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A Bre1-associated Protein, Large 1 (Lge1), Promotes H2B Ubiquitylation during the Early Stages of Transcription Elongation*[S]

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Transcription activation has been proposed to require both ubiquitylation and deubiquitylation of histone H2B. Here, we show that Lge1 (Large 1) is found in a complex containing Rad6·Bre1 and that it controls the recruitment of Bre1, a ubiquitin ligase, and Ubp8, a deubiquitylase, to promote ubiquitylation during the early steps in elongation. Chromatin immunoprecipitation experiments showed that Lge1 associates with promoter and coding regions of actively transcribed genes in a transcription-dependent manner. Disruption of Lge1 abolished ubiquitylation of histone H2B on lysine 123 and H3 methylation on lysines 4 and 79 and resulted in significant sensitivity to 6-azauracil and mycophenolic acid. In particular, in Lge1-deficient cells, Bre1 recruitment was attenuated, whereas recruitment of Ubp8 was facilitated. These alterations were coincident with changes in the interaction between Bre1·Ubp8 and RNA polymerase II phosphorylated at serine 5 of the C-terminal domain. We propose that Lge1 has a novel function in disrupting the balance between the recruitment of Bre1 and Ubp8, thus promoting transcription elongation.

Rad6, Bre1, and Lge1 form a complex that is required for histone H2B ubiquitylation in the budding yeast, *Saccharomyces cerevisiae* (1–3). Rad6 is a ubiquitin-conjugating enzyme, or E2, that is required for histone H2B ubiquitylation on lysine 123 (4). Bre1 is a ubiquitin ligase, or E3, that was first identified as an evolutionarily conserved RING finger protein required for both H2B ubiquitylation and H3 lysine 4 methylation *in vivo* (1). Lge1 was originally identified in a screen for mutants with defective cell size control (5). Madhani and colleagues (1) showed that the *lge1*Δ mutant shares most of the phenotypes observed in a *bre1*Δ mutant: deletion of either Lge1 or Bre1 results in synthetic lethality with the deletion of the histone variant *HTZ1* and reduces the levels of ubiquitylated H2B and H3 methylation at lysines 4 and 79.

Posttranslational modifications of core histones within eukaryotic chromatin play an important role in the regulation of chromatin structure and gene expression (6, 7), and histone ubiquitylation by Rad6·Bre1 has been implicated in gene expression (8). However, unlike other histone modifications, such as acetylation or methylation, the H2B ubiquitylation state is dynamic during transcription activation. Histone H2B is ubiquitylated on lysine 123 by the Rad6·Bre1·Lge1 complex and subsequently deubiquitylated by Spt-Ada-Gcn5-acetyltransferase (SAGA)[2]-associated Ubp8, a deubiquitylase (9, 10). In particular, this dynamic regulation is associated with factors involved in different stages of the transcription cycle. Ubiquitylation of H2B by Rad6·Bre1 requires early steps in transcription elongation, including interactions with the PAF complex, the BUR complex, and the elongation form of RNA polymerase II (RNApII) that has been phosphorylated on serine 5 of the C-terminal domain (CTD) by Kin28 (for review, see Ref. 10). Deubiquitylation of H2B is important for the recruitment of Ctk1, a kinase that is found in the elongation complex and phosphorylates serine 2 of the CTD of RNApII (11, 12). These findings provide strong evidence that histone H2B ubiquitylation and deubiquitylation are critically involved in gene activation. Although both Bre1 and Lge1 have similar effects on transcription and are required for ubiquitylation of histone H2B on lysine 123, the role of Lge1 in transcription activation is still not clear. Here, we provide evidence that Lge1 regulates the early steps in transcription elongation that are required for histone H2B ubiquitylation. Our results indicate that Lge1 disrupts the balance between Bre1 and Ubp8, controlling their interaction with RNApII phosphorylated at serine 5 of the CTD.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—Strains used in this study are listed in supplemental Table 1. Cells were grown at 30 °C in synthetic complete (SC) medium with appropriate amino acids and bases. For chromatin immunoprecipitation (ChIP) experiments, all yeast strains were grown at 30 °C to an A_{600} of 0.5–0.6. For *GAL10* inductions, cells were grown in SC medium to an A_{600} of 0.5–0.6 and then shifted to SC medium containing 2% raffinose. After 2 h, the 2% raffinose culture was switched to a medium containing 2% galactose and incubated for an additional 30 or 60 min. Between each shift, cells were washed twice with sterile water.

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1 and Tables 1 and 2.

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[2] The abbreviations used are: SAGA, Spt-Ada-Gcn5-acetyltransferase; RNApII, RNA polymerase II; CTD, C-terminal domain; SC, synthetic complete; ChIP, chromatin immunoprecipitation; 6-AU, 6-azauracil; MPA, mycophenolic acid; WT, wild-type; UAS, upstream activation sequence; TAP, tandem affinity purification.

Preparation of Yeast Whole Cell Extracts and Immunoblotting—Yeast whole cell extracts were prepared as described previously (12). Western blotting was performed using standard methods with antibodies against H3K4me3 (ab8580; Abcam), H3K36me3 (ab9050; Abcam), H3K79me3 (ab2621; Abcam), and histone H3 (ab1791; Abcam). The peroxidase anti-peroxidase antibody (P1291; Sigma) was used to detect the expression of TAP-tagged proteins.

Immunoprecipitations—Immunoprecipitation was performed as described previously using high ionic strength buffer to extract almost all of the RNApII in the cells (13). Whole cell extracts containing 1.5 mg of protein were incubated with 15 μl of preequilibrated IgG-Sepharose beads (GE Healthcare) for 5 h at 4 °C. After three washes in extraction buffer, the proteins bound to the beads were analyzed as described above using H14 (MMS-134R; Covance), H5 (MMS-129R; Covance), 8WG16 (MMS-126R; Covance), or peroxidase anti-peroxidase (P1291; Sigma) antibodies.

ChIP—ChIP procedures and quantification were performed as described previously (14–16). The sequences of oligonucleotides used in this study are listed in supplemental Table 2. Anti-H3 (ab1791; Abcam), anti-acetyl H3 (06-599; Millipore), and anti-Rpb1 (8WG16; Covance) were bound to protein A-Sepharose CL-4B (GE Healthcare) and used to precipitate chromatin. For precipitation of TAP-tagged proteins, 20 μl of IgG-Sepharose beads (GE Healthcare) was used. To control for amplification efficiency and label incorporation of different primers, the ratio of each gene-specific product to that of a nontranscribed region of chromosome V was calculated from the input sample signals. Signals for histone acetylation were normalized relative to the total H3 signal.

In Vivo Analysis of Histone Ubiquitylation—The relative levels of ubiquitylated histone H2B were analyzed as described previously (4) with minor modifications. In brief, 20% trichloroacetic acid-extracted histones from the indicated strains were resuspended in 0.7 ml of SDS-sample buffer, neutralized by adding 50 μl of unbuffered 2 M Tris, and centrifuged at 3,000 rpm for 10 min at room temperature. The clarified lysates were then immunoprecipitated with anti-FLAG M2-agarose (A2220; Sigma) and eluted into SDS-sample buffer by boiling for 5 min. After electrophoresis in a 15% SDS-polyacrylamide gel, ubiquitylated histones were probed with either anti-FLAG (F3165; Sigma) or anti-hemagglutinin (clone 12CA5; Roche Applied Science) antibodies.

6-Azauracil (6-AU) and Mycophenolic Acid (MPA) Plate Assay—Strains were transformed with a *URA3* plasmid before testing for sensitivity to 150 μg/ml 6-AU or 15 μg/ml MPA. For spotting analyses, cells were resuspended to an A_{600} of 0.1 and subjected to 10-fold serial dilutions, and 10 μl of each dilution was spotted. Images were obtained after 2–3 days of growth at 30 °C.

RESULTS AND DISCUSSION

To determine whether Lge1 is associated with actively transcribed gene regions, ChIP analysis was carried out. The strongly constitutively transcribed genes *PMA1* and *ADH1* were used to map the positions at which proteins were localized (Fig. 1*A*). Consistent with a previous report (17), a ubiquitin-conjugating enzyme, Rad6, showed weak cross-linking to promoter and coding regions. The ubiquitin ligase Bre1 was more intensely associated with chromatin at promoter and coding regions than Rad6, but in both cases, cross-linking decreased in the region downstream of the polyadenylation sites of *PMA1*, suggesting a role for the E2·E3 complex in transcription elongation by RNApII (Fig. 1*B*). One group has reported that Rad6 and Bre1 are localized on chromatin specifically at the promoter of the *PMA1* gene (2, 18). However, in accordance with the previous report (17), our finding confirms recruitment of the Rad6·Bre1 ubiquitylation machinery to the coding region of *PMA1*. As expected from its genetic interaction with Bre1 and its ability to affect histone ubiquitylation, the cross-linking pattern of Lge1 is very similar to that of Bre1. Overall, these results correlate with the patterns seen with *ADH1* (Fig. 1*C*).

A *GAL* gene was then examined to determine whether Lge1 associates with chromatin in a transcription-dependent manner. The *GAL10* gene was analyzed by changing the medium from glucose or raffinose to galactose to induce transcription (19). The cross-linking of Rpb1, the largest subunit of RNApII, to the promoter and coding region of *GAL10* increased to 4–8-fold when the cells were shifted to galactose and incubated for 60 min, indicating that *GAL10* was induced under this condition. Lge1 also showed a strong increase of up to 8–16-fold (Fig. 1*D*). Although Lge1 has previously been suggested to form a complex with Rad6·Bre1, these observations provide the first direct evidence for the transcription-dependent association of Lge1 with actively transcribed genes *in vivo*.

We next asked whether Lge1 affects histone modifications such as ubiquitylation or methylation. To explore ubiquitylation levels, a FLAG-tagged allele of the *HTB1* gene (encoding histone H2B) was immunoprecipitated in cells that contained a hemagglutinin-tagged *UBI4* gene (encoding ubiquitin) as well as the endogenous gene. Ubiquitylated histone H2B was detected by SDS-PAGE as a more slowly migrating form after immunoblotting with an anti-FLAG antibody (9, 20). As seen in Fig. 2*A*, histone ubiquitylation on lysine 123, which was easily detected in wild-type (WT) cells, was blocked by a point mutant in which lysine 123 of H2B was substituted for arginine (K123R). The absence of Rad6 also completely abrogated ubiquitylation, whereas the loss of the histone deubiquitylase, Ubp8, profoundly increased the modification as has been shown in previous reports (1, 2, 9, 19). Like cells deficient in Rad6, cells deficient in Lge1 showed undetectable levels of ubiquitylation, in agreement with a previous report (1).

H2B ubiquitylation by the Rad6·Bre1 complex is known to be required for H3 methylation on lysines 4 and 79, which is called the "trans-tail pathway" (1, 21–25). We therefore sought to examine H3 methylation on lysines 4, 36, and 79 using specific antibodies against trimethylated H3 in *lge1*Δ cells. As expected, H3 trimethylation on lysines 4 and 79 was abolished in cells lacking Rad6, Bre1, or Lge1 (Fig. 2*B*). These results generally correlated with the previous findings (18, 23, 25–27) except that methylation of lysine 79 was partially retained in *lge1*Δ cells (1). However, the level of methylation on lysine 36 remained unaffected in strains deficient in Rad6, Bre1, or Lge1, indicating that ubiquitylation is unrelated to this modification. The H2B K123R mutation also only affected H3 trimethylation on lysines

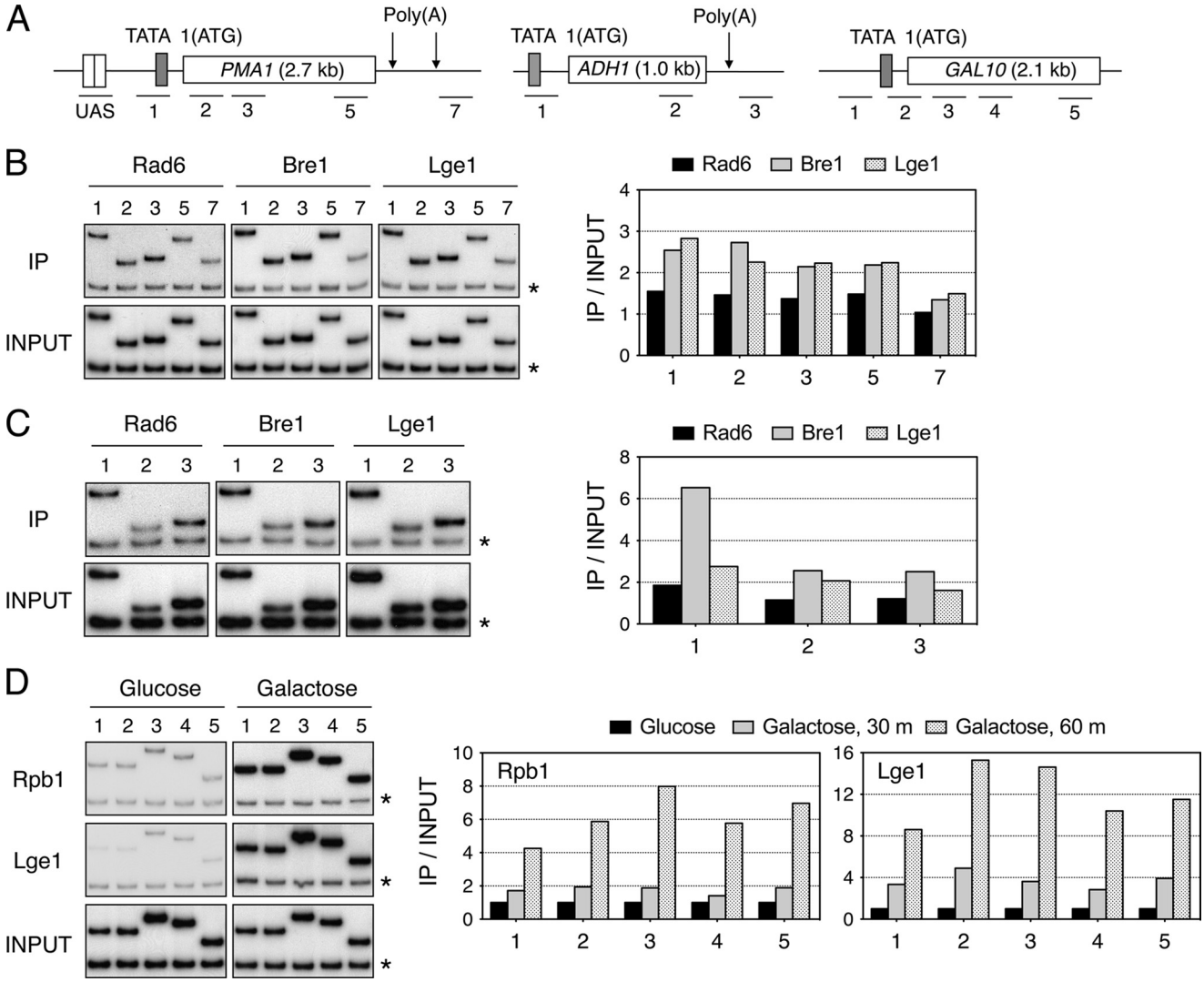

FIGURE 1. **Lge1 is recruited to actively transcribed genes.** *A*, schematic of the *PMA1*, *ADH1*, and *GAL10* genes. The UAS of the *PMA1* gene is indicated by an *open box* and used in later figures. The TATA/promoter region and open reading frame, starting with the initiation codon (+1), are represented by *gray* and *hatched boxes*. Major polyadenylation sites of the *PMA1* and *ADH1* genes are indicated by *arrows*. *Bars* and *numbers* below the genes show the relative positions of the PCR products used in the ChIP analysis and are used for identification in all later figures. *B*, occupancy of the indicated regions of *PMA1* by Rad6, Bre1, and Lge1. IgG-Sepharose was used for the immunoprecipitation (*IP*) of TAP-tagged proteins. *INPUT* was used to normalize the PCR amplification; the *asterisk* marks a nontranscribed PCR fragment indicated in all reactions as a background control. Quantitation of the ChIP experiments is shown in the *right panel*. *C*, ChIP analysis for the *ADH1* gene performed as in *B*. *D*, ChIP analysis of the *GAL10* gene performed using the Lge1-TAP strain grown under repressed (*Glucose*) and activated (*Galactose*) conditions using the primers indicated in *A*. The *asterisk* marks the same nontranscribed PCR fragment as in *B* and *C*. Each PCR signal was quantitated and normalized to the intergenic control and the INPUT DNA (*right panel*). The *y* axis shows the relative fold difference compared with the ChIP value obtained from cells grown in raffinose. This value was arbitrarily set to 1.

4 and 79 but not lysine 36. In both cases, the reduction was by about 50%, although deletions of *RAD6*, *BRE1*, or *LGE1* abolished the methylation. Recent studies suggest that H2B ubiquitylation is not sufficient to abolish H3 trimethylation (28, 29). In particular, Foster and Downs (28) reported that a strain bearing an *htb1-K123R* mutation (FY406 background) is still proficient for H3 trimethylation on both lysines 4 and 79. Although the original *htb1-K123R* mutant strain (Y131 background) lacked trimethylation of both lysines 4 and 79 (27–30), the dependence of H3 methylation upon H2B ubiquitylation remains to be confirmed. The YKH046 strain used in this study (31) has a *htb1-K123R* mutation in a different background from FY406 or Y131. In our experiment, the strain partially reduced H3 trimethylation on lysines 4 and 79. However, in contrast to the ambiguous results obtained from the *htb1-K123R* mutant strains, we and others have found that strains deficient in a gene required for H2B ubiquitylation such as *RAD6*, *BRE1*, or *LGE1* completely lost H3 methylation on lysines 4 and 79 (Fig. 2*B*) (1, 18, 23, 25–27). Therefore, it is likely that the Rad6·Bre1·Lge1 complex may have an additional target that is required for H3 methylation. In *ubp8*Δ cells, no apparent increase in methylation was observed despite a massive increase in ubiquitylation (Fig. 2, *A* and *B*). Collectively, these results confirm that Lge1 functions together with the Rad6·Bre1 complex to regulate histone ubiquitylation and subsequent methylation on lysines 4 and 79.

Lge1 is known to associate with Bre1, and it exhibits functions very similar to those observed for Bre1 (1, 3, 32, 33). However, several lines of evidences suggest that Lge1 may serve a particular function (or functions) distinct from that of Bre1. Unlike other proteins associated with Rad6 (Ubr1, Ubr2, or

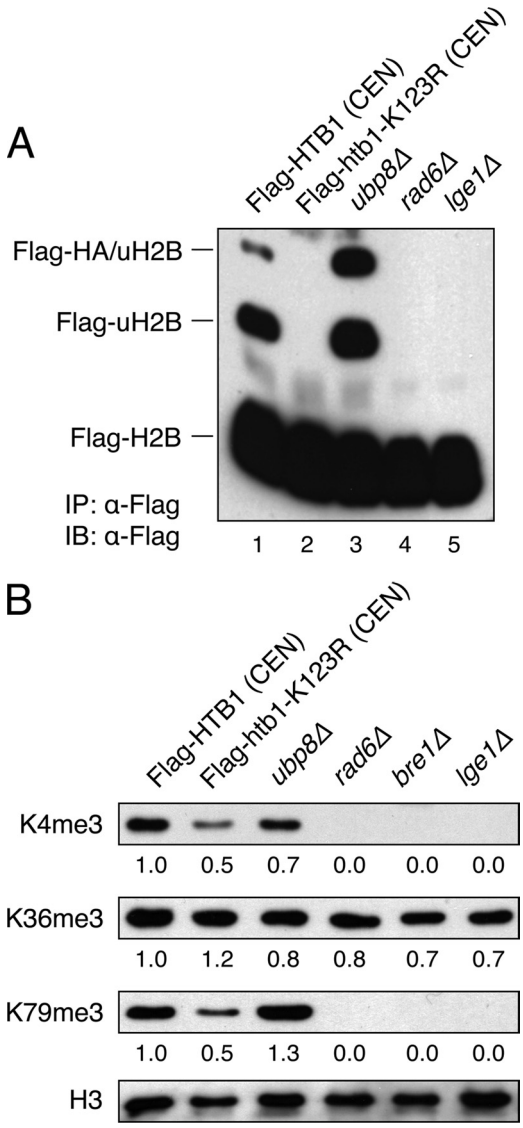

FIGURE 2. **Lge1 is required for H2B ubiquitylation on lysine 123 and H3 methylation on lysines 4 and 79.** *A*, ubiquitylation level of H2B is abolished in the *lge1*Δ strain. The *htb1–1/htb2–1* haploid strain containing both FLAG-*HTB1* on a CEN-*TRP1* plasmid and glyceraldehyde-3-phosphate dehydrogenase-regulated hemagglutinin (*HA*)-*UBI4* on a CEN-*URA3* plasmid was used as WT (YKH045). A strain containing a mutation in lysine 123 (*htb1*-K123R) was used as the negative control (YKH046). Histone H2B purified through FLAG immunoprecipitation (*IP*) from WT, *htb1*-K123R, *ubp8*Δ, *rad6*Δ, and *lge1*Δ strains (YKH045, YKH046, YKH047, SY082, and SY084, respectively) was probed (*IB*) with an anti-FLAG antibody to analyze unmodified or ubiquitylated H2B in cells. *B*, Lge1 regulates the methylation level of H3 on lysines 4 and 79 but not 36. Whole cell extracts from the indicated strains were subjected to SDS-PAGE and probed with antibodies directed against trimethylated H3 on lysine 4, 36, or 79. Histone H3 was used as a loading control. The *numbers* below the blots show the ratio of methylated H3 to total H3.

Rex4) or proteins containing a RING finger domain (Rad5 or Rad18), only Lge1 is required for H2B ubiquitylation and H3 methylation (2, 23). In addition, Northern blot analysis and microarray experiments have shown that induction of *PDR3* transcription in *S. cerevisiae* cells lacking a mitochondrial genome requires Lge1-mediated but not Rad6·Bre1-mediated H2B ubiquitylation. Furthermore, changes in gene expression in *RAD6*, *BRE1*, or *LGE1* deletions share some overlap but are distinct (34). We therefore attempted to define further the role of Lge1 in histone ubiquitylation during transcription. Although Lge1 forms a complex with Rad6·Bre1, we predicted that Lge1 could function in the regulation of ubiquitylation and transcription elongation independently of Rad6·Bre1. To test this idea, we first examined the sensitivity of *lge1*Δ cells to 6-AU or MPA (Fig. 3*A*). 6-AU or MPA treatments reduce intracellular nucleotide pools, reducing the availability of precursors and causing cells to grow slowly. When grown on SC plates containing 150 μg/ml 6-AU or 15 μg/ml MPA, cells lacking Lge1 showed sensitivities comparable with those of deletion mutants of Paf1, Rtf1, Rad6, or Bre1. These proteins are involved in transcription elongation (11, 17). In contrast, a deletion mutant of Rkr1, a nuclear RING domain protein shown to be involved in chromatin modification and ubiquitin-protein ligase activity *in vitro* (35), showed no sensitivity.

We then sought to determine whether Lge1 is required for the recruitment of the Rad6·Bre1 complex to the promoter and coding regions. ChIP was carried out in cells TAP-tagged with Rad6 or Bre1 and deficient in Lge1. As seen in Fig. 3*B*, a defect in the recruitment of Bre1 was observed in *lge1*Δ cells without changes in protein stability, whereas the association of the E2 enzyme Rad6 with chromatin remained unchanged. This shows that Lge1 seems to assist with recruitment of Bre1 to chromatin. Next, because the deubiquitylase Ubp8, a counterpart of Rad6·Bre1 in histone ubiquitylation, is also known to participate in transcription activation, we examined the recruitment of Ubp8 to active genes in *lge1*Δ cells. Recent studies have shown that Ubp8 is present in the SAGA and SALSA·SILK (SAGA altered, Spt8 absent; SAGA-like) complexes (31, 36–38) and is recruited to promoters simultaneously with Gcn5 to deubiquitylate histone H2B *in vitro* (9, 11, 30). Our ChIP analysis shows that Ubp8 is specifically localized to the upstream-activation sequence (UAS) region, about 0.9 kb away from the translation start site (ATG) of the *PMA1* gene (Fig. 3*C*). Surprisingly, we observed that the occupancy of Ubp8 in the UAS region was increased about 1.6-fold in cells lacking Lge1, even though protein expression levels of Ubp8 were unaffected. This indicates that Lge1 negatively regulates the recruitment of Ubp8, possibly preventing deubiquitylation during the early steps of transcription. Consistent with a previous report (31), Ubp8 still had an *in vivo* deubiquitylase activity level similar to that seen in another SAGA component, Sgf11 (Fig. 3*D*). As for Bre1, a small increase in the association of Lge1 with the UAS region was seen, supporting the idea that Ubp8 recruitment is regulated by Lge1 (supplemental Fig. S1). Collectively, our results show that Lge1 plays a role in transcription activation by controlling recruitment of Bre1 and Ubp8, which have opposing influences on the process of H2B ubiquitylation.

The finding that association of Ubp8 with chromatin was increased in *lge1*Δ cells raises the possibility that this mutation may also increase the binding of other SAGA subunits, including Gcn5. If this is the case, Lge1 mutation might affect histone acetylation. To address this question, ChIP analyses were carried out in cells deficient in one of the SAGA subunits, Sgf11, Gcn5, Taf6, and Spt8 (Fig. 4*A*). Sgf11 is required for association of Ubp8 with SAGA (30, 39). In cells lacking Lge1, the association of Sgfl1 with UAS of *PMA1* increased similarly to that of Ubp8 (Fig. 3*C*). Interestingly, we observed that the cross-linking of Gcn5, a histone acetyltransferase that modifies H3 (Lys^9/

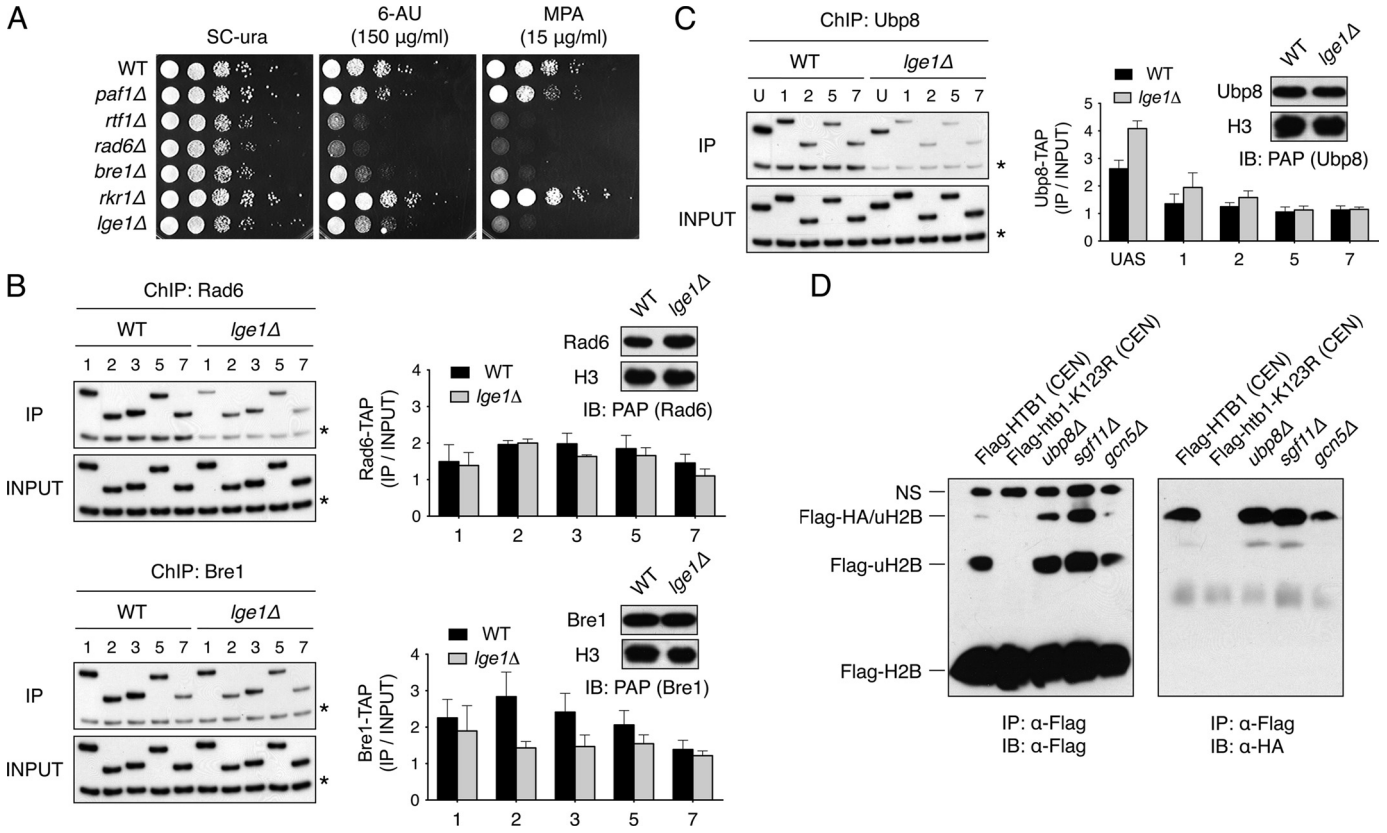

FIGURE 3. **Lge1 controls the recruitment of Bre1 and Ubp8 *in vivo* to promote transcription elongation.** *A*, sensitivity of the *lge1*Δ strain to 6-AU or MPA. Exponentially growing cells were transformed with a *URA3*-containg plasmid (pRS316) and spotted on SC (control), SC + 6-AU (150 μg/ml), or SC + MPA (15 μg/ml) plates in 10-fold dilutions. The growth of each strain was examined after 2 to 3 days at 30 °C. *B*, occupancy of Bre1 is attenuated in the *lge1*Δ strain. ChIP (*IP*) analyses carried out in WT or *lge1*Δ strains, both tagged with Rad6 or Bre1. All primer pairs used are described in Fig. 1*A*. Each quantitated result is graphed at the *right*. *Error bars* show the S.D. from three PCRs with two independent chromatin preparations. The *panels* inside each *graph* (*top right*) show Western blots (*IB*) of Rad6 or Bre1 in WT and *lge1*Δ strains. Histone H3 was used as a loading control. *C*, increased occupancy of Ubp8 in the *lge1*Δ strain. The recruitment of Ubp8 in the WT or *lge1*Δ strain was analyzed by ChIP as in *B*. *D*, ubiquitylation assay carried out in WT, *htb1*-K123R, *ubp8*Δ, *sgf11*Δ, and *gcn5*Δ strains. Histone H2B purified through FLAG immunoprecipitation (*IP*) from the indicated strains (YKH045, YKH046, YKH047, YKL142, and YKL143, respectively) was probed with anti-FLAG (*left panel*) or anti-hemagglutinin (*HA*; *right panel*) antibodies. *NS*, nonspecific band.

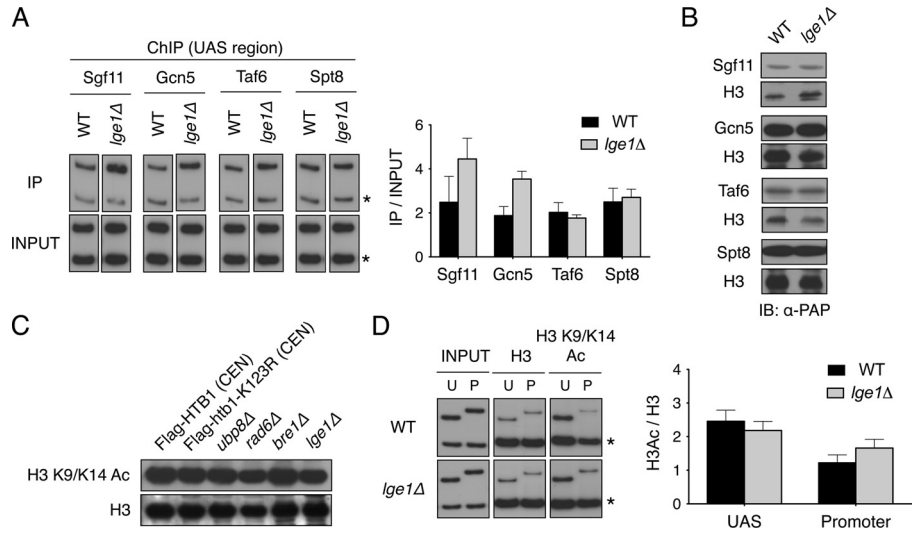

FIGURE 4. **SAGA subunits show different patterns of chromatin association in *lge1*Δ cells.** *A*, occupancy of SAGA subunits (Sgf11, Gcn5, Taf6, or Spt8) analyzed by ChIP (*IP*) in WT or *lge1*Δ strains, both tagged with each of the SAGA subunits. The UAS region of *PMA1* as shown in Fig. 1*A* was used. Each quantitated result is graphed at the *right* with *error bars* that show the S.D. from three PCRs with two independent chromatin preparations. *B*, Western blots (*IB*) of SAGA subunits in WT and *lge1*Δ strains. Histone H3 was used as a loading control. α-*PAP*, peroxidase anti-peroxidase antibody. *C*, global levels of Lys^9/Lys^{14} acetylation of histone H3 analyzed by Western blotting in the indicated strains described in Fig. 2*B*. *D*, H3 acetylation near the UAS (*U*) and promoter (*P*) regions of *PMA1* analyzed by ChIP in WT or *lge1*Δ strains. The result for acetyl-H3 was normalized compared with the total H3 signal, and the ratios are *graphed*. *Error bars* show the S.D. from three PCRs with two independent chromatin preparations.

Lys^{14}) and has been implicated in displacement of promoter nucleosomes during transcriptional activation (40), was also increased about 1.8-fold in *lge1*Δ cells. However, the occupancies of other SAGA subunits such as Taf6, a Taf histone fold module, or Spt8, a TBP-regulatory module (36, 39, 41), were not affected by loss of Lge1. Because the protein expression levels of each of the SAGA components remained unchanged by *lge1*Δ (Fig. 4*B*), our results show that Lge1 affects the association of modules within the SAGA complex with chromatin. These modules include a deubiquitylation module (Ubp8·Sgf11) and an acetylation module (Gcn5). We next asked whether histone H3 acetylation was affected by loss of Lge1 because the recruitment of Gcn5 to UAS was facilitated in *lge1*Δ cells. Fig. 4*C* shows that the

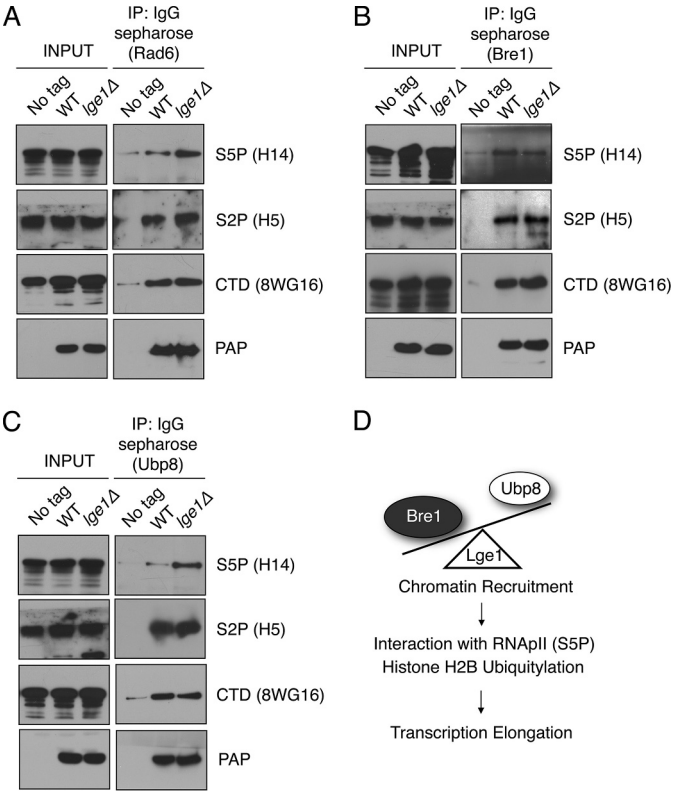

FIGURE 5. **Lge1 regulates the association of (de)ubiquitylating proteins with the serine 5 phosphorylated form of RNApII *in vitro*.** *A*, whole cell extracts prepared from cells tagged with Rad6 in WT (FY067) or *lge1*Δ (SY197) backgrounds immunoprecipitated (*IP*) with IgG-Sepharose followed by immunoblotting with antibodies directed against serine 5-phosphorylated CTD (H14), serine 2-phosphorylated CTD (H5), or the unmodified CTD (8WG16). The WT strain without a TAP tag (BY4741) was used as a negative control (*No tag*). *B* and *C*, immunoprecipitation experiments carried out as in *A* using strains tagged with Bre1 (*B*) or Ubp8 (*C*) in WT or *lge1*Δ backgrounds. Strains BY4741, FY068, and SY198 were used for *B*, and BY4741, FY089, and SY199 were used for *C*. *D*, proposed model for the regulation of transcription elongation by Lge1. See "Results" for details.

mutations that cause complete loss of H2B ubiquitylation (*htb1-K123R*, *rad6*Δ, *bre1*Δ, or *lge1*Δ*)*, or deletion *UBP8*, which increased the modification (1, 2, 9, 19) (Fig. 2*A*) had no effect on global levels of histone H3 Lys^9/Lys^{14} acetylation. However, the accumulation of Gcn5-associated H3 acetylation near the promoter of an actively transcribed gene, *PMA1,* slightly increased in *lge1*Δ cells (Fig. 4*D*). This result is in agreement with a recent report that deletions of *RAD6* or *BRE1* led to increased histone H3 and H4 acetylation in the promoter and 5′ regions of *YEF3* (42). Yet it has also been reported that Rad6-directed ubiquitylation of H2B is not required for Gcn5 association with the *GAL1* promoter (43). We propose that Lge1 has another role in regulating Gcn5-associated Lys^9/Lys^{14} acetylation of histone H3 and that this process is probably independent of histone H2B ubiquitylation.

Finally, because both Rad6 and Ubp8 have been known to associate with forms of RNApII phosphorylated at serine 5 and serine 2 of the CTD (11, 17), we sought to determine whether Lge1 is associated with the phosphorylated form of RNApII and can regulate interactions between the (de)ubiquitylating proteins and polymerase. Fig. 5 shows that Rad6·Bre1 as well as Ubp8 are associated with polymerase phosphorylated at either serine 2 or serine 5 of the CTD. In addition, the unphosphorylated form of RNApII also co-immunoprecipitated with the proteins. However, the whole cell extract from cells lacking Lge1 preferentially reduced the association of Bre1 with serine 5-phosphorylated polymerase, whereas that of Ubp8 was increased. The interaction of the (de)ubiquitylating proteins with polymerase phosphorylated at serine 2 of the CTD, the modification that increases during elongation (14), remained unchanged. Although the amount of co-immunoprecipitated Rad6 with serine 5-phosphorylated polymerase was increased (Fig. 5*A*), the results correlate with the ChIP results (Fig. 3, *B* and *C*) and show that Lge1 regulates the association between (de)ubiquitylating proteins and polymerase. These associations are required for the early steps of elongation and subsequent H2B ubiquitylation.

A model to explain dynamic regulation of H2B ubiquitylation during transcription is shown in Fig. 5*D*. In actively growing cells, both H2B ubiquitylation and deubiquitylation are required for transcription activation. To promote ubiquitylation, Lge1 begins to alter the ratio between bound Bre1 and Ubp8. Enhanced recruitment of the Bre1 protein would then lead to production of the hyperubiquitylated form of H2B. On the other hand, because H2B ubiquitylation and deubiquitylation are both required for transcription activation, it is possible that proteins that carry out functions opposite those of Lge1. The switches regulated by Rad6·Bre1-mediated ubiquitylation and/or Ubp8-mediated deubiquitylation are controlled in part by Lge1 and would thus be expected to exert control over the activity of RNApII in transcription elongation.

In the present study, we investigated the role of Lge1 in histone H2B ubiquitylation during the early steps of transcription. Our results suggest that Lge1 maintains ubiquitylation levels and promotes transcription activation both by facilitating the recruitment of Bre1 and by attenuating that of Ubp8. This may cause the preferential binding of Bre1 to RNApII phosphorylated at serine 5 of the CTD, an interaction that is required for H2B ubiquitylation and subsequent histone modifications such as H3 methylation on lysines 4 and 79. Thus, our findings imply that the Lge1-mediated switch to ubiquitylated H2B prior to the later stages of elongation is necessary for RNApII to proceed with transcription.

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