Actively Transcribed GAL Genes Can Be Physically Linked to the Nuclear Pore by the SAGA Chromatin Modifying Complex

Recent work has demonstrated that some actively transcribed genes closely associate with nuclear pore complexes (NPC) at the nuclear periphery. The Saccharomyces cerevisiae Mlp1 and Mlp2 proteins are components of the inner nuclear basket of the nuclear pore that mediate interactions with these active genes. To investigate the physical link between the NPC and active loci, we identified proteins that interact with the carboxyl-terminal globular domain of Mlp1 by tandem affinity purification coupled with mass spectrometry. This analysis led to the identification of several components of the Spt-Ada-Gcn5-acetyltransferase (SAGA) histone acetyltransferase complex, Gcn5, Ada2, and Spt7. We utilized co-immunoprecipitation and in vitro binding assays to confirm the interaction between the Mlp proteins and SAGA components. Chromatin immunoprecipitation experiments revealed that Mlp1 and SAGA components associate with the same region of the GAL promoters. Critically, this Mlp-promoter interaction depends on the integrity of the SAGA complex. These results identify a physical association between SAGA and the NPC, and support previous results that relied upon visualization of GAL loci at the nuclear periphery by microscopy (Cabal, G. G. Genovesio, A., Rodriguez-Navarro, S., Zimmer, C., Gadol, O., Lesne, A., Buc, H., Feuerbach-Fournier, F., Olivo-Marín, J.-C., Hurt, E. C., and Nehrbass, U. (2006) Nature 441, 770–773). We propose that a physical interaction between nuclear pore components and the SAGA complex can link the actively transcribed GAL genes to the nuclear pore.

Chromatin in the interphase nucleus is highly organized into discrete chromosome territories (1, 2), and the position of a particular genetic locus both within a chromosome territory and within the nucleus can influence its transcriptional state (3, 4). In the yeast Saccharomyces cerevisiae, specific genetic loci physically relocates from the nuclear interior to the nuclear periphery upon transcriptional activation (5–10). These transcribed genes interact with components of the nuclear pore complex (NPC)4 (5, 7–10), perhaps facilitating efficient mRNA processing and export. Although this phenomenon of locus association with the nuclear periphery has been reproducibly observed in yeast (5–10), critical questions remain unanswered regarding the mechanism of locus recruitment to the NPC. Transcription factors, chromatin modifying complexes, and the transcription machinery itself have each been independently implicated in targeting active loci to the NPC (8–12), perhaps suggesting a recruitment mechanism dependent upon transcription initiation. In addition, some interactions between components of the NPC and active loci are RNA-dependent (7), and mRNA processing and export factors have also been implicated in tethering loci to the NPC (9, 13, 14), suggesting that the interaction between active genes and the NPC may instead be dependent upon ongoing transcription and mRNA maturation. Recent, independent studies of GAL and HXX1 recruitment have yielded different results regarding the role of the transcription machinery and transcriptional co-activators in this process (8–10), raising the possibility that distinct mechanisms of recruitment may operate for individual loci. Notably, one caveat of these studies is the predominant use of microscopy to assess locus recruitment to the nuclear periphery, without biochemical confirmation of a physical association between a recruited locus and the NPC.

To further characterize the mechanism of gene recruitment to the NPC, we sought to identify proteins that physically interact with NPC components that had been linked to actively transcribed loci. These components of the NPC include the Mlp proteins, Nic96, Nup1, Nup2, Nup60, and Nup116 (5, 7, 9). We focused on the two Mlp proteins as they are localized to the nuclear basket of the NPC (15), have roles in mRNA processing and export (16–18), and have been implicated in the recruitment of multiple and diverse genes including the GAL genes, α factor-responsive genes, and many other genes that are highly

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4 The abbreviations used are: NPC, nuclear pore complex; ChIP, chromatin immunoprecipitation; SAGA, Spt-Ada-Gcn5-acetyltransferase; TAP, tandem affinity purification; UAS, upstream activating sequence; GFP, green fluorescent protein; PMSF, phenylmethylsulfonyl fluoride; GST, glutathione S-transferase; PBS, phosphate-buffered saline; qRT, quantitative real time PCR; TBP, TATA-binding protein; PAP, peroxidase anti-peroxidase.
SAGA Links Actively Transcribed GAL Genes to the NPC

TABLE 1

| Strain           | Description                                      | Ref./Source |
|------------------|--------------------------------------------------|-------------|
| FY23 (ACY192)    | MATα ura3–52 leu2Δ1 trp1Δ63                      | 48          |
| MLP1-TAP (ACY984)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 MLP1-TAP::HIS3 | Open biosystems |
| MLP2-TAP (ACY983)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 MLP2-TAP::HIS3 | Open biosystems |
| ADA2-TAP (ACY1016)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 ADA2-TAP::HIS3 | Open biosystems |
| GCN5-TAP (ACY1017)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 GCN5-TAP::HIS3 | Open biosystems |
| TBP-TAP (ACY1131)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 SPT15-TAP::HIS3| Open biosystems |
| ACY1121 (MLP1-TAP gcn5Δ)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 MLP1-TAP::HIS3 gcn5::KAN | This study |
| ACY1205 (MLP1-TAP sp7Δ)| MATα his3Δ1 leu230 met15Δ0 ura3Δ0 MLP1-TAP::HIS3 sp7Δ::KAN | This study |

Plasmids

| Description                  | Ref./Source |
|------------------------------|-------------|
| pAC1656                      | pGALI-TAP, 2µ, TRP1, AMPβ | This study |
| pAC1657                      | pGALI-CT-MLP1-TAP, 2µ, TRP1, AMPβ | This study |
| pPS892 (pAC403)              | pGALI-GST, 2µ, URA3, AMPβ | 49 |
| pAC2069                      | pGALI-GST-CT-MLP1, 2µ, URA3, AMPβ | This study |
| pAC1660                      | pGALI-GST-CT-MLP2, 2µ, URA3, AMPβ | This study |
| pAC2256                      | GCN5::GFP, 2µ, URA3, AMPβ | This study |
| pGEXX-3 (pAC736)             | GST, AMPβ, bacterial expression vector | Amersham |
| pAC1430                      | GST-CT-MLP1, AMPβ | 16 |
| pAC1682                      | GST-CT-MLP2, AMPβ | This study |
| pET28a (pAC762)              | HIS, KANβ bacterial expression vector | Novagen |
| pAC1781                      | HIS-TCN5, KANβ | This study |
| pAC1851                      | GST-ADA2, AMPβ | This study |

Strains, Plasmids, and Chemicals—All DNA manipulations were performed according to standard methods (28) and all media were prepared by standard procedures (29). All yeast strains and plasmids used are described in Table 1. All chemicals were obtained from Ambion (Austin, TX), Sigma, U. S. Biologicals (Swampscott, MA), or Fisher Scientific, unless otherwise noted.

**EXPERIMENTAL PROCEDURES**

**Strains, Plasmids, and Chemicals**—All DNA manipulations were performed according to standard methods (28) and all media were prepared by standard procedures (29). All yeast strains and plasmids used are described in Table 1. All chemicals were obtained from Ambion (Austin, TX), Sigma, U. S. Biologicals (Swampscott, MA), or Fisher Scientific, unless otherwise noted.

**Tandem Affinity Purification of Proteins**—The COOH-terminal globular domain of Mlp1 as a tandem affinity purification (TAP)-tagged protein in yeast, purified the associated proteins, and identified these proteins by mass spectroscopy. With this approach, we identified three components of the evolutionarily conserved SAGA complex, a histone acetyltransferase complex that regulates transcription of ~10% of the yeast genome (21, 22), including the GAL genes (23–25). SAGA interacts with genes specific transcriptional activators that recruit SAGA and additional transcription machinery to the promoters of target genes (26). In the case of the GAL genes, SAGA is recruited to the GAL upstream activating sequence (UAS) by the constitutively bound Gal4 protein when cells are grown in the absence of glucose and presence of galactose (27). The SAGA complex then facilitates recruitment of the general transcription factors and RNA polymerase II (27). As the SAGA complex regulates transcription of the GAL genes (23–25), and the GAL genes relocate to the nuclear periphery upon transcriptional activation (5), we hypothesized that a physical interaction between Mlp proteins and the SAGA complex might mediate the recruitment of the GAL genes to the NPC. We verified the interaction between the Mlp proteins and SAGA components, and we found that both Mlp1 and Mlp2 interact with the GAL UAS, the region of the GAL genes that interacts with the SAGA complex. This interaction between the Mlp proteins and the GAL UAS only occurs in the presence of galactose, when the GAL genes are active, and depends upon the integrity of the SAGA complex. These results suggest that the SAGA histone acetyltransferase complex at the GAL UAS may help to direct active GAL genes to the NPC, lending support to a model where chromatin modifying complexes facilitate interactions between the NPC and actively transcribed genes.
in the supernatant fraction was dialyzed (4 h, 4 °C) against dialysis buffer (20 mM K-HEPES, pH 8.0, 50 mM KCl, 0.2 mM EDTA, pH 8.0, 0.5 mM dithiothreitol, 0.5 mM PMSF, 2 mM benzamidine, 20% glycerol). This clarified lysate was used to bind TAP-tagged proteins to IgG-Sepharose beads (Amersham Biosciences). Beads were incubated with clarified lysate for 3 h at 4 °C, and TEV cleavage was performed with 30 units of TEV for 4 h at 4 °C followed by 1 h at 18 °C. Subsequent elution and purification was performed as described (31). Mass spectrometry identification was performed by the Emory University Microchemical and Proteomics Core Facility.

Co-purification of GST Fusion Proteins—Yeast cells were transformed with plasmids expressing fusion proteins (GST-CT-Mlp1 and GST-CT-Mlp2), or control vector (GST alone). Cultures were grown to log phase on 2% galactose minimal media and then harvested by centrifugation at 3000 × g. Cells were lysed in PBSMT (PBS, 5 mM MgCl2, 0.5% Triton X-100, pH 7.4) supplemented with protease inhibitor mixture (1 mM PMSF, 3 ng/ml pepstatin A, leupeptin, aproitin, and chymostatin). GST-tagged proteins were purified from protein extracts by incubation overnight at 4 °C with glutathione beads (Amersham Biosciences). The bound fraction was washed 3 times and eluted from beads with loading buffer (125 mM Tris-HCl, pH 6.8, 250 mM dithiothreitol, 5% SDS, 0.25% bromphenol blue, 25% glycerol). Lysate (25 μg of protein) and bound fractions were resolved by SDS-PAGE and analyzed by immunoblotting according to standard procedures (28). TAP-tagged proteins were detected with PAP antibody (Sigma). For experiments where nuclelease treatment was used, lysate prepared from a common culture was divided into four aliquots and aliquots were treated with RNase (10 units/ml), DNase (10 units/ml), both nucleases, or left untreated (control) for 10 min at 37 °C followed by incubation at 4 °C for 50 min.

Purification of Recombinant Proteins—Cell lysate preparation and the purification of recombinant proteins were performed as recommended by the resin manufacturer (Amersham Biosciences). Briefly, recombinant proteins were expressed in BL21 Escherichia coli by standard isopropyl 1-thio-β-d-galactopyranoside induction, and lysate was prepared from log phase cultures. GST and GST fusion proteins were purified from lysate by affinity chromatography on glutathione-Sepharose. His6-tagged Gcn5 (His-Gcn5) was purified with nickel-nitriiotriacetic acid-Sepharose.

In Vitro Binding Assay—For in vitro binding assays, 5 μg of GST or GST-fused protein was bound to glutathione-Sepharose in PBS buffer for 30 min at 25 °C. After three washes with 1 ml of PBS buffer, 1 μg of purified, recombinant His-Gcn5 fusion protein was added to a volume of 1 ml of Buffer A (20 mM Tris-HCl, pH 8.0, 0.5% Triton X-100). The mixtures were then incubated for 1 h at 4 °C. Unbound fractions were collected and the beads were washed three times with 1 ml of Buffer A containing 300 mM NaCl. The bound fraction was eluted by incubation with SDS sample buffer for 5 min at 95 °C. Bound and unbound fractions were separated by 12% SDS-PAGE followed by immunoblotting with anti-GST (Sigma) or anti-His (Santa Cruz) antibodies.

ChIP Analysis—ChIP was performed essentially as described (32). Briefly, 100-ml cultures were grown to log phase (A600 ~0.8–0.9). Formaldehyde was added to a final concentration of 1% for 20 min. Cross-linking was quenched by addition of 270 mM glycine for 10 min. Cells were washed twice with chilled TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) and once with lysis buffer (50 mM HEPES-KOH, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 0.1% SDS, 10% glycerol, 1 mM dithiothreitol, protease inhibitors mixture (0.2 mM PMSF, 1 mM benzamidine, 1 μg/ml pepstatin A)). The pellet was resuspended in lysis buffer and cells were lysed using glass beads. Chromatin was collected in the supernatant fraction and sheared by sonication to ~200-bp fragments. The chromatin solution was bound to prewashed, IgG beads for 2 h at room temperature. Immunoprecipitated chromatin was eluted from beads by heating for 10 min at 65 °C in elution buffer (25 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.5% SDS). To reverse cross-linking, samples were incubated with proteinase K for 1 h at 42 °C followed by overnight incubation at 65 °C. Samples were purified using Qiagen columns and analyzed by quantitative PCR using gene-specific primer pairs. The fraction of immunoprecipitated material for a specific fragment was calculated by dividing the amount of PCR product obtained from immunoprecipitated DNA by the amount obtained from total DNA (IP/Input).

RNA Isolation—Cells grown in 10-ml cultures of glucose or galactose were pelleted and washed twice with chilled diethyl pyrocarbonate water. Pellets were resuspended in 200 μl of LET buffer (100 mM LiCl, 25 mM Tris, pH 8, 20 mM EDTA), and then 200 μl of phenol and 100 μl of glass beads were added to the resuspension. Cells were subjected to a brief heat shock at 65 °C and lysed by bead beating for 4 min with 2-min intervals on ice. Debris was removed by brief centrifugation. The aqueous layer was transferred to a fresh tube containing 200 μl of phenol, vortexed, and centrifuged for 5 min. RNA was isolated from the aqueous layer by chloroform extraction and was precipitated at −80 °C for 30 min using 40 μl of 3× NaOAc and 1 ml of EtOH. The RNA pellet was collected by spinning the mixture at 12,000 × g for 10 min. The pellet was washed with 70% EtOH and dried on ice, then resuspended in RNase-free water for further analysis.

RT-PCR—Synthesis of cDNA from samples grown in glucose and galactose was performed with an Invitrogen SuperScript III Reverse Transcriptase kit. Amplification reactions to detect relative cDNA levels included 1 μl of cDNA and 300 nm gene-specific forward and reverse primers. Samples were amplified with 35 cycles of denaturation (95 °C, 30 s), annealing (60 °C, 30 s), and extension (72 °C, 1 min). Each PCR was performed in triplicate and products were analyzed by agarose gel electrophoresis.

Quantitative Real Time PCR—GAL transcript levels were determined by two-step quantitative real-time PCR (qRT-PCR) analysis. Synthesis of cDNA from cells grown in glucose and galactose was performed with the Quantitect reverse transcription kit (Qiagen). Amplification reactions to detect relative cDNA levels were performed with the Quantitect SYBR Green PCR kit (Qiagen) using the iCycler IQ Real-time PCR Detection System (Bio-Rad). Results were analyzed using the iCycler Optical System version 3.0a software, and data were normalized by the ΔΔCt method to a control transcript (33).
RESULTS

Previous studies have revealed that the Mlp proteins interact with actively transcribed loci at the NPC (5, 7). To further examine the interactions between the Mlp proteins and genetic loci, we expressed the COOH-terminal globular domain of Mlp1 in yeast cells as a COOH-terminal TAP-tagged protein. We then purified the CT-Mlp1-TAP protein through a standard two-step TAP purification (31) and identified specific bound proteins through mass spectrometric analysis of protein bands that were present in the bound fraction for CT-Mlp1-TAP, but not in the bound fraction for lysate from control cells expressing only the TAP tag. This analysis revealed a number of proteins that have previously been identified as Mlp-interacting proteins, including Nab2 (16). Among the other proteins identified in this analysis, we found three members of the SAGA histone acetyltransferase complex: the catalytic subunit, Gcn5 (34), a modulator of acetyltransferase activity, Ada2 (34–36), and a structural component, Spt7 (37). Recent structural analysis reveals that these members of the SAGA complex are all located in close proximity to one another in the complex within a region designated Domain III (38). The identification of three members of the SAGA complex, which are located within the same subcomplex, led us to hypothesize that Mlp1 could interact with the core components of the SAGA complex.

To confirm the results of our global binding analysis, we carried out several co-purification experiments to examine the interactions of Mlp1 and the homologous protein, Mlp2, with components of the SAGA complex. We first examined the interaction between the carboxyl-terminal domain of the Mlp proteins and the SAGA component, Gcn5. Each CT-Mlp was fused to GST and expressed from a galactose-inducible promoter in yeast cells that express a carboxyl-terminal TAP-tagged Gcn5 protein from the GCN5 genomic locus. Following induction, the yeast cells were lysed and the GST fusion proteins were purified on glutathione beads. The bound fractions were washed, then analyzed by immunoblotting and probed with PAP antibody to detect TAP-tagged Gcn5. As shown in Fig. 1A, Gcn5 is enriched in the bound fraction (B) of the GST-CT-Mlp1 and GST-CT-Mlp2 samples but not in the GST alone control sample. A control TAP-tagged protein did not co-purify with any of the GST proteins (data not shown). To address the question of whether Gcn5 can also interact with the full-length Mlp proteins, we expressed Gcn5-GFP in yeast strains expressing genomic carboxyl-terminal TAP-tagged Mlp1, Mlp2, or the control TAP tag alone. Cells were grown to log phase and then lysed, and the TAP-tagged proteins were purified on IgG beads. The lysate (L), unbound (U), and bound (B) fractions were analyzed by immunoblotting and probed for the Gcn5-GFP fusion protein (Fig. 1B). Results show that Gcn5-GFP is enriched in the bound fraction with both Mlp1-TAP and Mlp2-TAP, but not with the TAP tag alone (Fig. 1B). To extend our analysis to another member of the SAGA complex, we next examined the interaction between the Mlp proteins and Ada2. Each GST-CT-Mlp protein was expressed in yeast cells that express COOH-terminal TAP-tagged Ada2. Cells were grown to log phase, lysed, and GST fusion proteins were purified on glutathione beads. Fig. 1C shows that Ada2-TAP is enriched in the bound fraction (B) of both GST-CT-Mlp1 and GST-CT-Mlp2 samples but not the control GST protein. These results confirm that components of the SAGA complex can be co-purified with the Mlp proteins.

Because both the Mlp proteins and SAGA are implicated in gene expression (5, 7, 21, 22) and aspects of mRNA transcription and/or export (16–18, 20), one possibility is that the interaction between the Mlp proteins and SAGA components is mediated by either DNA or RNA. To address this point, we repeated the co-purification of GST-CT-Mlp proteins with Gcn5-TAP and treated samples with DNase and/or RNase before analyzing the
bound samples (Fig. 2A). Our results indicate that treatment with DNase, RNase, or both did not affect the interaction between Gcn5-TAP and GST-CT-Mlp1 (Fig. 2A) or GST-CT-Mlp2 (data not shown). The results of this experiment indicate that the interaction between Gcn5 and Mlp proteins does not depend on associated RNA or DNA, suggesting that this is a protein-protein interaction. To investigate whether the Mlp proteins could interact directly with components of the SAGA complex, we performed in vitro binding assays with Mlp proteins and the Gcn5 subunit of the SAGA complex (Fig. 2B). Purified, recombinant GST-CT-Mlp1, GST-CT-Mlp2, GST-Ada2, or GST alone was bound to glutathione-Sepharose beads and incubated with purified recombinant His-Gcn5. Samples were washed extensively, and bound and unbound fractions were analyzed with anti-His to detect co-purification of His-Gcn5 (upper panel). His-Gcn5 can be detected in the bound fractions for GST-CT-Mlp1 under all conditions. B, in vitro binding assay. Purified recombinant GST-CT-Mlp1, GST-CT-Mlp2, GST-Ada2, or GST alone was bound to glutathione-Sepharose beads and incubated with purified recombinant His-Gcn5. Samples were washed extensively, and bound and unbound fractions were analyzed with anti-His to detect co-purification of His-Gcn5 (upper panel). His-Gcn5 can be detected in the bound fractions for GST-CT-Mlp1 under all conditions.

FIGURE 2. The Mlp proteins can interact directly with Gcn5. A, the interaction between Gcn5 and the Mlp proteins does not depend on DNA or RNA. Yeast cells expressing Gcn5-TAP were transformed with the galactose-inducible GST-CT-Mlp1 plasmid. Expression of GST-CT-Mlp1 was induced with galactose and cell lysates were prepared. Prior to purification of GST-CT-Mlp1, lysates were treated with RNase, DNase, both RNase and DNase, or were left untreated as a control (Con), as described under “Experimental Procedures.” GST-CT-Mlp1 was then purified with glutathione beads and the bound fractions were analyzed with PAP antibody to detect co-purification of Gcn5-TAP. Gcn5-TAP can be detected in the bound fraction for GST-CT-Mlp1 under all conditions. B, in vitro binding assay. Purified recombinant GST-CT-Mlp1, GST-CT-Mlp2, GST-Ada2, or GST alone was bound to glutathione-Sepharose beads and incubated with purified recombinant His-Gcn5. Samples were washed extensively, and bound and unbound fractions were analyzed with anti-His to detect co-purification of His-Gcn5 (upper panel). His-Gcn5 can be detected in the bound fractions for GST-CT-Mlp1 under all conditions. B, in vitro binding assay. Purified recombinant GST-CT-Mlp1, GST-CT-Mlp2, GST-Ada2, or GST alone was bound to glutathione-Sepharose beads and incubated with purified recombinant His-Gcn5. Samples were washed extensively, and bound and unbound fractions were analyzed with anti-His to detect co-purification of His-Gcn5 (upper panel). His-Gcn5 can be detected in the bound fractions for GST-CT-Mlp1 under all conditions.

FIGURE 3. Mlp1 interacts with the UAS within the GAL genes. A, schematic of the GAL1/10 and GAL2 loci. GAL1/10 is located on chromosome II and GAL2 is located on chromosome XII. The schematic indicates the position of the UAS and TATA box. The regions amplified by the UAS-specific and TATA box-specific primers are indicated by the lines below the schematic. B, ChIP assay to detect Mlp1 interaction with the GAL2 locus. Yeast cells expressing genomically encoded TAP-tagged Mlp1, Gcn5, Ada2, or TBP or control cells with no TAP-tagged protein were analyzed by ChIP as described under “Experimental Procedures.” Cells were either grown in glucose (Glu) where the galactose genes are not induced or galactose (Gal) where the galactose genes are induced. Primers were designed to detect either the GAL2 UAS (left panel) or the GAL2 TATA box (right panel). The total input sample (Input) or the fraction of the TAP-tagged protein immunoprecipitated with IgG beads (IP) was analyzed by PCR. Mlp1 and SAGA components interact with the GAL2 UAS, whereas TBP does not. In contrast, TBP interacts with the TATA box but neither the SAGA components nor Mlp1 interact with this region.

We first tested whether Mlp1 interacts with the UAS region of both the GAL1/10 and GAL2 promoters. Fig. 3B (left panel) shows that the GAL2 UAS can be immunoprecipitated with Mlp1 in this assay. Similar results were obtained for the GAL1/10 locus (data not shown). As controls, both Gcn5 and Ada2, components of the SAGA complex (34), could also immunoprecipitate the UAS. In contrast, TATA-binding protein (TBP), which binds to the TATA box (40, 41) and not the UAS, did not immunoprecipitate the UAS region. As a control for the specificity of this interaction, we next examined whether Mlp1 interacts with the GAL2 TATA box, which is located ~230 base pairs from the GAL2 UAS. Results show that neither the SAGA components, Gcn5 and Ada2, nor Mlp1 interact with the TATA region (Fig. 3B, right panel). As a control, TBP does bind to the TATA region. These results suggest that Mlp1 and
the components of the SAGA complex bind to the same region of the chromatin upstream of GAL1/10 and GAL2.

To test whether the interaction of Mlp1 with the GAL UAS depends on the SAGA complex, we examined this interaction in cells that lack the SAGA complex. To disrupt the SAGA complex, we deleted the SPT7 gene, which encodes a protein required for the structural integrity of the SAGA complex (37). However, disruption of the SAGA complex due to deletion of the SPT7 gene also results in a severe slow-growth phenotype when galactose is the sole carbon source (37) and decreased recruitment of TBP to the GAL1 promoter (23), suggesting that transcription of the GAL loci might be decreased in spt7Δ cells. Thus a decreased association between the GAL loci and the Mlp proteins in spt7Δ cells could be due to either the physical absence of the SAGA complex at the GAL UAS or a decrease in transcription of the GAL genes. To differentiate between these two possibilities, we used semi-quantitative RT-PCR to analyze GAL transcript levels in spt7Δ cells and identify conditions under which GAL transcript levels could recover to near wild type levels. As shown in Fig. 4A, when spt7Δ cells were permitted to grow to an optical density (OD) of 0.9, the steady-state level of the GAL1 transcript in spt7Δ cells was approximately equal to the level in wild type cells grown to the same OD. Quantitation of RT-PCR data revealed only ~20% decrease in GAL1 or GAL2 transcript levels in spt7Δ cells as compared with wild type cells grown under these conditions (data not shown), suggesting that the level of transcription of these loci does not differ significantly in the absence or presence of SAGA when the cells are permitted to grow to this density. To further confirm that GAL transcript levels are similar under the conditions analyzed, we used qRT-PCR to assess GAL transcript levels in wild type, spt7Δ, and spt20Δ cells at early (0.3) and late (0.9) ODs. We included spt20Δ cells in this analysis as Spt20 is another SAGA subunit that contributes to the integrity of the SAGA complex (37). The qRT-PCR experiments revealed that both spt7Δ and spt20Δ cells display a severe GAL1 transcript defect compared with wild type at the early OD (Fig. 4B), consistent with previous reports (24, 42). However, both spt7Δ and spt20Δ cells show recovery of GAL1 transcript levels when allowed to grow to OD 0.9, with the GAL1 transcript in spt7Δ cells approaching wild type levels (Fig. 4B). Similar results were obtained for GAL2 transcript levels (data not shown). These results are consistent with a previous report that loss of the Ada2 and Sus1 components of the SAGA complex does not drastically affect the transcript levels produced from the GAL genes under similar experimental conditions (9).

Having identified conditions that should allow us to distinguish between a requirement for SAGA-dependent transcription or the physical presence of the SAGA complex, we next employed ChIP analysis to compare the interaction of Mlp1 with either the GAL1/10 (Fig. 4, C and D) or the GAL2 UAS (Fig. 4D) in wild type versus spt7Δ cells, under conditions where GAL transcript levels were near wild type in spt7Δ cells. Results indicate that the loss of the intact SAGA complex decreases the interaction of Mlp1 with the GAL1/10 UAS significantly (Fig. 4C). Quantitative analysis of the ChIP data reveals that loss of the SAGA complex leads to a greater than 75% decrease in the association of Mlp1 with either the GAL1/10 or the GAL2 UAS (Fig. 4D). This result is consistent with previous reports that loss of components of the SAGA complex results in loss of association of active GAL genes with the nuclear periphery (9).

The primary catalytic function associated with the SAGA complex is the histone acetyltransferase activity of the Gcn5 component (34). Deletion of GCN5 results in a catalytically inactive yet structurally intact SAGA complex (37, 43). To determine whether histone acetyltransferase activity was important to link the SAGA-dependent GAL loci to the nuclear pore, we tested whether deletion of GCN5 decreases the interaction of Mlp1 with the GAL1/10 locus using ChIP. As shown in Fig. 5, the ChIP data revealed no significant decrease in the association of the Mlp1 with the GAL1/10 locus upon deletion of GCN5. When the data were quantitated, results revealed only ~10% decrease in the association of Mlp1 with the GAL1/10 locus in gcns5Δ cells as compared with wild type cells. These results confirm that the acetyltransferase function of SAGA is
SAGA Links Actively Transcribed GAL Genes to the NPC

FIGURE 5. Gcn5 is not required for the interaction between GAL genes and the nuclear periphery. The ChIP interaction between Mlp1-TAP and the GAL1/10 UAS was analyzed in wild type (WT) cells and gcn5Δ cells, which both express Mlp1-TAP. As a negative control (Control), the ChIP interaction was also analyzed in cells that do not express Mlp1-TAP. Cells were grown in glucose (Glu) or galactose (Gal) and the ChIP interaction between Mlp1-TAP and the GAL1/10 was analyzed as described under “Experimental Procedures.” The Input is indicated in the lower panel and the IP (GAL1/10 UAS) is in the upper panel.

not required to link actively transcribed genes to the nuclear pore, which is consistent with previous reports that Gcn5 is not required for expression of the GAL genes (23, 24), integrity of the SAGA complex (43), or visual detection of the GAL genes at the nuclear periphery (9).

DISCUSSION

Our results reveal a SAGA-dependent, physical association between the Mlp proteins at the NPC and the actively transcribed GAL UAS, as determined by ChIP analysis. Whereas these results support and complement previous reports that components of the SAGA complex are required for visual detection of localization of the GAL genes to the nuclear periphery (9), they are significant in identifying the SAGA complex as required for a physical association between NPC components and the actively transcribed GAL genes. Furthermore, our results define the requirement for SAGA in the interaction between the NPC and the active GAL genes as physical rather than functional through two separate findings. First, we determined that the acetyltransferase activity of the SAGA complex is not required for the physical association between the Mlp proteins and the GAL genes, which is consistent with previous reports (9). Second, we found that the physical interaction between the Mlp proteins and the GAL genes is significantly reduced in the absence of the SAGA complex, even under conditions where transcription of the GAL genes is likely to be largely unaffected. Taken together, these findings indicate that it is the physical presence of the SAGA complex at the GAL promoter that is required for association of the GAL genes with the NPC. Our findings are complemented by recent results suggesting that Mlp1 is an important connection between the NPC and actively transcribed genes (14), which suggests that the Mlp/SAGA link analyzed here could be a key element of locus recruitment to the NPC.

Previous reports have implicated diverse aspects of transcription and mRNA processing in the recruitment of active loci to the NPC (8–14), including transcription factors, chromatin modifying complexes, the pre-initiation complex, and mRNA processing and export factors. As the SAGA complex regulates only ~10% of the yeast genome (21, 22), our data together with these previous reports strongly suggests that distinct mechanisms of recruitment exist for individual loci. Indeed, a previous study found that altering the induction mechanism of the yeast HKX1 locus with an artificial promoter abrogated recruitment of the locus to the NPC (10). Furthermore, we find that both the SAGA complex (Ref. 9 and this study) and the mRNA export factor Sac3 (13) are required for GAL locus recruitment to the NPC indicates that the interactions that link an activated locus to the NPC may depend upon a host of functionally diverse transcription and mRNA processing factors, some of which are locus-specific. Our data suggests that chromatin modifying complexes, which are recruited to individual loci through specific transcriptional activators, may be important components of this combinatorial, physical interaction between active loci and the NPC.

As the recruitment of actively transcribed genes to the NPC has been thus far documented only in S. cerevisiae, a major question is whether this phenomenon also occurs in higher eukaryotes. The evolutionary conservation of both the SAGA (human TBP-free TAF-containing complex (TFTC), p300- and CBP-associated factor (PCAF), and SPT3-TAF31-GCN5 acetyltransferase (STAGA) complexes (44–47)) and the components of the NPC including the Mlp proteins (human translocated promoter region (Tpr) (15)) implies that locus recruitment to the NPC could in principle occur in higher eukaryotes by mechanisms similar to those in yeast. Furthermore, the Drosophila Mlp homolog, Mtor, is required for proper localization of the MSL histone acetyltransferase complex to the male X chromosome, where the MSL complex up-regulates transcription of X-linked genes on the single copy of the male X chromosome at the nuclear periphery (12). These results tantalizingly hint that the interactions between the NPC and actively transcribed genes may represent an evolutionarily conserved mechanism for gene regulation across the eukaryotic lineage.

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