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Sus1, a Functional Component of the SAGA Histone Acetylase Complex and the Nuclear Pore-Associated mRNA Export Machinery

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Summary

Gene expression is a coordinated multistep process that begins with transcription and RNA processing in the nucleus followed by mRNA export to the cytoplasm for translation. Here we report the identification of a protein, Sus1, which functions in both transcription and mRNA export. Sus1 is a nuclear protein with a concentration at the nuclear pores. Biochemical analyses show that Sus1 interacts with SAGA, a large intranuclear histone acetylase complex involved in transcription initiation, and with the Sac3-Thp1 complex, which functions in mRNA export with specific nuclear pore proteins at the nuclear basket. DNA macroarray analysis revealed that Sus1 is required for transcription regulation. Moreover, chromatin immunoprecipitation showed that Sus1 is associated with the promoter of a SAGA-dependent gene during transcription activation. Finally, mRNA export is impaired in *sus1* mutants. These data provide an unexpected connection between the SAGA histone acetylase complex and the mRNA export machinery.

Introduction

In eukaryotic cells, certain steps of gene expression are restricted to the nucleus while other steps take place in the cytoplasm. As a consequence of this compartmentalization, an enormous amount of RNA and protein traffic moves between the nucleus and cytoplasm. Studies over the past several years have revealed that nucleocytoplasmic transport occurs by a variety of mechanisms that have in common the use of specific transport receptors (Weis, 2002). These receptors can pass through the nuclear pore complexes via transient interactions with phenylalanine-glycine (FG)-repeat containing nucleoporins. For mRNA export, a conserved heterodimeric export receptor (mRNA-exporter), called Mex67-Mtr2 in yeast and Tap-p15 (NXF1-NXT1) in metazoans, is essential for mRNA export (Conti and Izaurralde, 2001; Reed and Hurt, 2002).

Another key player in mRNA export is the conserved nuclear protein Yra1/Aly, which acts upstream of the mRNA-exporter and is thought to couple intranuclear steps in mRNP biogenesis with mRNA export (Reed and Hurt, 2002). In yeast, Yra1 interacts with Mex67 (Sträßer and Hurt, 200; Stutz et al., 2000; Zenklusen et al., 2001). Aly, the metazoan counterpart of Yra1, was also shown to interact directly with TAP, suggesting a conserved role for Yra1/Aly in mRNA export (Stutz et al., 2000; Rodrigues et al., 2001). Moreover, Aly promotes splicing-dependent export of mRNA in *Xenopus* oocytes (Zhou et al., 2000), and microinjection of anti-Aly antibodies blocks mRNA export (Rodrigues et al., 2001).

Another feature of Yra1/Aly is that it associates with Sub2/Uap56, a conserved intranuclear DEAD-box RNA helicase with multiple functions (for review, see Reed and Hurt, 2002; Jensen et al., 2003). Work from several laboratories indicated that the export factors Yra1 and Sub2 are cotranscriptionally recruited to pre-mRNAs (for review, see Jensen et al., 2003; Reed, 2003; Stutz and Izaurralde, 2003). As an example for cotranscriptional targeting of an mRNA binding protein to the nascent transcript, the shuttling RNA binding protein Npl3p was shown to associate with the gene and thus may function in mRNP assembly during transcription (Lei et al., 2001). Moreover, Yra1 and Sub2 associate with the four THO complex members Hpr1, Tho2, Mft1, and Thp2 to form the TREX complex that is recruited to actively transcribed genes (Jensen et al., 2001; Zenklusen et al., 2002; Libri et al., 2002; Lei and Silver, 2002; Sträßer et al., 2002). Current models assume that the TREX complex is recruited to the elongating RNA polymerase II complex via its transcription (THO) members, whereas the mRNA export factors (Sub2 and Yra1) recruit the Mex67-Mtr2 export receptor (Sträßer and Hurt, 2001; Jensen et al., 2003; Stutz and Izaurralde, 2003). Interestingly, mutation of any of the TREX components results not only in inhibition of nuclear mRNA export, but also sequestration of heat shock mRNAs at their sites of transcription (Jensen et al., 2001; Libri et al., 2002). Moreover, the TREX complex is linked to the nuclear exosome, which plays a role in many RNA processing and degradation steps (Libri et al., 2002). Thus, correct mRNP assembly is monitored by the exosome, resulting in retention and destruction of aberrant mRNPs at their sites of transcription (Jensen et al., 2003). Recently, the Dbp5 RNA helicase was shown to associate with the transcription factor TFIIH (Estruch and Cole, 2003). Previous work revealed that Dbp5 is concentrated at the cytoplasmic fibrils of the nuclear pore complex, suggesting an involvement in a terminal step during mRNA export (Estruch and Cole, 2003 and references therein). Thus, Dbp5 appears to have multiple roles during gene expression.

Although there is growing evidence for transcription-coupled mRNA export, the physical nature of this coupling is not known. Here, we identified a novel factor, Sus1, which is physically associated with SAGA, a histone acetylase complex, and the Sac3-Thp1 complex,

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which is involved in mRNA export (Fischer et al., 2002; Lei et al., 2003; Gallardo et al., 2003). Taken together, the data suggest that Sus1 connects the mRNA export machinery to the SAGA complex.

Results

Synthetic Lethality with a *yra1*-Mutated Allele Identifies *SUS1* as a Novel Gene in Yeast

The Yra1 protein is an essential component of the conserved mRNA export machinery (Sträßer and Hurt, 2000, 2001; Stutz et al., 2000; Zhou et al., 2000; Luo et al., 2001; Rodrigues et al., 2001). To identify mRNA export factors, we performed a synthetic lethal (sl) screen using the *yra1-ΔRRM* mutant allele (Stutz et al., 2000; Sträßer and Hurt, 2001). Three synthetic lethal mutants (sl9, sl15, sl302) were isolated, each of which was complemented by a yeast genomic DNA fragment (500 nt) located in an intergenic region between the *YSA1* and *SSN6* genes (Figure 1A). Northern analyses using this DNA fragment as a probe detected an RNA of ~320 nt in both wild-type (Figure 1B) and in the three sl mutant cells (data not shown). This RNA is absent in cells in which the chromosomal *YSA1-SSN6* locus is disrupted (Figure 1B). The RNA from the *YSA1-SSN6* intergenic locus was cloned by RT-PCR and contains 2 introns and 3 exons (Figure 1A). We designated the gene encoding this pre-mRNA *SUS1* (for sl gene *u*pstream of Ysa1), which is located between the *YSA1* and *SSN6* genes on the "Watson" strand of chromosome 2 (Figure 1A; GenBank accession number AY278445). This gene had not been previously annotated in the yeast database. The *SUS1* gene encodes a conserved protein of 96 amino acids (Figure 1C).

SUS1 Interacts Genetically with Several mRNA Export Factors

To verify the genetic interaction between *YRA1* and *SUS1*, the *yra1-ΔRRM* allele was combined with the null allele of *SUS1* (*sus1Δ*) in a haploid yeast strain. The *sus1Δ* strain alone is viable, but exhibits slower growth at 23°C and 30°C and is temperature sensitive for growth at 37°C (Figure 1D). The *sus1Δ/yra1-ΔRRM* cells were not viable (Figure 1E). In contrast, another *YRA1*mutant allele, *yra1-ΔN*, is not synthetic lethal with *sus1Δ*, suggesting an allele-specific interaction (data not shown). To determine whether *SUS1* interacts genetically with other essential components of the conserved mRNA export machinery (*SUB2*, *MEX67*), pairwise combinations of mutant alleles were made in haploid strains. This analysis revealed that *sus1Δ* is synthetically lethal with *sub2-85* and *mex67-5* mutant alleles (Figure 1E).

Moreover, we performed a synthetic lethal screen with the *sus1Δ* strain to identify additional factors that interact with *SUS1*. This analysis identified two sl mutants that were complemented by *DBP5* or *NAB2* (data not shown). Both *DBP5* and *NAB2* are required for nuclear mRNA export (Hodge et al., 1999; Schmitt et al., 1999). Thus, *SUS1* interacts genetically with several key components of the mRNA export machinery.

SUS1 Is Required for Nuclear Poly (A)$^+$ RNA Export

The observation that *SUS1* interacts genetically with the mRNA export machinery suggests that Sus1 plays a role in mRNA export. To test this possibility, we carried out in situ poly (A)$^+$ RNA hybridization with *sus1Δ* cells. This analysis showed a significant defect in export of poly (A)$^+$ RNA after a 90 min shift to the restrictive temperature (Figure 2A). At semipermissive temperatures (e.g., 30°C), mRNA export is also impaired in the *sus1Δ* strain (Figure 2A). Moreover, mRNA export is defective in all three sl mutants, sl9, sl15, sl302 (Figure 2A and data not shown), and the mRNA export defect of the *sus1* sl mutants is complemented by the recombinant *SUS1* gene (Figure 2A). We conclude that *SUS1* is a newly identified component of the nuclear mRNA export machinery.

Sus1 Interacts Physically with the mRNA Export Factors Thp1 and Sac3 and the SAGA Complex

To identify proteins that associate with Sus1 in yeast, the *SUS1* gene was TAP-tagged at its 3′ end by homologous integration. The Sus1-TAP cells grow normally and express a fusion protein of 30 kDa (Figure 3A). Sus1-TAP was affinity-purified from a whole-cell lysate by two consecutive affinity columns (IgG-Sepharose and Calmodulin-Sepharose). After the second purification, ~20 proteins were specifically enriched (Figure 3A, lane 1). Unexpectedly, mass spectrometry revealed that most of these proteins are components of the SAGA complex, which functions in histone acetylation and is required for the expression of a subset of Pol II genes (Lee et al., 2000). The SAGA proteins identified in the Sus1-TAP eluate include Tra1, Spt3, Spt7, Spt8, Spt20, Ada1, Ada2, Ada3, Taf5, Taf6, Taf9, Taf10, Taf12, Gcn5, Sgf29, Sgf73, and Ubp8, which are essentially all of the reported SAGA components (Grant et al., 1997, 1998; Gavin et al., 2002; Sanders et al., 2002; Wu and Winston, 2002). Moreover, histones H3 and H2B, which are specifically modified by SAGA, and Cdc31 are present in the affinity-purified Sus1 preparation. The significance of Cdc31 association with Sus1 is currently under investigation (T.F., unpublished data).

Remarkably, two other proteins detected in the Sus1-TAP pulldown were identified as Thp1 and Sac3 (Figure 3A, lane 1). Both of these proteins are required for mRNA export and interact physically and genetically with the general mRNA export receptor Mex67 (Fischer et al., 2002; Gallardo et al., 2003; Lei et al., 2003). Moreover, Sac3-Thp1 require the nucleoporin Nup1p to dock at the nuclear site of the nuclear pore complex, a step that is crucial for nuclear mRNA export (Fischer et al., 2002).

Since the Sus1-TAP pulldown contains both transcription and export components, we sought to determine whether each of these types of components interacts separately with Sus1 or are in the same complex. First, we determined whether Sus1 is a specific component of the SAGA complex. Therefore, the SAGA subunits Ada2 and Taf6 were TAP-purified from yeast expressing myc-tagged Sus1 (Figure 3A, lanes 2 and 3). This analysis revealed that Sus1-myc together with the other SAGA proteins is associated with Ada2 and Taf6, respectively. We conclude that Sus1 is a component of the SAGA complex.

Next, we examined whether Sus1 is present in the purified Sac3-Thp1 complex. Previously, Sac3 was detected in a Thp1-TAP purification (Fischer et al., 2002;

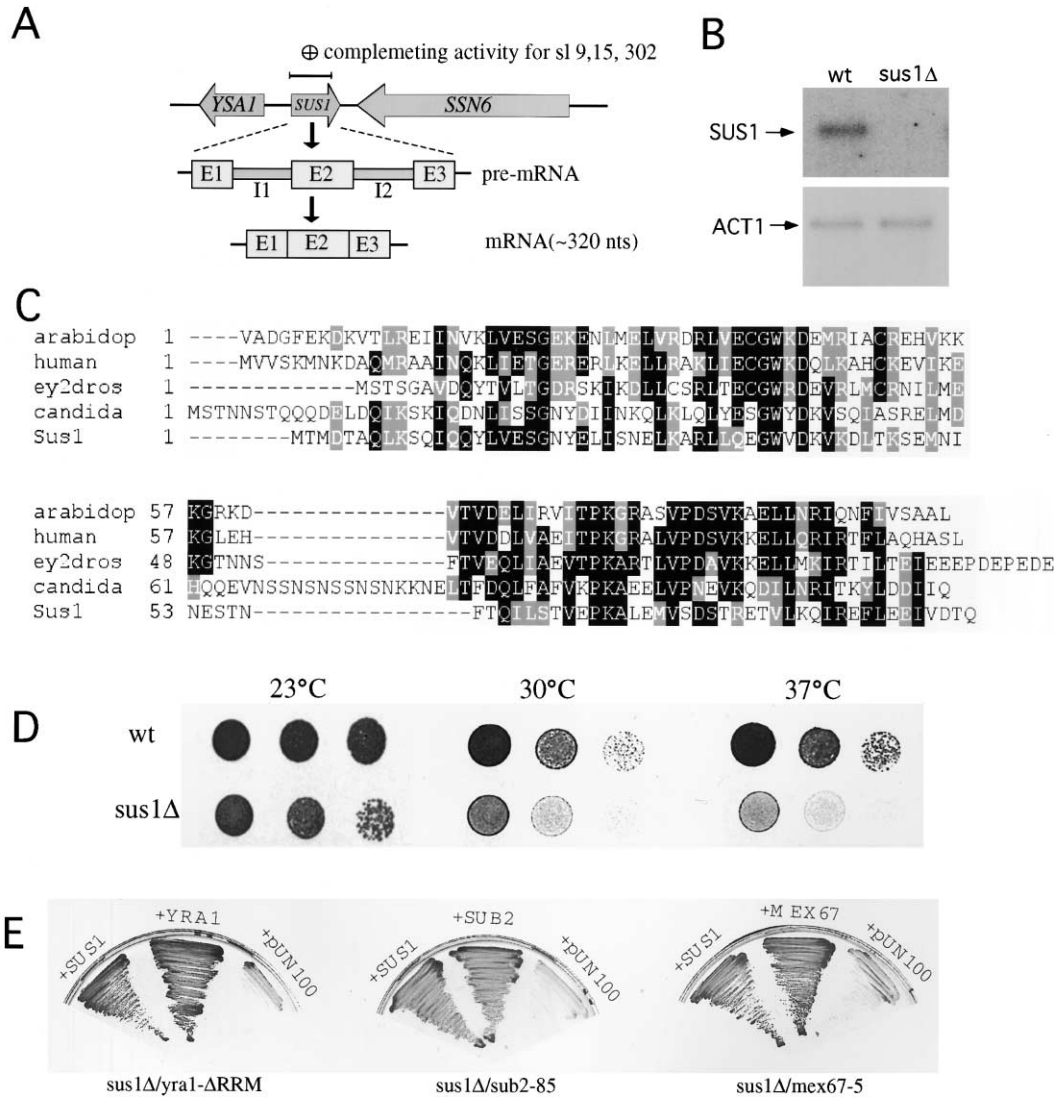

Figure 1. SUS1 Interacts Genetically with Genes Encoding Components of the Conserved mRNA Export Machinery

(A) A drawing of the *SUS1* gene locus on the "Watson strand" of chromosome 2 between the *YSA1* and *SSN6* genes. *SUS1* pre-mRNA consists of three exons (E1, E2, E3) and two introns (I1, I2), and is spliced to generate *SUS1* mRNA (E1-E2-E3).

(B) Detection of the *SUS1* mRNA by Northern analysis. Total RNA from wild-type and *sus1*Δ was analyzed by agarose-formaldehyde gel electrophoresis. The oligonucleotides used for the Northern were specific for either *SUS1* or actin (*ACT1*) mRNA. The *SUS1* RNA has an apparent size of approximately 320 nt.

(C) Amino acid sequence of Sus1 and alignment with orthologs from *Arabidopsis* (arabidop), human (human), *Drosophila* (ey2dros), and *Candida tropicalis* (candida).

(D) Growth of the *sus1*Δ and wild-type yeast cells. Cells were diluted in 10^{-1} steps, and equivalent amount of cells were spotted onto YPD plates. It was grown for 3 days at the temperatures indicated.

(E) Synthetic lethality of *sus1*Δ with *yra1-ΔRRM*, *sub2-85*, and *mex67-5*. Double shuffle strains *sus1*Δ/*yra1*Δ, *sus1*Δ/*sub2*Δ, and *sus1*Δ/*mex67*Δ were transformed with an empty plasmid (pUN100) or plasmids encoding *SUS1*, *YRA1*, *SUB2*, and *MEX67*, respectively. Transformants were streaked onto 5-FOA containing plates, which were incubated at 23°C for 5 days. No growth indicates synthetic lethality.

Gallardo et al., 2003). Sus1, however, may not have been detected in this purification due to its low molecular weight of ~10 kDa. Strikingly, Sus1 is readily detected as a ~42 kDa Coomassie-stainable band in a Thp1-TAP purification using a larger form of Sus1 tagged with a 13-mer tandem myc cassette (Figure 3B, lane 1). We conclude that Sus1 is also in a complex with Thp1 and Sac3.

The data so far suggested that Sus1 is associated with two complexes that function in mRNA export and transcription, respectively. This finding raises the possibility that the two complexes (SAGA and Sac3-Thp1) become physically connected to function in transcription-coupled mRNA export. Notably, low levels of the SAGA component Tra1 were previously detected in the Thp1 purification (Fischer et al., 2002). This prompted us to test whether purified Thp1 contains further SAGA components. As shown by Western analysis, Taf12, Spt20 (Figure 3B, lanes 1 and 2), and Ada2-myc (Figure 3C, lane 1) are all associated with Thp1. This interaction

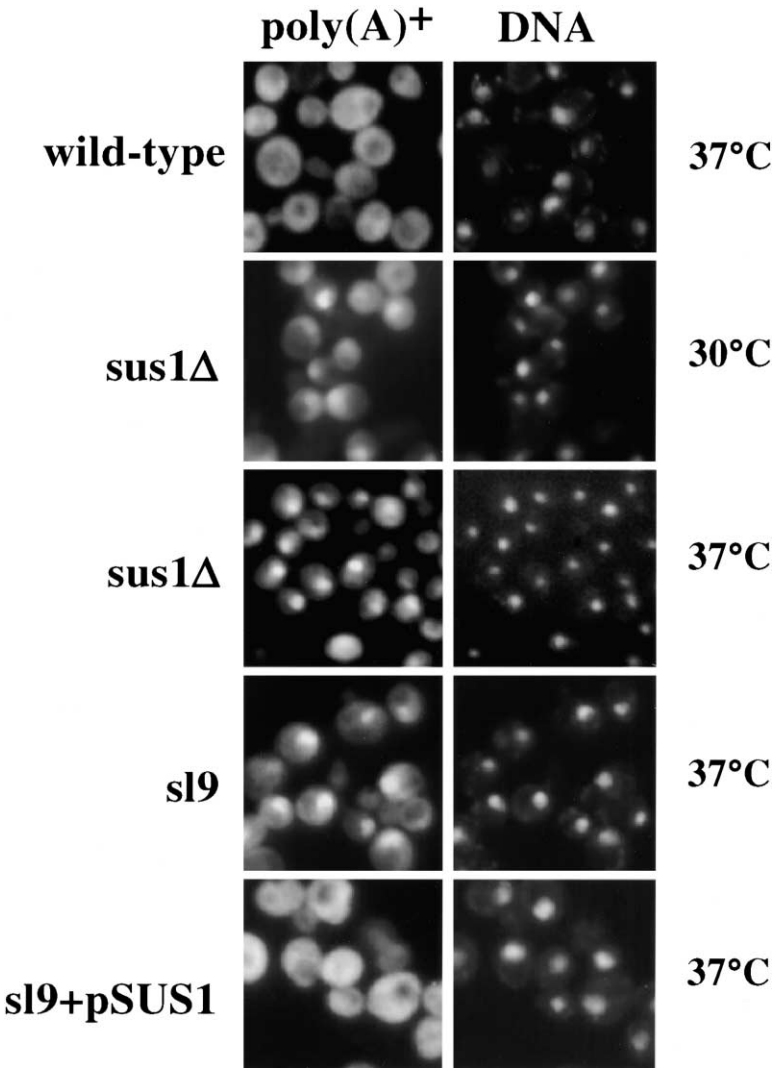

Figure 2. *sus1* Mutants Are Defective in mRNA Export

Wild-type, *sus1*Δ, and sl9 strains, transformed with an empty or *SUS1*-containing plasmid (pSUS1), were grown at 23°C shifted for 90 min to 37°C. *sus1*Δ cells were also analyzed at 30°C. The localization of poly $(A)^+$ RNA was assessed by in situ hybridization with a Cy3-labeled oligo(dT) probe. DNA was stained with DAPI.

is specific since TAP-purified nucleoporin Nup82 does not contain these SAGA components (Figure 3B, lanes 3 and 4). In a reciprocal experiment, when TAP-purified Ada2 was probed for the presence of Thp1-myc by Western, a significant signal for Thp1 was seen (Figure 3C, lane 3). Taken together, these data showed that SAGA components are present in the Sac3-Thp1-Sus1 complex, and vice versa Thp1 (Sac3 was not tested) is associated with a bona fide SAGA component, Ada2.

To demonstrate in another way that Thp1 coenriches with the SAGA complex, TAP-purified Sus1 was analyzed by gel filtration chromatography. As shown in Figure 4, a significant pool of Sus1 is soluble in yeast and found in fractions 19–21 of the Superose 6 column. Another fraction of Sus1 cofractionates with Thp1 and Sac3, which all co-peak in fractions 13–14 (~400 kDa). Consistent with its size of 1.8 MDa (Grant et al., 1997; Wu and Winston, 2002), SAGA elutes as a large complex from the column (fraction 9). Notably, Western analysis detects Thp1-myc and Sus1-CBP in this fraction as well (Figure 4). Thus, gel filtration confirmed that Sus1 exists in two assemblies, the Sac3-Thp1 and the SAGA complexes. Moreover, Thp1 can also be found in the SAGA complex. In light of these observations that mRNA export proteins can associate with the SAGA complex, we tested whether SAGA mutants are impaired in poly $(A)^+$ RNA export. However, no nuclear accumulation of mRNA was observed in *gcn5*, *ada2, ada3*, or *spt7* mutants (data not shown). Thus, bona fide SAGA components are not involved in mRNA export.

The observation that Sus1 is associated with a complex that has acetyltransferase activity prompted us to test for acetylated proteins in purifications that contain Sus1. To do this, TAP-purified complexes (Sus1, Thp1, Ada2, Taf6) were examined by Western analysis using an anti-acetyl-lysine antibody that recognizes proteins acetylated at lysine residues (e.g., histones, CBP, p53, and PCAF; see Experimental Procedures). This analysis suggests that Sac3 is acetylated both in the Thp1-TAP and Sus1-TAP preparations (Figure 5). In addition, Sus1-TAP and Ada2-TAP preparations contain other acetylated proteins on the Western blot, two of which could be Taf5 and Spt7 (Figure 5). In contrast, Thp1 and Sus1, or other prominent bands in the TAP preparations (e.g., Tra1) are not reactive with the anti-acetyl antibody (Figure 5). Peptide mass fingerprint analysis (MALDI-TOF)

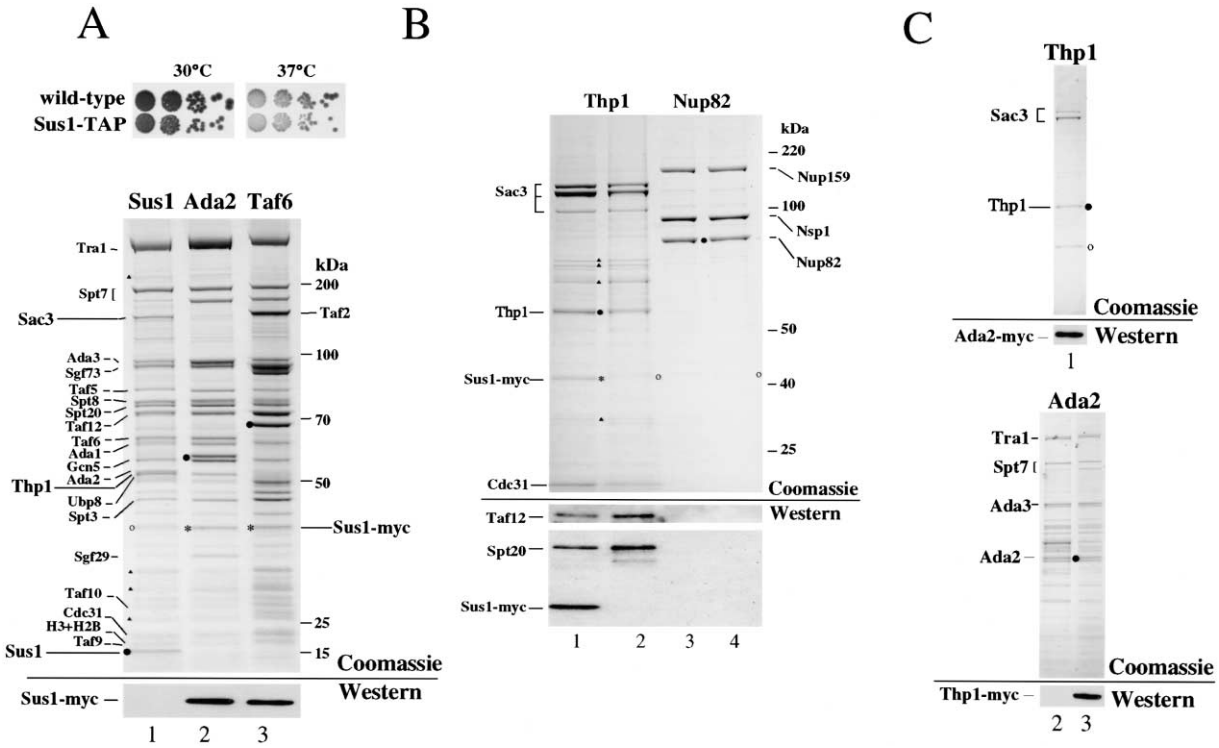

Figure 3. Sus1 Is Present Both in the SAGA Transcription Complex and the Sac3-Thp1 mRNA Export Complex

(A) Growth of *SUS-TAP* and wild-type cells, which were plated in 10^{-1} dilutions on YPD plates at the indicated temperatures (upper panel). TAP-tagged Sus1 (lane1), Ada2 (lane 2), and Taf 6 (lane 3) were isolated from yeast lysates by the TAP-method (middle pannel). The highly purified second EGTA eluates are shown. Strains Ada2-TAP and Taf6-TAP in addition contained a chromosomally modified *SUS1* gene, which was tagged with the 13 myc cassette at the 3′ end. Purified proteins were separated on a SDS 4%–12% gradient polyacrylamide gel and visualized by Coomassie staining (upper panel). The Sus1 protein migrates at approximately 15 kDa on the gel (note that Sus1 carries the approximately 5 kDa long CBP-tag). Copurifiying proteins were identified by mass spectrometry and are indicated. Marked with closed circles are the bait proteins (Sus1, Ada2, Taf6). The Sus1-myc band (marked with an asterisk) was verified by mass spectrometry. Please note that Sus1-myc comigrates with a bacterial porin contaminant derived from the calmodulin beads (marked by an open circle), whose intensity varies from preparation to preparation. Other bands identified by mass spectometry are most likely contaminants (indicated by triangles from top to botton: fatty-acyl-CoA synthase, Rps0, Rpl13, Rpl16). Western analysis of the TAP-purified Sus1, Ada2, and Taf6 using antibodies against myc (lower panel) was used.

(B) TAP-tagged Thp1 (lanes 1 and 2) and TAP-tagged Nup82 (lanes 3 and 4) were isolated from yeast expressing Sus1-13myc (lanes 1 and 3) or Sus1 (lanes 2 and 4) by two step affinity purification. Purified proteins were separated on an 4%–12% gradient gel and visualized by Coomassie staining (upper panel). The copurifiying protein band at approximately 40 kDa was identified by mass spectrometry to be Sus1-myc. Western analysis of the TAP-purified Thp1 (lanes 1 and 2) and Nup82 (lanes 3 and 4) using antibodies against the SAGA components Spt20 and Taf12 and anti-myc to detect Sus1 (lower panel). Other bands identified by mass spectometry are most likely contaminants (indicated by triangles from top to botton: Ssa2, Pab1, Keratin, Rpl10A).

(C) TAP-tagged Thp1 with chromosomally integrated Ada2-myc (lane 1), TAP-tagged Ada2 without (lane 2), and with integrated Thp1-myc (lane 3) were isolated from yeast by the tandem-affinity purification method. Western analysis (lower panel) using anti-myc antibodies reveals Ada2-myc (lane 1) and Thp1-myc (lane 3).

of Sac3 preparations revealed a peptide mass of 1471.81 Da that correlates with the mass of the tryptic Sac3 peptide EVVNSSVISIVKR (amino acids 737–749) carrying an acetyl group. Masses that correspond to the unmodified peptide or the peptide EVVNSSVISIVK were not detected in these preparations. These findings are consistent with the possibility that K748 of Sac3 is acetylated.

Sus1 Is Located Both in the Nucleoplasm and at the Nuclear Pores

The finding that Sus1 is in physical contact with the SAGA complex, which is distributed throughout the nucleus, and also with the nuclear pore-associated Sac3-Thp1 complex suggested that Sus1 may be present in both locations. To test this possibility, Sus1 was tagged at the C terminus with GFP by chromosomal integration. Sus1-GFP cells grow normally, indicating that the GFP tag does not impair the Sus1 function (Figure 6A). As shown in Figure 6B, Sus1-GFP does indeed have an intranuclear location with a concentration around the nuclear periphery. The pool of Sus1-GFP at the nuclear periphery appears to be connected to the nuclear pore complexes (NPCs) because Sus1-GFP co-clusters with NPCs in the pore-clustering *nup133*Δ mutant (Figure 6B). Interestingly, Sus1-GFP is no longer detected at the nuclear periphery in *sac3*Δ cells, suggesting that Sac3 is required for association of Sus1 with the nuclear pores (Figure 6B). We conclude that Sus1 via Sac3 can associate with the nuclear pores complexes.

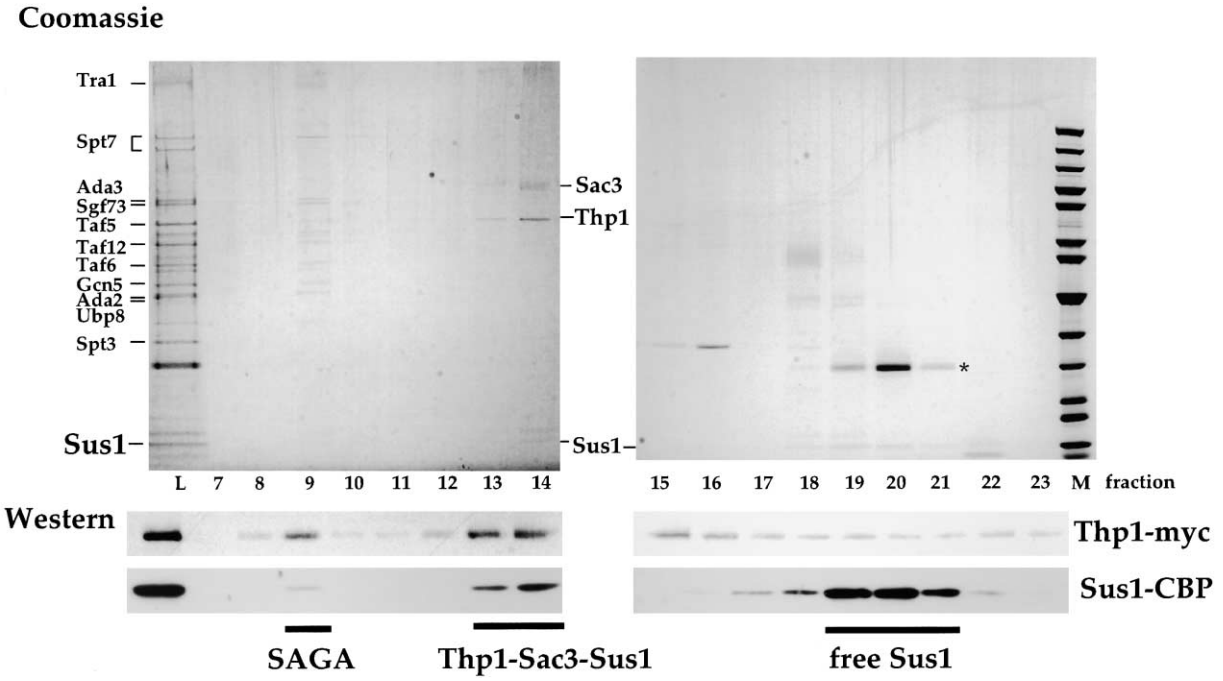

Figure 4. Separation of Sus1-Containing Complexes by Gel Filtration Chromatography

TAP-tagged Sus1 was TAP-purified from a yeast strain that carries integrated Thp1-myc. The final EGTA-eluate was concentrated by ultrafiltration and applied on a Superose 6 column. Fractions from the column were analyzed by SDS-PAGE and Coomassie staining, and Western blotting using anti-myc and anti-CBP antibodies to detect Thp1 and Sus1, respectively. Shown are also the EGTA-eluate input fraction (L) and a protein standard (M). Only the most relevant bands are indicated. The star (*) indicates the position of DNase.

Sus1 Plays a Role in Transcription Regulation

As shown above, Sus1 functions in mRNA export. To determine whether Sus1, like other SAGA components, plays a role in transcription regulation, we analyzed the expression profile of the ~6000 yeast genes by DNA macroarrays in the *sus1*Δ strain. In a recent genome-wide analysis, gene expression was found to be affected in several SAGA mutants (Lee et al., 2000). Expression of ~7.5% of the genes is altered (416 decreased and 35 increased) in *spt20*, 1.8% in *gcn5*, and 1.5% in *spt3* mutants (ChIP database; see http://staffa.wi.mit.edu/chipdb/public/index.html). Only a modest overlap is observed between genes that are repressed in several SAGA mutants (Lee et al., 2000; see also Table 1B). Our genome-wide analysis showed that expression of ~9% of yeast genes is altered (341 decreased and 208 increased) in the *sus1*Δ strain (Table 1A and Supplemental Table S2 at http://www.cell.com/cgi/content/full/116/1/75/DC1). As observed with other SAGA mutants, the overlap of genes whose expression is affected in both *sus1*Δ and SAGA mutants is moderate (Table 1B). Thus, our data show that Sus1, like other SAGA components, is involved in a complex regulation of a subset of yeast genes.

To confirm these findings, we analyzed *PHO84* transcript levels in *sus1*Δ cells by Northern analyses. DNA macroarray revealed that *PHO84* is the most significantly decreased transcript (~50-fold) in *sus1*Δ cells (see Supplemental Table S2 on *Cell* website). Consistent with these results, Northern analysis shows that *PHO84* transcript levels are dramatically reduced in *sus1*Δ cells, as well as in *gcn5*Δ strains (Figure 7A). The levels of *PGK1* mRNA, whose expression is not SAGA dependent, were not reduced in the *sus1*Δ and *gcn5* mutants (Figure 7A). We also analyzed the levels of *PHO84* transcripts in the *sac3*Δ and a bona fide mRNA export mutant, *mex67-5* (Segref et al., 1997). However, neither *mex67-5* nor *sac3*Δ cells exhibit decreased levels of *PHO84* mRNAs at permissive and restrictive temperatures (Figure 7A). Thus, the transcriptional defect observed in *sus1*Δ cells appears not to be due to impaired mRNA export.

To show that another SAGA-dependent promoter is controlled by Sus1, we analyzed *GAL1* transcript levels in *sus1*Δ cells by Northern. This analysis revealed that *GAL1* mRNA levels are decreased in *sus1*Δ, but not in *gcn5*Δ cells (Figure 7A). The observation that *GAL1* transcripts are not decreased in *gcn5*Δ cells is consistent with an earlier report, which showed that the SAGA-dependent *GAL1* promoter does not require Gcn5 for activation (Bhaumik and Green, 2001). We conclude that Sus1 plays a direct role in transcription of a subset of yeast genes.

Sus1 Is Recruited to the *GAL1* Promoter

To determine whether Sus1 is physically associated with SAGA-dependent genes, we carried out chromatin immunoprecipitation (ChIP) assays using a Sus1 myc-tagged strain and the SAGA-dependent *GAL1* promoter (Bhaumik and Green, 2001; Larschan and Winston, 2001). This analysis revealed that Sus1 is specifically recruited to the *GAL1* gene after induction with galactose (Figure 7B, lanes 1–3). A similar pattern of association with the *GAL1* promoter was observed for Ada2-

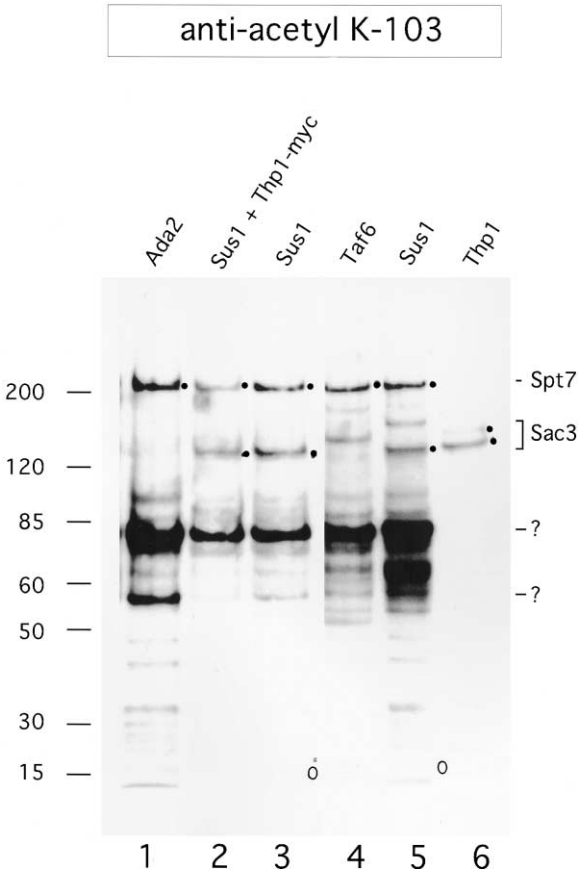

Figure 5. In Vivo Acetylation of Sac3

TAP-tagged Ada2 (lane 1), Sus1+Thp1-myc (lane 2), Sus1 (2 different preparations, lanes 3 and 5), Taf6 (lane 4), and Thp1 (lane 6) were isolated from yeast lysates by the TAP method. The highly purified second EGTA eluates were separated by SDS-PAGE (see Figure 3) and blotted onto nitrocellulose. The positions of Spt7 (closed circle), the Sac3-doublet band (closed circle), and Sus1 (open circle) were marked on the Ponceau-stained nitrocellulose membrane before Western analysis was performed using a monoclonal antibody against acetyl-lysine. Note that the marked Spt7 and Sac3 bands react with the anti-acetyl lysine antibody. The strongly acetylated bands, which are marked by question marks, were not identified.

myc (Figure 7B, lanes 4–6) and PolII (Figure 7B, lanes 7–9). To determine where Sus1 interacts along the gene, eight different regions of the *GAL1* gene were used for ChIP assays. As shown in Figure 7C, Sus1 is associated with the *GAL1* promoter region, but not with the middle and 3′ end of the gene. This association is characteristic of SAGA components (Larschan and Winston, 2001). Moreover, Sus1-myc is not recruited to the *PMA1* gene, whose expression is not dependent on the SAGA complex (data not shown). These results, together with the DNA macroarray (Table 1) and the biochemical data (Figures 3 and 4), indicate that Sus1 functions in transcription together with the SAGA complex.

Discussion

In this study, we report the identification of a protein Sus1 that is associated with the SAGA histone acetylase complex, a large intranuclear assembly that functions in transcription initiation, and with the Sac3-Thp1 complex, which has a nuclear pore location and a role in nuclear mRNA export. Consistent with the biochemical data, Sus1 shows a dual location in the nucleoplasm and at the nuclear pores. Moreover, Sus1 has roles both in transcription regulation and nuclear mRNA export. Thus, Sus1 could function in transcription-coupled mRNA export.

Although we cannot rule out that Sus1 has separate roles in transcription and mRNA export, we favor the model that Sus1 is a bridging factor that recruits the export machinery to active genes or vice versa at the earliest steps of gene expression. Experimental evidence for these possibilities comes from the isolation of a "supercomplex" that contains both SAGA components, Sus1 and Thp1 (Sac3 was not tested). We do not know how and when this supercomplex is formed in the cell. One possibility is that Sus1, which is present in the SAGA complex, recruits the Sac3-Thp1 complex to promoters upon SAGA-dependent gene activation. Subsequently, when the 5′ end of the pre-mRNA emerges from RNA polymerase II, the Sac3-Thp1-Sus1 complex would be in close proximity to nascent transcripts and thus could bind to them.

A role for Sus1 in transcription-coupled mRNA export could be also envisaged in a different way, in which actively transcribed (e.g., SAGA-regulated) genes are tethered to the nuclear pores via Sus1, which is both a subunit of the SAGA complex and the NPC-associated Sac3-Thp1 complex. We have previously shown that the Sac3-Thp1 complex functions at the nuclear basket in conjuction with Nup1 and Nup60 to recruit the mRNA export machinery to the inner site of the nuclear pore complex (Fischer et al., 2002). Interesting in this context is a recent study, which showed that chromatin boundary activities (BAs) that establish a nonsilenced domain within a gene locus are nuclear export receptors (Ishii et al., 2002). Strikingly, these export factors block spreading of heterochromatin by physical tethering of the gene locus to the nucleoporin Nup2, which is located at the nuclear basket. Thus, physical tethering of genomic loci to the nuclear basket of the NPC can alter gene expression. Moreover, another study showed that also desilencing activities (DAs) can induce euchromatic island formation that blocks spreading of heterochromatin. Interestingly, yeast Gcn5, a SAGA subunit, and many mammalian transcription factors can function as "DAs" (Ishii and Laemmli, 2003). Accordingly, transcription factors are suggested to perform a dual function, acting as transcription factor in the classical sense and functioning as blockers/desilencers of heterochromatin.

According to these models discussed above, active genes may be physically tethered to the nuclear pore complex via chromatin bound SAGA complexes, which interact with the Sac3-Thp1 complex. This complex could be bound to the inner site of the nuclear pore complex via the nuclear basket protein Nup1. This tethering would ultimately bring nascent transcripts into the vicinity of the NPC-associated mRNA export machinery. In this way, transcription-coupled mRNA export would be established directly at the nuclear pores and thereby specifically target these transcripts for export. It is conceivable that regulated (e.g., cell cycle) mRNAs or lower

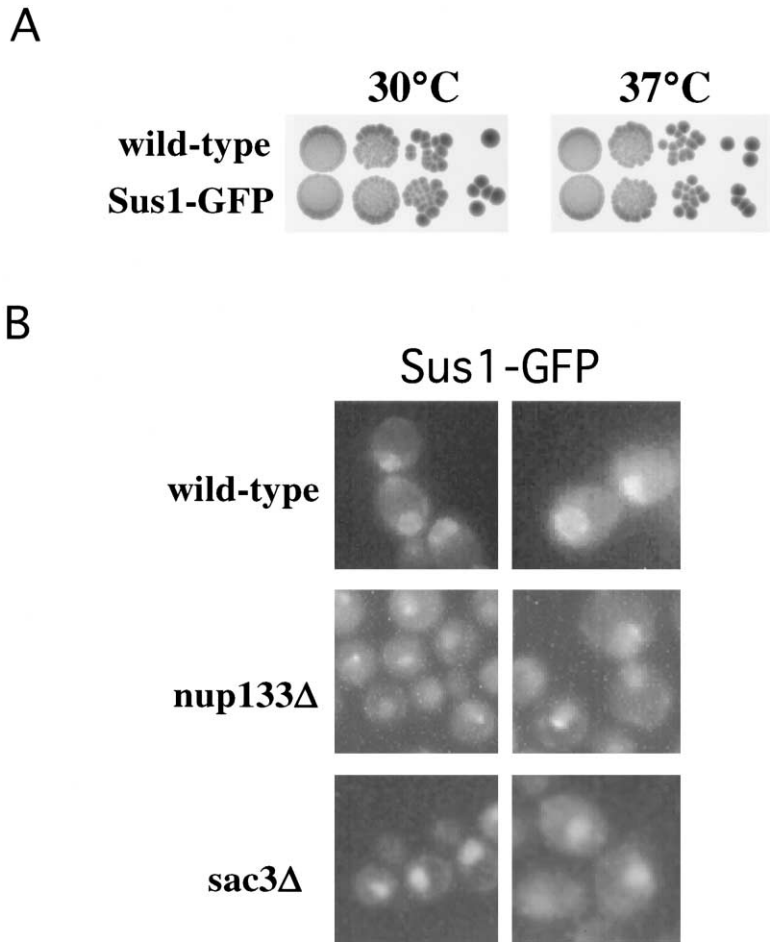

Figure 6. Sus1 Is Localized in the Nucleus and at the Nuclear Pores

SUS-GFP cells expressing Sus1-GPF were grown in 10^{-1} dilutions on YPD plates at 30°C and 37°C (A) and analyzed in the fluorescence microscope for subcellular protein location (B). The in vivo location of the chromosomally integrated Sus1-GFP is shown for wild-type cells, the *sac3*Δ, and *nup133*Δ strains. The *nup133*Δ strain is known to have a NPC-clustering phenotype (Doye et al., 1994).

abundance transcripts may use this export route to effectively compete with transcripts derived from highly or constitutively expressed genes, which would be expressed inside the nucleus. In support of this possibility, *SAC3* is functionally linked to the cell cycle (Bauer and Kölling, 1996) and cell cycle genes (e.g., *CDC28* and *CDC23;* Jones et al., 2000).

How the various steps in Sac3-Thp1-Sus1-mediated

Table 1. Genes Repressed or Induced in *sus1*Δ and SAGA Mutants

A

Mutant	sus1	spt20	spt3	gcn5	taf9	taf6	taf12	taf5
Number of genes increased	208	35	8	11	22	42	51	164
Number of genes decreased	341	416	79	108	1050	1574	661	269
% of genes regulated	9	7.5	1.5	1.8	18	26	12	7.2

Number of genes, whose expression is increased or decreased in the *sus1*Δ strain (see Supplemental Table S2 on *Cell* website) and in several SAGA mutants. Except for sus1Δ, the numbers were obtained from the ChipDB (http://staffa.wi.mit.edu/chipdb/public/index.html).

B

Mutant	Overlapping Genes
spt3+gcn5	13
spt3+spt20	45
spt20+gcn5	26
sus1+spt3	9
sus1+gcn5	17
sus1+spt20	34
spt3+spt20+gcn5	8
sus1+spt20+spt3+gcn5	3

Overlap between genes, which are commonly repressed in *sus1*Δ and several SAGA mutants. Numbers were obtained by using the "compare set of genes" tool from http://staffa.wi.mit.edu/chipdb/public/index.html for *spt3*, *spt20*, and *gcn5* mutants and comparing them with our *sus1*Δ DNA macroarray data (for a detailed list, see Supplemental Table S2 online).

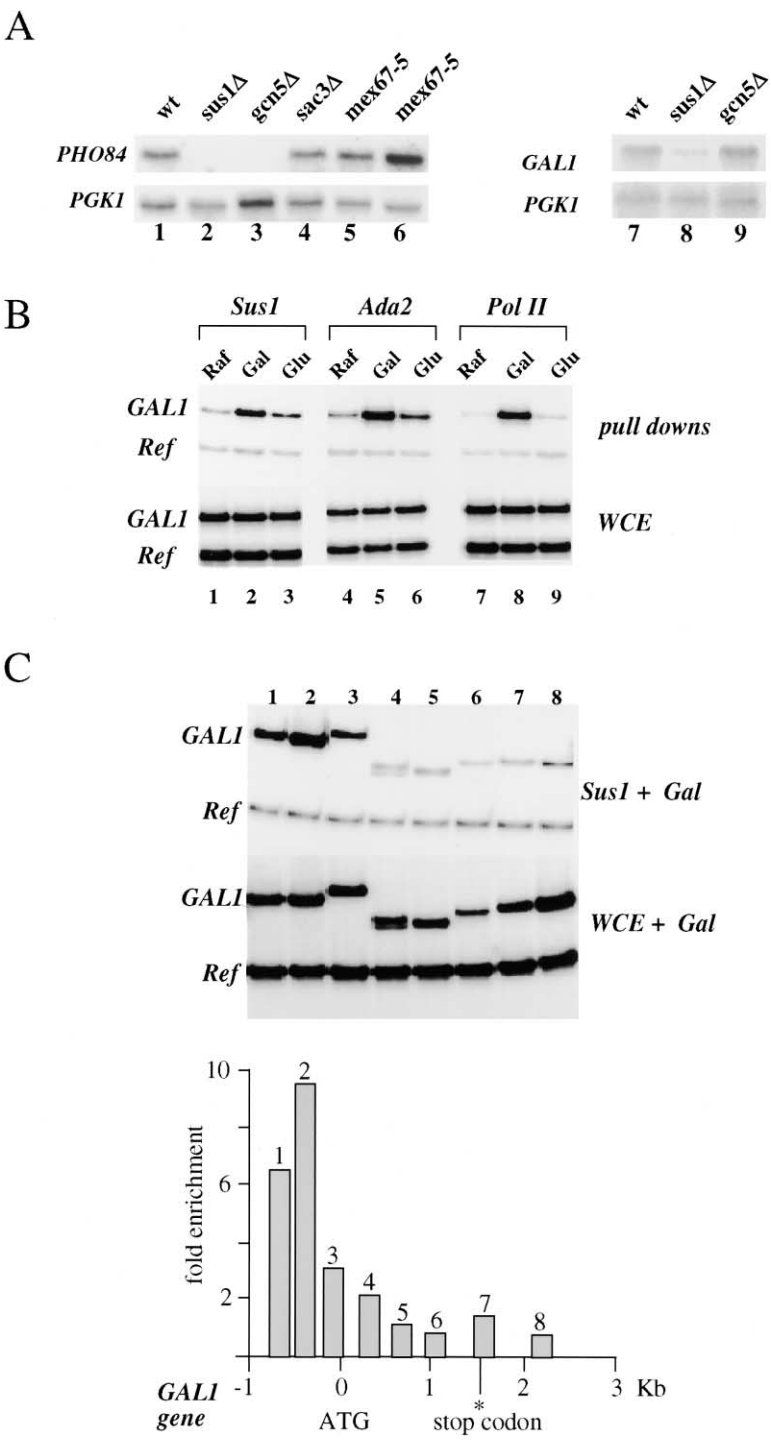

Figure 7. Sus1 Is Required for Gene Expression

(A) Northern analysis of total RNA to detect *PHO84* (lanes 1-6) and *GAL1* transcripts (lanes 7-9) in *sus1Δ*, *gcn5Δ*, *sac3Δ*, *mex67-5*, and wild-type cells in comparison to the *PGK1* signal. RNA loading was also controlled by rRNA levels (data not shown). Cells were grown at 30°C, except for *mex67-5*, which was grown at 23°C (lane 5) or shifted for 1 hr to 37°C (lane 6).

(B) Sus1 associates with the *GAL1* promoter upon transcription activation. Cells containing Sus1-myc or Ada2-myc were grown in raffinose (lanes 1, 4, 7), raffinose followed by 3 hr galactose induction (lanes 2, 5, 8), or after galactose induction followed by 30 min of glucose incubation (lanes 3, 6, 9). Chromatin immunoprecipitations were then carried out with anti-myc or anti-Pol II antibodies, and PCR analysis was performed using the indicated primer sets (see Experimental Procedures).

(C) Sus1 specifically associates with the *GAL1* promoter region. Cells containing Sus1-myc were grown in raffinose followed by 3 hr of galactose induction. Chromatin immunoprecipitations were then carried out with a anti-myc antibody and PCR was performed with the indicated primers. Immunoprecipitations were quantified and displayed as the fold enrichment of Sus1 association with the *GAL1* gene relative to that with an intergenic region.

nuclear mRNA export are controlled is not known. Since the data suggest that Sac3 is acetylated, acetylation/deacetylation could be one means to regulate association of Sac3-Thp1-Sus1 with the nuclear envelope and/or supercomplex formation with the SAGA complex. Significantly, the human Sac3 homolog MCM3AP contains an acetyltransferase motif in the C domain, which is typically found in the superfamily of Gcn5-related N-acetyltransferases (Takei et al., 2001). Yeast Sac3 does not have such a motif, and the Sac3 acetylases remain to be identified.

Ubiquitinylation could affect Sac3-Thp1-Sus1-dependent mRNA export. A role of the ubiquitin ligase Tom1 in SAGA-dependent transcription (ubiquitination of Spt7) and mRNA export has been already reported (Saleh et al., 1998; Duncan et al., 2000). Notably, Tom1 is involved in nuclear mRNA export of a subset of mRNA transcripts, which associate with the mRNP protein Nab2 (Duncan et al., 2000; Green et al., 2002). In our studies, we have found a synthetic lethal interaction between *SUS1* and *NAB2*, and a genetic interaction between *NAB2*, *SAC3*, and *THP1* has also been reported (Gallardo et al., 2003). Thus, Tom1-dependent ubiquitination could coordinate

SAGA-dependent transcription and Sus1- Sac3-Thp1-dependent mRNA export.

Our data also show that Sus1 interacts genetically with the essential mRNA export receptor Mex67-Mtr2 and the coupling proteins Yra1 and Sub2. However, these export factors do not appear to interact physically with Sus1. Thus, it remains unclear how Sus1-dependent mRNA export is linked to the conserved mRNA export machinery. In previous studies, we identified a complex designated TREX that couples mRNA export to transcription elongation. The TREX complex contains Yra1 and Sub2, as well as the THO complex (Tho2, Hpr1, Mft1, Thp2), which functions in transcription elongation (Strässer et al., 2002). ChIP analyses revealed that the TREX complex is recruited to the middle and 3′ part of the gene, but not to the promoter (Lei et al., 2001; Lei and Silver, 2002; Strässer et al., 2002; Zenklusen et al., 2002). In contrast, Sus1 is specifically associated with a transcription complex located at the promoter, and ChIP analysis shows that Sus1 is associated with the promoter but not the middle and 3′ part of the gene. This reciprocal association of Sus1 and TREX raises the possibility that Sus1 is involved in recruiting the export components Sac3 and Thp1 during transcription activation, whereas TREX is involved in recruitment of Yra1 and Sub2 during transcription elongation. Thus, all of the export components that are recruited during transcription may associate in an mRNP that is targeted to the nuclear pore complex. Whether these are different or identical transcripts remains to be shown.

The function of Sus1 is likely to be conserved. Among the SAGA components associated with Sus1 is the histone-like Taf9. The *Drosphila* Sus1 homolog e(y)2 is a ubiquitous transcription factor that interacts with the counterpart of yeast Taf9 in a large complex (Georgieva et al., 2001). Future work will show whether Sus1 homologs in metazoans function in transcription-coupled mRNA export.

Experimental Procedures

Yeast Analysis

The *yra1*Δ *ade2 ade3* strain used in the sl screen was transformed with the *ARS/CEN-TRP1* plasmid pRS314 that contained both the *yra1-*Δ*RRM* allele and the *SUB2* wild-type gene. The rationale of having an increased *SUB2* gene dosage was to counterselect for sl mutants that are complemented or suppressed by *SUB2*. The sl screen was performed as previously described (Strässer et al., 2002). The *sus1* knockout strain (*sus1*Δ) was generated by disrupting with a kanamycin cassette the intergenic region (in total 550 nt) between *YSA1* (150 nt 5′of the ATG start codon) and *SSN6* (300 nt 3′ of the stop codon) by homologous recombination. The *gcn5*Δ, *spt7*Δ, *ada2*Δ, and *ada3*Δ strains were received from EUROSCARF. *SUS1* was cloned by PCR amplification of the *SUS1* gene locus from chromosomal DNA creating a NotI site 150 nt 5′ upstream of the *SUS1* start codon and a XhoI site 180 nt 3′ downstream of the stop codon. For identification of mutations in the 3 sl mutants, DNA sequencing revealed that sl9 has a point mutation in the branchpoint of the first intron (TACTGAC to CACTGAC), whereas sl15 and sl302 have point mutations, which generate premature stop codons (S74→stop, S48→stop, respectively). *SUS1* cDNA was retrotranscribed from total yeast RNA using appropriate primers. The obtained cDNA was amplified by PCR, cloned into the pCR2.1-TOPO vector (Invitrogen). The GenBank accession number of *SUS1* is AY278445.

Plasmids pUN100-*YRA1*, pUN100-*MEX67,* pRS314-*SUB2*, pRS314-*yra1-*Δ*RRM*, pRS314-*mex67-5*, and pRS314-*sub2-85* were described previously (Strässer et al., 2002). The TAP-tag, GFP-tag, or 13myc tag were chromosomally integrated at the 3′ end (C-terminal tagging) of the *SUS1, THP1, ADA2, TAF6*, and *NUP82* genes by homologous recombination (Rigaut et al., 1999; Fischer et al., 2002). The synthetic lethal screens with strains *yra1-*Δ*RRM* and *sus1*Δ, oligo(dT), in situ hybridization, and GFP localization were performed as described (Strässer and Hurt, 2000).

Protein Purification and Mass Spectrometry

TAP-tagged proteins were purified essentially as described (Rigaut et al., 1999). Mass spectrometry was performed as described (Baßler et al., 2001). Gel filtration analysis was performed on an Ettan LC (Amersham Biosciences) using a Superose 6 column. For gel filtration separation, Sus1-TAP was affinity-purified as described above, and after elution with EGTA from the calmodulin beads, it was treated with 20 units/ml DNase I (Rnase free). Western analysis was performed using polyclonal anti-Spt20, anti-Taf12, and anti-myc antibodies.

DNA Macroarray, Northern, and Western Analyses

DNA macroarray construction will be described elsewhere (J.E.P.-O., personal communication). They were used as previously described (Hauser et al., 1998). Total RNA from logarithmically growing *sus1*Δ and isogenic wild-type cultures (for each strain three independent experiments were performed) was retrotranscribed into cDNA using ^{33}P-dCTP. Image and statistical analyses were done, respectively, with *ArrayVision* and *ArrayStat* softwares. A detailed description of the protocols is given in Supplemental Data on the *Cell* website. Northern analysis was performed as described (Rodríguez-Navarro et al., 2002). Western analysis using acetylated-lysine monoclonal antibody (AC-K-103) was performed as described by the instruction of the company (Cell Signaling Technology, www.cellsignal.com).

Chromatin Immunoprecipitation

Cells containing Sus1-myc or Ada2-myc were grown in SC-trp medium containing 2% raffinose prior to 2% galactose or 4% glucose treatments as indicated. Forty milliliters culture were treated with 1% formaldehyde and subjected to immunoprecipitation and PCR analysis essentially as described (Kuras and Struhl, 1999; Strässer et al., 2002), except that 15% of each whole-cell extract (WCE) was used for immunoprecipitation with polyclonal myc antibodies (Upstate Biotechnology), and 5% of each WCE was used for immunoprecipitation with anti-Rpb1 antibodies (8WG16, Covance). The *GAL1* primers used in Figure 7B amplify *GAL1* promoter region #2 indicated in Figure 7C. The reference primers amplify an intergenic region around nt11000 on chromosome V. Other primers used for the *GAL1* gene are also indicated.

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Accession Numbers

The *SUS1* sequences have been deposited in GenBank with the accession number AY278445.