatin (Figure 2B). Thus, Sus1 and Ubp8 are required for concomitant recruitment to *GAL1* promoter, which is consistent with their physical interaction.

To further understand how Sus1 binds to chromatin, we analyzed whether SAGA integrity is necessary for promoter recruitment of Sus1. Therefore, we carried out ChIP assays

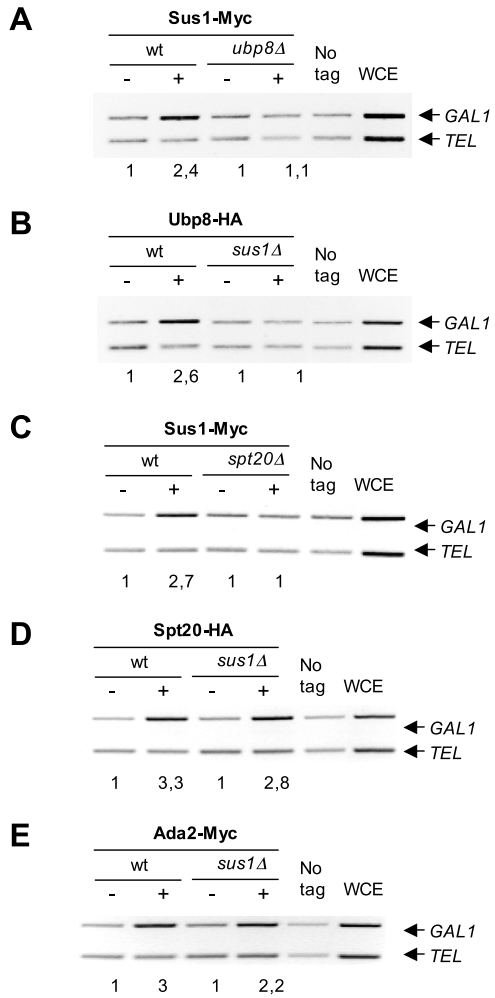

Figure 2. Sus1 and Ubp8 recruitment to the *GAL1* promoter depend on each other and on SAGA. (A) Recruitment of Sus1-myc to the *GAL1* gene locus is inhibited in *ubp8*Δ cells. Cross-linked cell extracts derived from wild-type or *ubp8*Δ strains, grown in either glucose (−) or galactose (+), were immunoprecipitated using anti-myc antibodies. PCR analysis was then performed using the appropriate primers to detect binding of Sus1-myc to the *GAL1* promoter or a *TEL* region. Control lanes show DNA amplified from extracts without tagged Sus1 (no tag) or before immunoprecipitation (whole-cell extract; WCE). Numbers indicate the mean of binding quantified from two independent experiments. (B) Recruitment of Ubp8-HA to *GAL1* depends on Sus1. Wild-type and *sus1*Δ strains containing chromosomally tagged Ubp8-HA were grown and analyzed as described in A and immunoprecipitated using anti-HA antibodies. PCR analysis was then performed using the appropriate primers to detect binding of Ubp8-HA to the *GAL1* promoter or telomeric region. (C) Recruitment of Sus1-myc to *GAL1* depends on SAGA integrity. Wild-type and *spt20*Δ strains containing chromosomal Sus1-myc were grown and analyzed as described in A. (D) Recruitment of Spt20-HA to *GAL1* is not affected by *sus1* deletion. Wild-type and *sus1*Δ strains containing chromosomal Spt20-HA were grown and analyzed as described in A. (E) Recruitment of Ada2-myc to *GAL1* does not depend on Sus1. Wild-type and *sus1*Δ strains containing chromosomal Ada2-myc were grown and analyzed as described in A.

of Sus1 in wild-type and *spt20*Δ cells. Sus1 was not present at the *GAL1* promoter in an *spt20* mutant (Figure 2C), which is known to have a severely disrupted SAGA structure (Grant *et al.*, 1997; Sterner *et al.*, 1999). Thus, we conclude that Sus1 cannot be targeted to the *GAL1* promoter independently but requires a functionally intact SAGA complex. These data are complementary to a recent report demonstrating that Ubp8 recruitment to chromatin equally depends on *SPT20* (Shukla *et al.*, 2006).

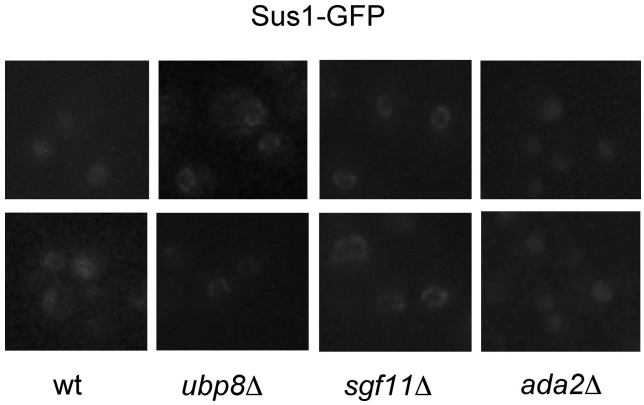

Figure 3. Wild-type intranuclear localization of Sus1 shifts upon *ubp8* and *sgf11* deletion and persists in *ada2*Δ. In vivo localization of chromosome tagged Sus1-GFP was analyzed by fluorescence microscopy in wild-type, *ubp8*Δ, *sgf11*Δ, and *ada2*Δ strains.

Considering the potential role of Sus1 in histone modification, we next asked whether it could also be involved in SAGA targeting to chromatin. To test this possibility, we performed ChIP assays of two different SAGA subunits in wild-type and *sus1*Δ cells. Although a slight reduction in the binding of Spt20 and Ada2 to the *GAL1* promoter was observed for the *sus1* mutant (Figure 2, D and E), we conclude that Sus1 is dispensable for SAGA recruitment to chromatin. These results corroborate the recent findings from Shukla *et al.* (2006) in which it was shown that Ubp8 does not affect Spt20, Taf10, or Taf12 recruitment to the *GAL1* upstream-activating sequence.

Intranuclear Localization of Sus1 Depends Upon Ubp8 and Sgf11

Sus1 is bound to two different complexes: the SAGA complex, which shows a predominantly intranuclear localization, and the Sac3 complex, which is associated with the nuclear pore. Accordingly, Sus1 is located in the nucleoplasm with a modest concentration at the nuclear periphery in wild-type cells (Rodríguez-Navarro *et al.*, 2004). Interestingly, the localization of Sus1-GFP at the nuclear periphery significantly increased in *ubp8*- or *sgf11*-deleted cells (Figure 3). In addition, we analyzed the preferential Sus1 localization in the SAGA mutant *ada2*. As expected, deletion of Ada2 did not cause a change in the intranuclear localization of Sus1 (Figure 3). These data suggest that Sus1 is redistributed to the nuclear envelope, possibly the NPC (i.e., the Sac3 complex), when binding to the SAGA complex and that recruitment to chromatin are inhibited.

Sus1 Is Involved in H2B Deubiquitinylation and H3 Methylation

The data presented in this study together with the finding that Ubp8 and Sgf11 are involved in histone H2B deubiquitinylation (Ingvarsdottir *et al.*, 2005; Lee *et al.*, 2005) suggested to us that Sus1 could also be important for the deubiquitinylation of histones. Hence, *SUS1* was disrupted in a strain that expressed FLAG-tagged H2B to facilitate detection of ubiquitinylated and unmodified H2B by Western blot analysis (Robzyk *et al.*, 2000). As anticipated, although sim-

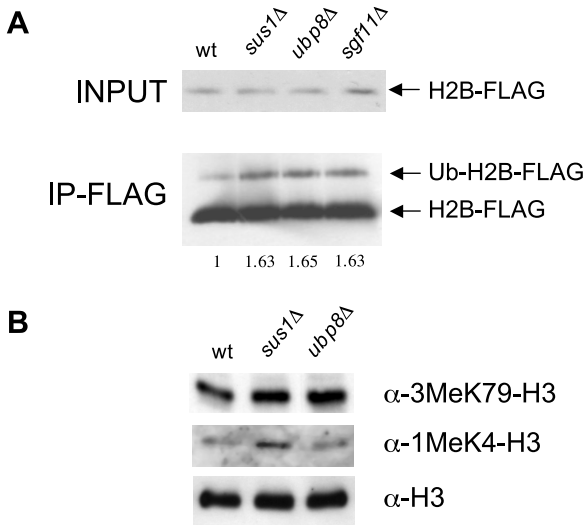

Figure 4. Global levels of H2B deubiquitinylation and H3 methylation are increased in *sus1*Δ cells. (A) Analysis of cell extracts (INPUT) and immunoprecipitated H2B-FLAG (IP-FLAG) derived from wild-type (wt), *sus1*Δ, *ubp8*Δ, and *sgf11*Δ cells by SDS-PAGE and Western blotting by using anti-FLAG antibodies to detect unmodified H2B-FLAG and ubiquitinylated uH2B-FLAG. (B) Global levels of H3 K4 monomethylation and K79 trimethylation are increased in cells lacking *SUS1*. Analysis of cell extracts derived from *sus1*Δ, *ubp8*Δ, or wild-type (wt) cells by Western blotting by using anti-H3 (1MeK4-H3 and 3MeK79-H3). Anti-H3 antibodies were used to demonstrate equal loading.

ilar levels of unmodified H2B were detected in whole-cell extracts, the amount of ubiquitinylated H2B increased significantly in a *sus1*Δ strain compared with wild type (Figure 4A). In addition, we found that ubH2B levels in *sus1*Δ are similar to those present in *ubp8*Δ and *sgf11*Δ, reinforcing the functional link between the three members of the SAGA subcomplex. Therefore, we conclude that Sus1 is required for H2B deubiquitinylation.

Because H2B monoubiquitinylation is a prerequisite for H3 K4 methylation (Dover *et al*., 2002; Sun and Allis, 2002), we analyzed the role of Sus1 in H3 methylation. H3 K4 monomethylation and H3 K79 trimethylation levels in total yeast extract were compared in wild-type and *sus1*Δ cells. These analyses revealed that the amount of H3 methylation at K4 and K79 residues is increased in cells lacking *SUS1* (Figure 4B). Thus, Sus1 acts in the same pathway as Ubp8 with respect to H2B deubiquitinylation and H3 methylation.

Coenrichment of Histones During SAGA Purification Depends on Sus1 and Ubp8

Notably, Sus1-TAP copurifies Coomassie-stainable amounts of histones H2A, H2B, H3, and H4 (Rodríguez-Navarro *et al*., 2004). To investigate the role of Sus1–Ubp8–Sgf11 for the interaction of SAGA with histones, we compared the amount of coenriched histones H3 and H4 during affinity purification of Sus1-TAP from wild-type and *ubp8*Δ or *sgf11*Δ cells. Whereas similar levels of H3 and H4 were present in the lysates of each strain, coprecipitation of these histones with Sus1-TAP after the TEV and EGTA elutions was largely abolished when either *UBP8* or *SGF11* was disrupted (Figure 5A). To find out whether purification of a bona fide SAGA subunit also leads to coenrichment of histones, we tested affinity-purified Ada2-TAP. As expected, Ada2 significantly coenriched H3 and H4 (Figure 5B). Interestingly, the copurification of histones H3 and H4 was drastically reduced when Ada2-TAP was isolated from *sus1*Δ or *ubp8*Δ cells (Figure 5B). In contrast, Ada2-TAP still coprecipitated the other SAGA subunits in the *sus1*Δ or *ubp8*Δ mutants (e.g., Tra1 or Ada3; also see Figure 1, A and B). Together, the data show that Sus1, Sgf11, and Ubp8 are required for the copurification of histones with the SAGA complex. Thus, it is possible that this subcomplex establishes an affinity between SAGA and its histone substrates on a biochemical level.

sgf11 Deletion Enhances the mRNA Export Defect of a sus1 Deletion Strain

The physical and functional interaction between Sus1, Sgf11, and Ubp8 prompted us to analyze whether *Sgf11* and *Ubp8* could also play a role in mRNA export. Therefore, we compared growth and mRNA export of the three mutants. At elevated temperatures (37°C), *sus1*Δ cells grow very slowly and exhibit an mRNA export defect (Figure 6A; also see Rodríguez-Navarro *et al*., 2004). In contrast, *sgf11*Δ and *ubp8*Δ cells are neither impaired in growth nor in nuclear mRNA export at 37°C (Figure 6A). Next, we constructed a *sus1*Δ/*sgf11*Δ double mutant. We speculated that this strain could exhibit an enhanced mRNA export defect, because Sus1 and Sgf11 coprecipitate even in the absence of *ubp8* (see above). Indeed, deletion of *SGF11* in a *sus1*Δ strain caused a synergistically enhanced mRNA export at 30°C and exacerbated the growth defect of *sus1*Δ at 39°C (Figure 6B). Interestingly, the *ubp8*Δ*sus1*Δ double mutant did not show any synthetic effect regarding mRNA export and growth (Figure 6B). These data suggest that Sgf11 has a function more closely related to Sus1, including a role in nuclear mRNA export.

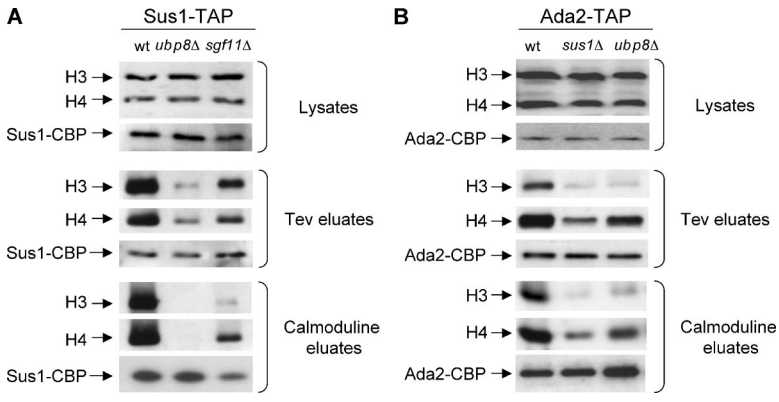

Figure 5. Sus1, Sgf11, and Ubp8 are required for coenrichment of histones during SAGA purification. (A) The presence of histones H3 and H4 was monitored during Sus1-TAP purification in wild-type, *ubp8*Δ, and *sgf11*Δ cells. Western blotting by using anti-H3, anti-H4, and anti-CBP antibodies to detect Sus1-CBP was performed from lysates (top), TEV eluates (middle), and calmodulin eluates (bottom). (B) Ada2-TAP was affinity purified from wild-type, *sus1*Δ, and *ubp8*Δ strains. The lysates (top), TEV eluates (middle), and calmodulin eluates (bottom) were analyzed in the same way as for A.

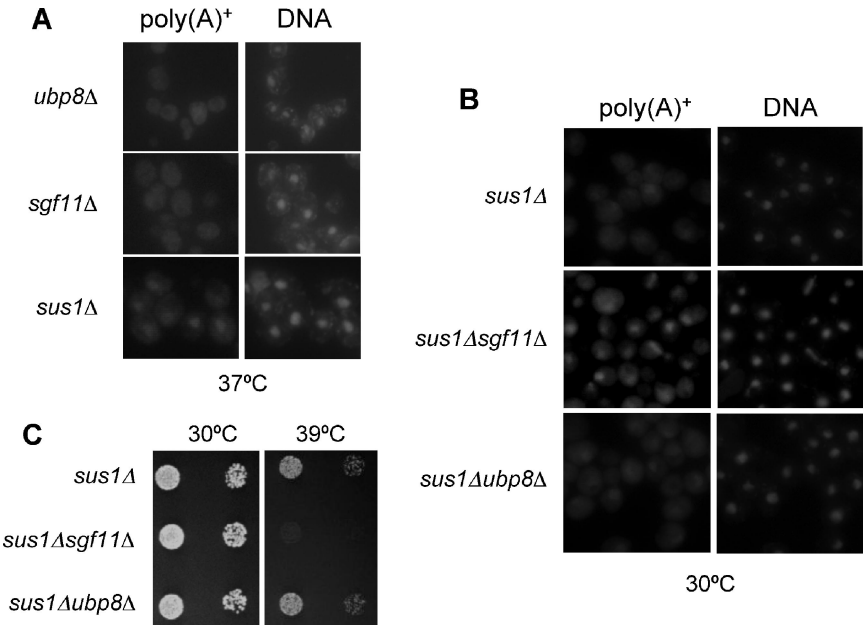

Figure 6. *sus1*Δ/*sgf11*Δ double mutant exhibits enhanced growth defect and impaired mRNA export. (A) Analysis of nuclear mRNA export in *ubp8*Δ, *sgf11*Δ, and *sus1*Δ cells after shifting to 37°C for 90 min. The localization of poly(A)$^+$ RNA was assessed by in situ hybridization using Cy3-labeled oligo(dT) probes. DNA was stained with 4,6-diamidino-2-phenylindole. (B) Double disruption strain *sus1*Δ/*sgf11*Δ, but not *sus1*Δ/*ubp8*Δ, exhibits an enhanced mRNA export defect. The localization of poly(A)$^+$ RNA in *sus1*Δ/*sgf11*Δ and *sus1*Δ cells, grown at 30°C, was assessed by in situ hybridization. (C) *sgf11* deletion specifically exacerbates the *sus1* growth defect. *sus1*Δ, *sus1*Δ*sgf11*Δ, and *sus1*Δ*ubp8*Δ cells were diluted in 10^{-1} steps, and equivalent amount of cells were spotted onto YPD plates. Cells were grown for 3 d at the indicated temperatures.

DISCUSSION

In this study, we have investigated how Sus1, a protein with roles in transcription and mRNA export, is connected to the SAGA complex. To date, it was not clear whether Sus1 is part of a known SAGA module or whether it forms a distinct platform for the association with the NPC-associated mRNA export machinery. Here, we show that Sus1 is essential for Ubp8 recruitment to SAGA and chromatin. Sus1, together with Ubp8 and Sgf11, is required for deubiquitinylation of histone H2B and thereby affects the methylation level of H3. Thus, Sus1 acts at the interface of chromatin modification and mRNA export.

Sus1, Ubp8, and Sgf11 are physically and functionally linked as a module in the SAGA complex. Previous studies have described that Ubp8 depends on a putative zinc finger domain in its N terminus to bind to SAGA (Ingvarsdottir *et al*., 2005). However, stable association also requires the small 11-kDa subunit Sgf11 (Ingvarsdottir *et al*., 2005; Lee *et al*., 2005). Our work extends these findings and demonstrates that Sus1 contributes yet another surface for SAGA association, because its absence disrupts the interaction of Ubp8 and Sgf11 with the SAGA complex. The interdependence of Sus1, Ubp8, and Sgf11 for SAGA association could be explained in at least two ways. One possibility is that the SAGA interaction is cooperative such that all three subunits undergo a sequential or concerted conformational change before SAGA incorporation. Alternatively, Sus1, Ubp8, and Sgf11 could form a common SAGA binding surface that only displays sufficient SAGA affinity if all three members are present. Interestingly, we found that Sus1 is able to bind to Sgf11 in the absence of Ubp8, and no association of Sus1 to Ubp8 was observed when Sgf11 was deleted. In addition, Ubp8 could be dissociated from Sus1–Sgf11 by an increase in salt concentration. This implies that Sus1 directly interacts with Sgf11, and this dimer may form a template for Ubp8. Nevertheless, we cannot exclude that the Sus1–Ubp8–Sgf11 deubiquitinylating module comprises additional factors, which are less tightly bound. Whether the heterotrimeric subcomplex is preassembled or undergoes a step-by-step incorporation during SAGA biogenesis remains to be shown.

Loss of Sus1 causes an increase in the levels of H2B ubiquitinylation and H3 methylation. We therefore conclude that Sus1 works in the same pathway as Ubp8. Ubp8 activity is context (i.e., SAGA) dependent, because it is not able to deubiquitinylate H2B in vivo or in vitro on its own (Lee *et al*., 2005). The H2B ubiquitinylation level increases at a given promoter during gene activation and declines shortly afterward (Henry *et al*., 2003). How this sophisticated interplay of ubiquitin addition and removal is regulated in time and space is not well understood, even though the enzymatic components have been defined. Is Ubp8 constitutively active, or can it be stimulated by other factors? SAGA association could be required either for targeting the enzymatic activity or to relieve a certain form of Ubp8 inhibition. The increase in H2B ubiquitinylation upon Sus1 deletion could be a simple consequence of Ubp8 dissociation. This would imply that Sus1 exerts its function only by anchoring Ubp8 and Sgf11 to SAGA. However, it is also possible that Sus1 acts as a direct regulator of Ubp8 activity. Concomitantly with an increase in global H2B ubiquitinylation, we found that H3 methylation is also affected in a *sus1* deletion mutant. The observed increase in K4 monomethylation and K79 trimethylation could possibly be correlated with alterations in gene expression (9% of the yeast genes) that we previously measured in *sus1*Δ cells by DNA macroarray analysis (Rodríguez-Navarro *et al*., 2004).

The three-dimensional architecture of SAGA has an elongated, curved shape and comprises five domains. The Taf subunits together with Spt7 and Ada1 constitute a core structure. This core structure is surrounded by domains that function in TBP regulation, activator binding, and histone acetylation. Distinct regulatory functions are spatially separated from each other and illustrate the principle of SAGA modularity (Wu *et al*., 2004; for review, see Timmers and Tora, 2005). The precise position of Sus1, Ubp8, or Sgf11 within SAGA is not known. However, we assume that this subcomplex is probably not a core structural component because deletion of *sus1*, *ubp8*, or *sgf11* did not perturb the overall integrity of SAGA. This distinguishes the Sus1 subcomplex from SAGA subunits, such as Spt20, Spt7, or Ada1.

Deletion of any of these proteins exhibits a strong disruptive effect on SAGA structure (Roberts and Winston, 1996; Grant *et al*., 1997; Horiuchi *et al*., 1997; Roberts and Winston, 1997; Sterner *et al*., 1999; Wu and Winston, 2002). Interestingly, the dissociation of Ubp8 upon Sus1 deletion also did not affect SAGA composition when Ada2 or Taf6 were used as baits for purifications, suggesting a peripheral location of Sus1 within a separate module.

Our ChIP analyses suggest an in vivo relevance for Sus1 function, because it is necessary for Ubp8 recruitment to chromatin. Conversely, Ubp8 is important for Sus1 binding to chromatin. Our study together with recent reports demonstrates that Sus1, Ubp8, and Sgf11 are dispensable for SAGA recruitment to the *GAL1* promoter, consistent with the biochemical dispensability of the module for SAGA integrity (Ingvarsdottir *et al*., 2005; Lee *et al*., 2005; Shukla *et al*., 2006). Along this line, we found an altered nuclear localization of Sus1 when it was uncoupled from SAGA by *ubp8* or *sgf11* deletions. This shift in Sus1 localization suggests interdependence between the association of Sus1 with SAGA and its location within the Sac3 complex at the nuclear pore.

Coenrichment of histones in a SAGA purification critically depends on the presence of all members of the subcomplex. This was unexpected, because several other mechanisms have been described by which SAGA can be targeted and bound to DNA. The 400-kDa protein Tra1, for example, directs SAGA to a promoter via its interaction with transcription activators. In addition, several histone-fold-containing subunits, such as Taf6, Taf12, and Ada1, seem to be involved (Brown *et al*., 2001; Hall and Struhl, 2002; Klein *et al*., 2003; Bhaumik *et al*., 2004). Our biochemical data suggest that the deubiquitinylating module may have an intrinsic affinity for histones (possibly nucleosomes). This affinity could be important for orienting the Ubp8 catalytic site with respect to the monoubiquitinated lysine at position 123 of H2B and might be involved in stabilizing the SAGA-nucleosome interaction once it has been formed. In contrast, the Sus1 subcomplex does not seem to be strictly required for chromatin targeting in vivo when analyzed by ChIP. The discrepancy between our TAP purifications and the ChIP assay may be due to major differences between these techniques. However, it seems also possible that our biochemical observations reflect an additional SAGA function that is independent of its role at the promoter of genes.

Sus1, together with other protein complexes, could couple chromatin modification, transcription initiation, and nuclear mRNA export machineries at the level of the NPC. However, it is not yet clear how Sus1 could connect histone modification and mRNA export. To strengthen the idea that the Sus1 module is indeed linked to both transcription and mRNA export, we tested whether deletion of Sus1 would have a synthetically enhanced mRNA export defect in combination with Sgf11, its putative direct partner. We did observe an enhanced defect when deletions of *sgf11* and *sus1* were combined. This opens the possibility that Sgf11, a bona fide SAGA component, could have a role in both processes. Interestingly, we have shown that *ubp8* deletion does not cause an exacerbation of the *sus1* single deletion phenotype. This result suggests that Sgf11 has a function more closely related to Sus1.

Our study has defined a critical mRNA export factor, Sus1, as an important part of a SAGA-deubiquitinylating module. Thus, the interaction between SAGA and the NPC-associated mRNA export machinery could involve the deubiquitinylating module. This insight may help to develop a more accurate view of how transcription and mRNA export are mechanistically coupled.

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