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Association of the Histone Methyltransferase Set2 with RNA Polymerase II Plays a Role in Transcription Elongation*

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The *Saccharomyces cerevisiae* protein, Set2, has recently been shown to be a histone methyltransferase. To elucidate the function of Set2, its associated proteins were identified using tandem affinity purification and mass spectrometry. We found that Set2 associates with RNA polymerase II. The interaction between the Set2 protein and RNA polymerase II requires the WW domain in Set2 and phosphorylation of the carboxyl-terminal domain of the largest subunit of RNA polymerase II. Set2 directly binds to the carboxyl-terminal domain with phosphorylated Ser^2 in the heptapeptide repeats. *set2* deletion mutant is sensitive to 6-azauracil, a property often associated with impaired transcription elongation. Together, our results suggest that Set2 through association with the elongating form of RNA polymerase II plays an important role in transcription elongation.

Transcription of protein-coding genes by RNA polymerase II is a highly regulated process in eukaryotes and involves the cooperative assembly of multicomponent protein complexes (1, 2). The carboxyl-terminal domain (CTD)[1] of the largest subunit of RNA polymerase II is phosphorylated in a manner correlated with ongoing transcription. Before transcription initiation, RNA polymerase II with an unphosphorylated CTD is recruited to the promoter by association with general initiation factors and the mediator complex. Shortly after initiation, the CTD becomes partially phosphorylated, and phosphorylation of CTD stimulates dissociation of initiation factors from RNA polymerase II and promotes the recruitment of pre-mRNA 5′-end capping enzymes. Further phosphorylation of CTD during transcription elongation permits it to interact with a variety of elongation, splicing, and 3′-end processing factors (3–6).

The CTD consists of multiple tandem repeats (26 in yeast and 52 in mammals) of an evolutionarily conserved heptapeptide with the consensus sequence Tyr^1-Ser^2-Pro^3-Thr^4-Ser^5-Pro^6-Ser^7 (7). Phosphorylation of the CTD occurs primarily at Ser^2 and Ser^5 of the heptapeptide, and phosphorylation at each site may play distinct roles (8). It was found that in budding yeast, Ser^5 is strongly phosphorylated at initiation and early elongation phase, whereas Ser^2 is predominantly phosphorylated during the elongation phase (9, 10). Genetic and biochemical evidence indicates that the CTD is the docking site for some RNA polymerase II-interacting proteins and phosphorylation of Ser^2 or Ser^5 of the CTD affects its interaction with other proteins (6).

A characteristic feature of yeast mutants defective in transcription elongation is their sensitivity to the uracil analog 6-azauracil (6AU), which inhibits enzymes in the nucleotide metabolism pathway and leads to depletion of UTP and GTP in yeast cells (11, 12). Nucleotide depletion affects the elongation efficiency of RNA polymerase II. Thus, 6AU is a useful tool for identifying proteins important for elongation. It has been shown that mutations in the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2), factors important for elongation such as PPR2 (TFIIS), SPT4, SPT5, ELP1 (elongator), and PAF1, and the CTD kinase CTK1 which phosphorylates Ser^2 (8, 13) render yeast sensitive to 6AU (11, 14–19).

Transcription elongation requires RNA polymerase II passing through chromatin and this is thought to require changes in the modification of histones (20–22). Histone methylation has been recently recognized as an important mechanism for regulation of chromatin structure and gene transcription. Site-specific methylation of histones is involved in regulation of DNA methylation, recruitment of chromatin-associated proteins, heterochromatic assembly, transcriptional activation, and repression (23–26).

In budding yeast (*Saccharomyces cerevisiae*), lysine residues 4, 36, and 79 of histone H3 are subjected to methylation by Set1, Set2, and Dot1, respectively (27–33). Methylation of H3 at lysine 4 and lysine 79 by Set1 and Dot1, respectively, mediates gene silencing and is regulated by ubiquitination of histone H2B (28, 32–35). In contrast, methylation of Lys^{36} by Set2 is unaffected by ubquitination of histone H2B (35). Although Set2-LexA could repress transcription of the LacZ reporter gene when tethered to a heterologous promoter (31), the *in vivo* role of Set2 in transcription is still unclear and needs further investigation.

To elucidate the function of Set2, we sought to identify proteins associated with Set2 by using tandem affinity purification (TAP) and mass spectrometry (36). In this study, we report that the histone methyltransferase Set2 in *S. cerevisiae* physically interacts with RNA polymerase II. Set2 interacts with RNA polymerase II through the putative WW domain in Set2. The interaction between Set2 and RNA polymerase II depends on phosphorylation, and Set2 associates with elongating RNA polymerase II with Ser^2-phosphorylated CTD. Furthermore, deletion of *SET2* results in sensitivity to 6AU, suggesting that Set2 is required for transcription elongation. These results indicate that Set2 is involved in the regulation of transcription elongation.

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[1] The abbreviations used are: CTD, carboxyl-terminal domain; 6AU, 6-azauracil; TAP, tandem affinity purification; GST, glutathione-*S*-transferase; CBP, calmodulin-binding peptide.

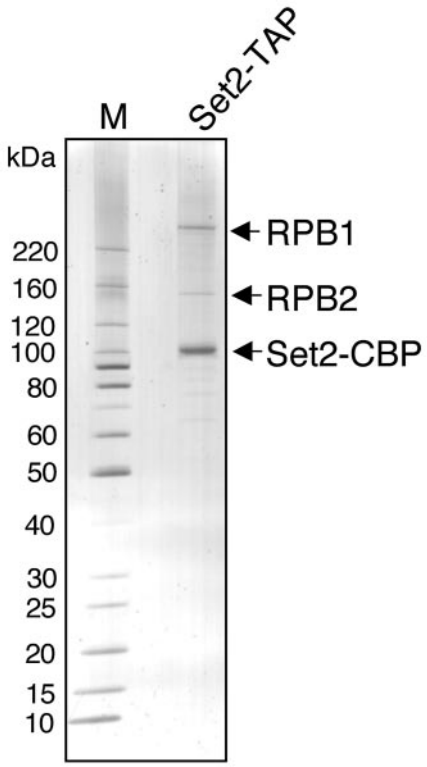


FIG. 1. **Set2 interacts with RNA polymerase II.** SET2 was tagged with TAP tags and affinity-purified through IgG agarose and calmodulin agarose columns. The purified proteins were resolved by SDS-PAGE and stained with silver. Proteins were identified by tandem mass spectrometry. The molecular weight markers are shown on the *left*. CBP, calmodulin-binding peptide, a remnant of the original TAP tag.

EXPERIMENTAL PROCEDURES

Construction of TAP-tagged Strains—The yeast strain used for making TAP-tagged constructs was SF10 (BJ5459, *MAT*a *ura3-52 trp1 lys2-801 leu2 1 pep4*::*HIS3 prb11.6R can1*). The *SET2-TAP* and *RPB2-TAP* constructs were generated by integration of the TAP tag immediately before the stop codon of the genes (carboxyl-terminal tagging), as described previously (36). *SET2-WW*Δ*-TAP* construct was made by integration of the TAP tag immediately after the codon for Leu^{472} of the Set2 protein, which excluded the carboxyl-terminal region (amino acid residues from 473 to 733) containing the putative WW domain of the Set2 protein. Transformations of yeast strains were performed using the lithium acetate method (37). TAP-tagged constructs were confirmed by PCR, Western blotting (detecting the IgG-binding sites of TAP tag using peroxidase anti-peroxidase complex), and sequencing of the tagged protein using tandem mass spectrometry (see below).

Purification of TAP-tagged Proteins—Yeast pellets (10–20 g) from the TAP-tagged strains grown to an OD_{600} of around 2.0 were resuspended in about 1 volume of extraction buffer (20 mM Hepes, pH 8.0, 350 mM NaCl, 10% glycerol, 0.1% Tween-20, 10 mM NaF, 5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 2 mM benzamidine, 1 μg/ml of bestanin, leupeptin and pepstatin A, 10 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride), lysed by agitation with glass beads using a BioSpec bead beater, and then centrifuged at 47,000 × *g* for 1 h at 4 °C as described (27, 38). The TAP-tagged protein in the supernatant was purified using IgG-agarose (Sigma) and calmodulin-Sepharose (Stratagene) essentially as previously described (36) except that cleavage by tobacco etch virus protease was performed at 4 °C overnight. Purified proteins were separated on a 5–20% gradient SDS-polyacrylamide gel and stained with silver. For the binding assay of Set2 to phosphorylated CTD and synthesized CTD peptides, Set2-CBP was obtained by elution of the calmodulin-Sepharose (~200 μl of beads) with five × 200 μl of elution buffer, combining the last four fractions (discard the first fraction), and concentrating them using Centricon (Millipore).

Identification of Protein and Methylation Sites by Mass Spectrometry—Protein bands were excised from SDS-PAGE gels and digested with sequencing-grade trypsin (Promega) as described (39). Digested samples were pressure loaded onto a fused silica microcapillary C18 column (Magic beads, Michrom BioResources) packed in-house (75 μm inner diameter, 10 cm long). An Agilent 1100 high-pressure liquid chromatography (HPLC) system (Agilent Technologies) was used to deliver a gradient across a flow splitter to the column. Eluting peptides from the column were ionized by electrospray ionization and analyzed by an LCQ-Deca XP ion-trap mass spectrometer (ThermoFinnigan). Peptide ions were dynamically selected by the operating software for fragmentation. The peptide fragmentation spectra were searched against the nonredundant protein data base using the Sequest computer algorithm (40). For searching methylation sites, a differential modification of +14 Da (monomethylation), +28 Da (dimethylation), or +42 Da (trimethylation/acetylation) on lysine was included in the search parameters.

In Vitro Histone Methyltransferase Assays—Methyltransferase assays were carried out by incubating purified Set2-TAP in 30 μl of incubation buffer (50 mM Tris, pH 8.5, 10 mM $MgCl_2$, 1 mM dithiothreitol), containing 10 μg of calf thymus histones (Roche) as substrate and 1 μCi of *S*-adenosyl-L-[methyl-^{3}H]methionine (60 Ci $mmol^{-1}$, Amersham) as methyl donor. After incubation for 60 min at 30 °C, reactions were stopped by boiling in SDS loading buffer, and proteins were separated by 5–15% SDS-PAGE and visualized by Coomassie staining and fluorography.

Binding Assay for Phosphorylated CTD—Equal amounts of Set2-TAP preparations were resolved in parallel on a 5–20% gradient SDS-PAGE gel and proteins in the gel were electrophoretically transferred onto Protran nitrocellulose membrane (Schleicher & Schuell). Blots were incubated in 5% nonfat dry milk in buffer A (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween-20, 5 mM NaF, and 1 mM phenylmethylsulfonyl fluoride) for 1 h at room temperature with rocking, and then the blots were incubated with CTD monoclonal antibodies H5 (Covance, 1:1000 dilution), H14 (Covance, 1:500 dilution), or 8WG16 (a gift of S. Buratowski, 1:500 dilution) for 2 h at room temperature. After four washes with buffer A, the blots were incubated with goat anti-mouse IgM or IgG horseradish peroxidase conjugate (Pierce) for 1 h. The blots were washed four times with buffer A and then developed using Super-Signal West Pico Chemiluminescent Substrate (Pierce) and Biomax film (Kodak).

Recombinant GST-CTD and GST were produced in *Escherichia coli* and purified by glutathione agarose column using standard techniques (41). Phosphorylation of CTD using HeLa nuclear extract as a source of kinase activity was performed as described (42). Briefly, purified GST-CTD or GST (10 μg) was incubated with or without HeLa nuclear extract (100 μg) in buffer B (20 mM Hepes (pH 7.9), 10 mM $MgCl_2$, 2 mM dithiothreitol, 2 mM ATP, 2 μg/ml of BSA, 20 mM β-glycerophosphate, 2 mM benzamidine, 0.01 μg/μl of aprotinin, 0.01 μg/μl of pepstatin A, 0.02 μg/μl of leupeptin, and 80 μM phenylmethylsulfonyl fluoride) for 30 min at 25 °C. The mixture was then incubated with preequilibrated glutathione agarose beads for 30 min at room temperature with rocking. After three washes with buffer C (20 mM Hepes (pH 7.6), 1 mM EDTA, 10% glycerol, 100 mM NaCl, 1 mM dithiothreitol, 0,03% Nonidet P-40, 5 mM NaF, and 1 mM phenylmethylsulfonyl fluoride), the glutathione agarose beads were incubated with purified Set2-TAP in buffer C for 1 h at room temperature with gentle rocking. The beads were washed four times with buffer C and then boiled in SDS sample buffer for 5 min. The supernatants were run on a 5–15% SDS-PAGE gel, and proteins in the gel were visualized by silver staining and identified by tandem mass spectrometry. Sequence analysis by tandem mass spectrometry of the CTD was accomplished chymotrypsin digestion because no trypsin cleavage sites were present.

The procedure used for assay of Set2 binding to synthesized CTD peptides is basically as described (10). Biotinylated CTD peptides $(YSPTSPS)_4$, $(YS\text{-}PO_4\ PTSPS)_4$, and $(YSPTS\text{-}PO_4\ PS)_4$ (a gift of S. Buratowski (13)) were immobilized on avidin beads (Pierce). After washing the beads three times with buffer D (20 mM Hepes, pH 7.9, 0.1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 100 mM NaCl, 0.1% Nonidet P-40, and 1 mM β-glycerophosphate), equal amounts of purified Set2-TAP in buffer D were added to the CTD peptide-bound beads. After incubation for 1 h at room temperature, beads were washed four times with buffer D. Bound proteins to the beads were separated by SDS-PAGE and identified by mass spectrometry as above.

6-AU Sensitivity Assay—All yeast strains used for the 6-AU sensitivity assay contained a *URA3*$^+$ allele in the genome. The *URA3* marker in pRS306 was amplified by PCR and used to replace *ura3-52* in the SF10 strain. The *set2* deletion mutant generated from the *Saccharomyces* Genome Deletion Project was obtained through Invitrogen (record No. 1257). To generate a *set2* deletion mutant in the SF10 strain containing a *URA3*$^+$ allele, KanMX4 flanked by noncoding *Set2* sequences were amplified by PCR using the genomic DNA from the *set2*

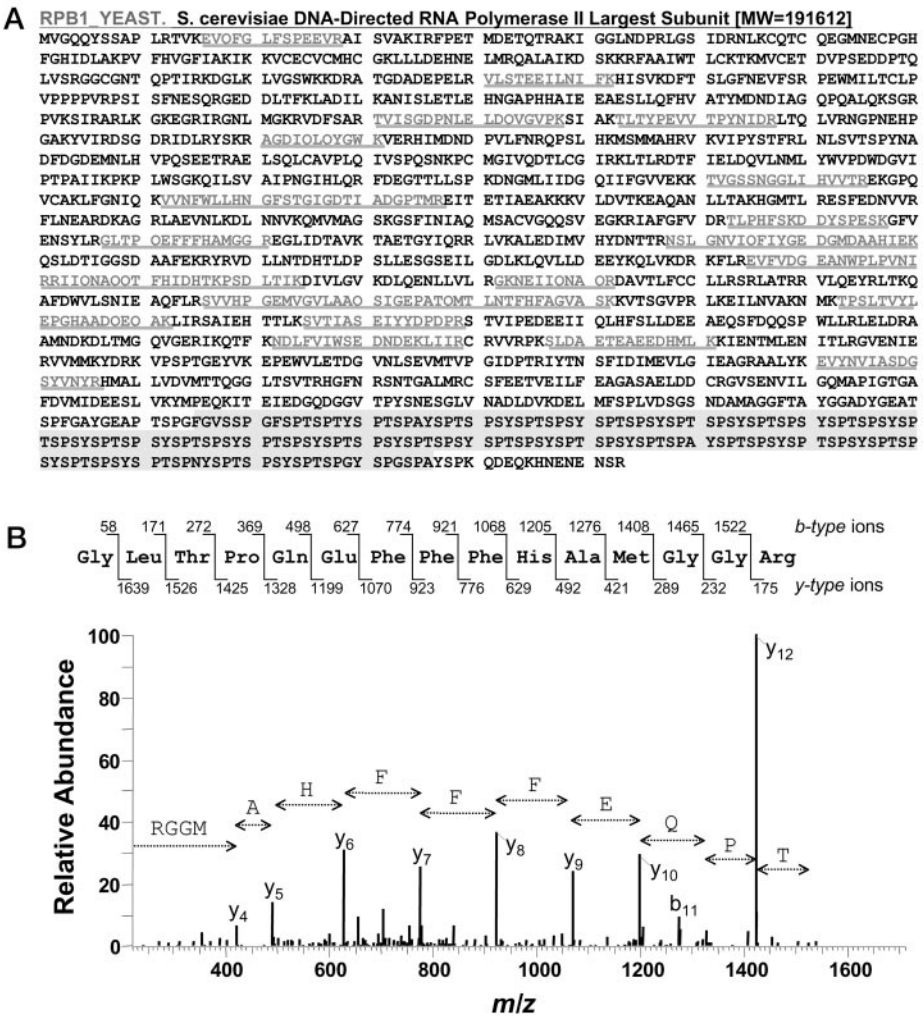


FIG. 2. **Nanoscale microcapillary liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of RPB1 band from Fig. 1A**. *A*, eighteen peptides (*underscored*) from RNA polymerase II subunit 1 were unambiguously matched to acquired tandem mass spectra. The carboxyl-terminal domain (CTD) of the protein is also highlighted. *B*, tandem mass (MS/MS) spectrum of a peptide from *panel A* derived by collision-induced dissociation of the $(M+2H)^{2+}$ precursor, m/z 848.7. Fragment ions in the spectrum represent mainly single-event preferential cleavage of the peptide bonds, resulting in the sequence information recorded from both the N and C termini (*b*- and *y*-type ions, respectively) of the peptide simultaneously. This spectrum was computer-searched with the Sequest program (40) and was matched to the tryptic peptide shown.

deletion strain 1257 with primers: 5′-AACTGCATAGTCGTGCTGTC-3′ and 5′-GCCCCAAATATGCATGTCTG-3′. The resulting PCR product was then used for transformation of the SF10 strain containing a *URA3*$^+$ allele, and positive transformants were selected by geneticin (200 mg/liter) in YPD medium. Media containing 6-AU were prepared by supplementing SD-Ura media with 100 μg/ml 6-azauracil (product no. 12,329-3, Aldrich). Overnight cultures were diluted 1:25 and grown to log phase at 30 °C. Five microliters of 10-fold serial dilutions of each culture in water were spotted on SD-Ura plates with or without 6-AU. Plates were incubated at 30 °C for 3 to 4 days and photographed using a Nikon digital camera.

RESULTS

Set2 Interacts with RNA Polymerase II—To identify proteins that associate with the histone methyltransferase Set2, the tandem affinity purification (TAP) approach was used. In this approach, two protein A repeats and a calmodulin-binding peptide (CBP), linked by the tobacco etch virus protease recognition site, are fused to the protein of interest and used for sequential affinity purification on resins containing immobilized immunoglobulin G and calmodulin. Because expression of the targeted gene is still controlled by its own endogenous promoter, protein complexes isolated through the target protein are expressed at their natural levels (36).

We targeted Set2 at its carboxyl terminus with a TAP tag and purified Set2-TAP as outlined above. Consistent with previous reports (31), the purified Set2-TAP has methyltranferase activity against histone H3 (data not shown), indicating that Set2-TAP is functional. After affinity purification of Set2-TAP, the proteins were resolved on an SDS-PAGE gel and stained by silver (Fig. 1). Peptide sequencing by tandem mass spectrometry indicated that the largest and the second largest subunits of RNA polymerase II (RPB1 and RPB2) and Set2 were found in the preparation (Fig. 1). As an example, the amino acid sequence analysis of RPB1 is shown in Fig. 2. These results suggest that Set2 associates with RNA polymerase II. As a further test, RPB2 was tagged with a TAP tag, and its associated proteins were identified by tandem mass spectrometry. We found 11 subunits of the RNA polymerase II as well as Set2 (Fig. 3). These data indicate that Set2 interacts with RNA polymerase II.

Interaction of Set2 with RNA Polymerase II Requires the WW Domain in Set2 and Phosphorylation of RNA Polymerase II—The Set2 protein contains a putative WW domain (Fig. 4*A*, also see Ref. 31). WW domains, named for two signature tryptophan (W) residues that are spaced 20–22 amino acids apart, are small modules found in a number of unrelated proteins and have been implicated in binding to proteins with proline-rich sequences (43). Some WW domain-containing proteins interact with RNA polymerase II through their WW domains (43–45). To determine whether the putative WW domain in Set2 is involved in the interaction of Set2 with RNA polymerase, we constructed a TAP-tagged version of Set2 that lacked the WW domain. As shown in Fig. 4*B*, Set2 with deleted WW domain could not interact with RPB1 and RPB2, indicating that the WW domain in Set2 protein is required for the interaction between Set2 and RNA polymerase II. In addition, when the RPB2-TAP complex isolated from the IgG-agarose column was treated with alkaline phosphatase and then run through the calmodulin affinity column, Set2 could not be detected in the

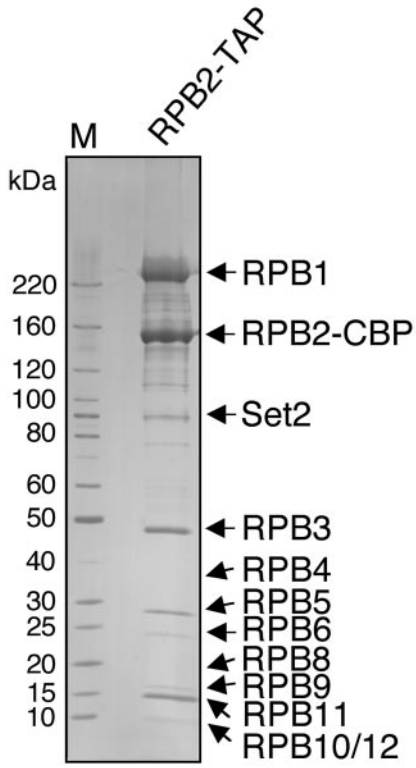


FIG. 3. **Association of RNA polymerase II with Set2.** RPB2 was tagged with TAP tags and affinity-purified through IgG agarose and calmodulin agarose columns. The purified proteins were resolved by SDS-PAGE, stained by silver, and identified by tandem mass spectrometry. The molecular weight markers are shown on the *left*. CBP, calmodulin-binding peptide, a remnant of the original TAP tag.

complex (Fig. 4*C*), suggesting that phosphorylation of RNA polymerase II is important for its interaction with Set2 protein. Furthermore, it suggests that the absence of phosphatase inhibitors during purification steps may result in an inability to detect the interaction between Set2 and RNA polymerase II.

Set2 Binds Directly to the Phosphorylated CTD of RPB1—The CTD of the largest subunit of RNA polymerase functions as a platform for interaction with other proteins that associate with RNA polymerase II (6). To determine whether the association of Set2 with RNA polymerase II is through the CTD, *in vitro* binding assays using purified Set2-TAP and recombinant GST-CTD were carried out. GST-CTD was phosphorylated in HeLa nuclear extract in the presence of ATP (phosphorylation of GST-CTD was confirmed by immunoblotting using anti-phosphorylated CTD antibody H5, data not shown). In parallel, GST protein was treated by incubation of HeLa nuclear extract and ATP. Unphosphorylated and phosphorylated GST-CTD proteins as well as GST untreated or treated with HeLa nuclear extract in the presence of ATP were immobilized onto glutathione agarose. After incubation with purified Set2-TAP, the glutathione bead-bound proteins were resolved on an SDS-polyacrylamide gel. One band was found in the unphosphorylated GST-CTD sample (Fig. 5*A, lane 1*), whereas two bands were found in the phosphorylated GST-CTD sample (Fig. 5*A, lane 2*). Peptide sequencing (chymotrypsin digestion) by mass spectrometry confirmed that the band found in the untreated sample (Fig. 5*A, lane 1*) and the higher band (Fig. 5*A, lane 2*) in the GST-CTD sample treated with HeLa nuclear extract were GST-CTD (phosphorylation of GST-CTD by HeLa nuclear extract resulted in mobility shift of GST-CTD), whereas the lower band found only in phosphorylated GST-CTD sample was Set2 (Fig. 5*A, lane 2*). No bands were found in the corresponding regions of the gel from GST untreated or treated with HeLa nuclear extract (Lanes 3 and 4, Fig. 5*A, lanes 2* and *4*). These results indicate that Set2 interacts only with phosphorylated GST-CTD.

To further determine which phosphorylation sites in CTD are important for Set2 binding, equal amounts of purified Set2-TAP protein complex were resolved in parallel on an SDS-PAGE gel, transferred onto a nitrocellulose membrane, and probed with CTD monoclonal antibodies H5, H14, and 8WG16, which recognize CTD with phosphorylated Ser^2, CTD with phosphorylated Ser^5, and unphosphorylated CTD, respectively. As shown in Fig. 5*B*, RPB1 pulled down by Set2 was mainly recognized by H5 but not by H14 or 8GW16, indicating that Set2 mainly associates with RPB1 with CTD phosphorylated at Ser^2 positions.

In addition, when purified Set2-TAP was incubated with immobilized biotinylated CTD peptides, Set2 was found to bind to CTD peptide phosphorylated on Ser^2 but not phosphorylated on Ser^5 or unphosphorylated CTD peptide (Fig. 5*C*). Together, these results indicate that Set2 interacts with RNA polymerase II through binding to Ser^2-phosphorylated CTD of the largest subunit of the RNA polymerase II.

Set2 Deletion Mutant Shows Transcription-Elongation Defects—In budding yeast, Ser^2 of CTD is predominantly phosphorylated during the elongation phase (9, 10); therefore, Set2 may be associated with elongating RNA polymerase II by binding to phosphorylated Ser^2 of CTD (Fig. 5). Mutants defective in transcription elongation render yeast cells sensitive to 6-AU; therefore, sensitivity to 6-AU has been used as an assay for involvement in transcription elongation (46). To determine whether Set2 is important for transcription elongation, we tested the Δ*set2* strain for sensitivity to 6-AU. As shown in Fig. 6, the Δ*set2* strain grew more slowly on medium containing 6-AU than did the parent strain. In contrast, the Δ*set2* strain grew like the parent strain on medium lacking 6-AU (Fig. 6). These results suggest that Set2 play a role in transcription elongation *in vivo*.

DISCUSSION

In yeast, the ubiquitin ligase Rsp5 binds both unphosphorylated and phosphorylated CTD through its WW domains (45, 47), whereas the splicing factor Prp40 and the prolyl isomerase Ess1 only associate with the phosphorylated CTD through their WW domains (44, 45). Furthermore, there is little difference in the affinity of Ess1 for CTD peptides phosphorylated at Ser^2 or Ser^5 (48). Our results demonstrate that Set2 binds preferentially to CTD with phosphorylated Ser^2 and the WW domain in Set2 is required for the binding of Set2 to RPB1.

Whereas Ser^5 of CTD is strongly phosphorylated at initiation and early elongation phase, Ser^2 is predominantly phosphorylated during the elongation phase (9, 10). The yeast CTD kinase I phosphorylates the CTD on Ser^2 of the heptapeptide repeats. This is distinct from the sites of phosphorylation by transcription factor IIH and Srb10, which reside mostly at positions Ser^5, and Ser^5 and Ser^2, respectively (49). CTD kinase I is composed of three subunits termed Ctk1, Ctk2, and Ctk3. Deletion of the gene *CTK1*, encoding the catalytic subunit of CTD kinase I, causes a loss of phosphorylation of the CTD at Ser^2 of the heptad repeat (8, 13). Furthermore, *in vitro* studies using a HeLa nuclear extract showed that yeast CTD kinase I could promote transcription elongation but had little effect on initiation (50). These studies thus suggested a role for CTD kinase I in transcription elongation. Phosphorylation of CTD on Ser^2 may allow RNA polymerase II to associate with other factors such as Set2 that allow efficient elongation.

Consistent with the above hypothesis, we found that Δ*set2* is sensitive to 6-azauracil (Fig. 6). Mutations in genes that encode

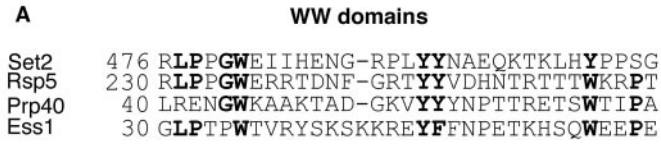
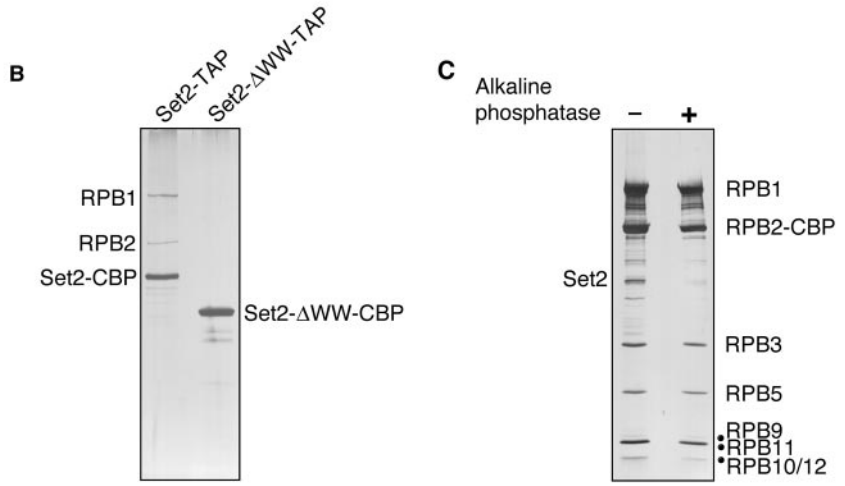
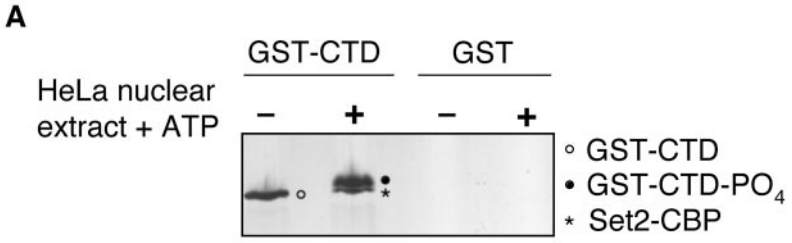
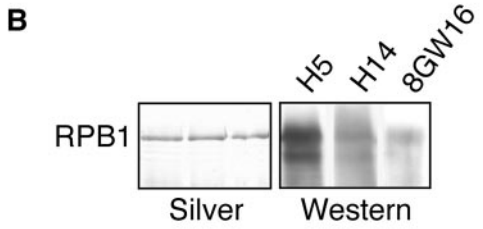


FIG. 4. **Set2 interaction with RNA polymerase II requires the WW domain in Set2 and phosphorylation of RNA polymerase II.** *A*, the putative WW domain was aligned with the WW domains of three other yeast proteins with conserved amino acids in **bold**. *B*, proteins associated with Set2-TAP or Set2-ΔWW-TAP were purified as described. No RNA polymerase II was associated with Set2-ΔWW-TAP. *C*, proteins associated with RPB2-TAP were isolated through IgG affinity chromatography and then treated or untreated with alkaline phosphatase. The proteins were run through calmodulin affinity column. The resulting purified proteins were resolved by SDS-PAGE, stained with silver, and identified by tandem mass spectrometry. CBP, calmodulin-binding peptide, a remnant of the original TAP tag.







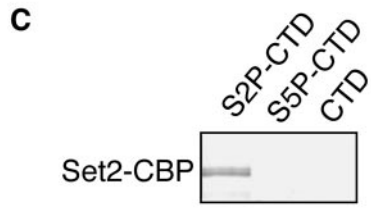


FIG. 5. **Set2 binds directly to the phosphorylated CTD.** *A*, GST-CTD or GST was incubated with HeLa nuclear extract in the presence of ATP. The treated GST-CTD or GST and untreated GST-CTD or GST were immobilized onto glutathione agarose beads and incubated with purified Set2-CBP. Bound proteins were resolved by SDS-PAGE, stained by silver, and identified by tandem mass spectrometry. Phosphorylation of the CTD (GST-CTD-PO_4) by HeLa nuclear extract caused a shift in the migration of the protein. *B*, equal amounts of purified Set-TAP complex were resolved in parallel on SDS-PAGE gel. After transfer onto a nitrocellulose membrane, the blots were detected with H5, H14,and 8WG14 antibodies. *C*, biotinylated CTD peptides $(YSPTSPS)_4$, $(YS\text{-}PO_4\ PTSPS)_4$, and $(YSPTS\text{-}PO_4\ PS)_4$ were immobilized on avidin beads. After washing the beads, equal amount of purified Set2-CBP was added. After incubation for 1 h and then washing, bound proteins to the beads were separated by SDS-PAGE and stained with silver. CBP, calmodulin-binding peptide, a remnant of the original TAP tag.

elongation factors and elongation-defective forms of RNA polymerase II are 6-azauracil-sensitive (11, 14–17). Interestingly, deletion of the gene *CTK1*, which encodes the catalytic subunit of the CTD kinase I being responsible for phosphorylation of Ser^2 of the CTD heptad repeat, resulted in the yeast sensitive to 6-azauracil (19). Together, these data suggest that phosphorylation of Ser^2 of the CTD heptad repeat creates a binding site for the Set2 protein. Methylation of histone H3 at Lys^{36} by Set2 is therefore likely to be important.

During transcription elongation, phosphorylation of Ser^2 increases (9, 10), and this may create the binding sites for Set2 to associate with RNA polymerase II. Association of Set2 with

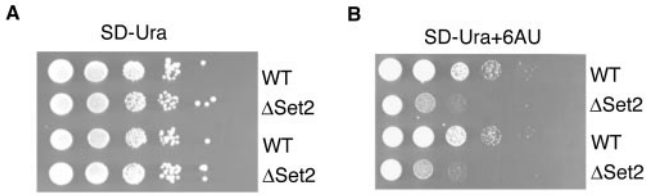


FIG. 6. ***set2* deletion mutant has transcription elongation defect phenotype.** 10-fold serial dilutions of the *set2* deletion mutant strain (Δ*set2*) or its parental strain (*WT*) were plated on medium lacking uracil (SD-Ura) in the absence (*A*) or presence (*B*) of 100 μg/ml 6AU. The cells were grown at 30 °C for 3–4 days.

elongating RNA polymerase II may allow Set2 to methylate histone H3 and then affect local nucleosome structure. It is also possible that Set2 affects transcription elongation by targeting non-H3 protein components associated with the elongating RNA polymerase II, and this affects elongation, although we did not detect any methylation of the largest and second largest subunits of RNA polymerase II treated by Set2 (data not shown). In the future, it would be interesting to determine the consequence of H3 Lys^{36} methylation on transcription elongation as well as to see whether Set2 can methylate other nuclear proteins.

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