MOLECULAR AND CELLULAR BIOLOGY, June 2003, p. 4207–4218 Vol. 23, No. 12
0270-7306/03/$08.00+0 DOI: 10.1128/MCB.23.12.4207–4218.2003

Methylation of Histone H3 by Set2 in *Saccharomyces cerevisiae* Is Linked to Transcriptional Elongation by RNA Polymerase II

Nevan J. Krogan,[1,2,3] Minkyu Kim,[4] Amy Tong,[1,2] Ashkan Golshani,[1] Gerard Cagney,[1,2,3] Veronica Canadien,[5] Dawn P. Richards,[5] Bryan K. Beattie,[5] Andrew Emili,[1,2,3] Charles Boone,[1,2] Ali Shilatifard,[6] Stephen Buratowski,[4] and Jack Greenblatt[1,2,3]*

Banting and Best Department of Medical Research[1] and Toronto Yeast Proteomics Organization,[3] University of Toronto, Toronto, Ontario, Canada M5G 1L6; Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8[2]; Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115[4]; Affinium Pharmaceuticals, Toronto, Ontario, Canada, M5J 1V6[5]; and Department of Biochemistry and Cancer Center, St. Louis University School of Medicine, St. Louis, Missouri 63104[6]

Received 13 January 2003/Returned for modification 27 February 2003/Accepted 20 March 2003

Set2 methylates Lys36 of histone H3. We show here that yeast Set2 copurifies with RNA polymerase II (RNAPII). Chromatin immunoprecipitation analyses demonstrated that Set2 and histone H3 Lys36 methylation are associated with the coding regions of several genes that were tested and correlate with active transcription. Both depend, as well, on the Paf1 elongation factor complex. The C terminus of Set2, which contains a WW domain, is also required for effective Lys36 methylation. Deletion of *CTK1*, encoding an RNAPII CTD kinase, prevents Lys36 methylation and Set2 recruitment, suggesting that methylation may be triggered by contact of the WW domain or C terminus of Set2 with Ser2-phosphorylated CTD. A *set2* deletion results in slight sensitivity to 6-azauracil and much less β-galactosidase produced by a reporter plasmid, resulting from a defect in transcription. In synthetic genetic array (SGA) analysis, synthetic growth defects were obtained when a *set2* deletion was combined with deletions of all five components of the Paf1 complex, the chromodomain elongation factor Chd1, the putative elongation factor Soh1, the Bre1 or Lge1 components of the histone H2B ubiquitination complex, or the histone H2A variant Htz1. *SET2* also interacts genetically with components of the Set1 and Set3 complexes, suggesting that Set1, Set2, and Set3 similarly affect transcription by RNAPII.

In *Saccharomyces cerevisiae* RNA polymerase II (RNAPII) initiates transcription in concert with general transcription factors and a 20-subunit mediator complex (16, 38, 59). During initiation it becomes phosphorylated by the Kin28 subunit of the general transcription factor TFIIH on Ser5 of the heptapeptide repeats, YSPTSPS, in the carboxy-terminal domain (CTD) of its largest subunit, Rpb1, resulting in recruitment of the mRNA-capping enzyme to the transcription complex (23, 32, 49, 69). Ser5 phosphorylation declines during the early stages of elongation and is replaced by Ser2 phosphorylation, mediated mainly by the cyclin-dependent kinase, Ctk1, during the later stages of elongation by RNAPII (10, 23). General transcription factors and mediator dissociate from the transcription complex or remain behind at the promoter and are replaced by various elongation factors during chain elongation by RNAPII (25, 43). Among these elongation factors are Spt4/Spt5, Spt6/Iws1, and Spt16/Pob3, whose patterns of genetic interactions suggest that they participate in transcription on chromatin templates (66). Another elongation factor is the Paf1 complex, which consists of Paf1, Rtf1, Cdc73, Ctr9, and Leo1 and associates with RNAPII (25, 34, 52). Chromatin immunoprecipitation (ChIP) experiments have shown that all of these polypeptides are associated with transcribed regions (25, 43).

* Corresponding author. Mailing address: Banting and Best Department of Medical Research, University of Toronto, 112 College St., Toronto ON, Canada M5G 1L6. Phone: (416) 978-4141. Fax: (416) 978-8528. E-mail: jack.greenblatt@utoronto.ca.

Histone methylation by SET domain-containing histone lysine methyltransferases has important roles in chromatin structure and function (44). Lysines 4, 9, 27, and 79 are well-studied sites of methylation on histone H3, while lysine 20 is the only known methylated lysine in histone H4 (55, 64). There are at least six proteins with recognizable SET domains in *S. cerevisiae*. Set1, a component of an eight-protein complex (COMPASS), methylates Lys4 of histone H3 (7, 24, 33, 36, 46). Recruitment of COMPASS to the early transcribed region, as well as histone H3 Lys 4 trimethylation in this region, requires both Ser5 phosphorylation of the RNAPII CTD by Kin28 and the Rtf1, Paf1, and Ctr9 subunits of the Paf1 complex (26, 37, 48). Methylation of H3 Lys4 by Set1, as well as methylation of H3 Lys79 by Dot1, also requires ubiquitination of histone H2B by Rad6 and Bre1 (13, 19, 58, 67).

Set2 methylates Lys36 of histone 3 (54). Members of the mammalian nuclear receptor-binding SET-domain-containing family (NSD1, NSD2, and NSD3) contain a SET domain that is highly related to that of yeast Set2. NSD genes have been implicated in acute myeloid leukemia (21, 47, 53), whereas the NSD2 gene maps to the region associated with Wolf-Hirschhorn syndrome (47), which is characterized by mental retardation and developmental defects.

In this study, we found that Set2 interacts physically with RNAPII. Similar observations have been made recently by other groups (28, 29, 68). We used ChIP assays to show that Set2 is recruited and methylates histone H3 Lys36 in the coding regions of actively transcribed genes. Synthetic growth de-

fects were observed when a *set2* deletion was combined with deletions of known or suspected transcriptional elongation factors, including Chd1, Soh1, and members of the Paf1 elongation complex, as well as components of the Set1 and Set3 complexes. A deletion of *set2* also results in slight sensitivity to the drug 6-azauracil (6-AU) and a decrease in β-galactosidase from a *lacZ* reporter plasmid, implicating Set2 in transcriptional regulation. Finally, recruitment of Set2 and Lys36 histone H3 methylation relies on components of the Paf1 complex as well as the RNAPII CTD kinase Ctk1. These results suggest that binding of Set2 to RNAPII and histone H3 methylation occur during transcriptional elongation and depend on both the Paf1 elongation factor and the phosphorylation state of the CTD. The involvement of SET domain proteins in transcriptional elongation would not be entirely unexpected, because a translocation that joins part of the human elongation factor ELL to part of the SET domain protein MLL leads to myelogenous leukemia (12).

MATERIALS AND METHODS

Yeast strains used in this study. The following yeast strains were used in this study: NJK581 *MAT***a** *ura3-1 leu2-3,112 his3-11,15 trp1*Δ *ade2-1 can1-100 set2-TAP*::*TRP1* and NJK742 *MAT***a** *ura3-1 leu2-3,112 his3-11,15 trp1*Δ *ade2-1 can1-100 set2*(Δ*476-733*)*-TAP*:*TRP1*. Strains with the following genes replaced by a kanamycin resistance cassette were obtained from Research Genetics (http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions3.html): *CDC73*, *RTF1*, *CTK1*, and *SET2*. Tandem affinity purification (TAP)-tagged versions of Set2 were then made in these backgrounds (4, 17), as follows: YSB971, *MAT***a** *ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 set2-TAP*::*HIS3 ctk1*Δ::*KanMX*; YSB972, *MAT***a** *ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 set2-TAP*::*HIS3 rtf1*Δ::*KanMX*; and YSB973, *MAT***a** *ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 set2-TAP*::*HIS3 cdc73*Δ::*KanMX*. Sensitivity to 6-AU was tested by plating strains harboring pRS316 (51) onto plates lacking uracil and containing 25 μg of 6-AU per ml.

ChIP assays. ChIP assays were performed essentially as described earlier (23). Yeast strains were grown at 30°C to an optical density at 595 nm (OD_{595}) of 0.4 to 0.6. For the ChIP with Rpb3 on the *lacZ* gene, both wild-type and *set2*Δ strains harboring p416*GAL1-lacZ* were incubated until they reached an OD_{595} of approximately 1 in medium containing 2% glucose. Cells were harvested, washed three times with sterile water, and divided into two aliquots. The cells were reinoculated into media containing either 2% glucose or 2% galactose. After 4 h at 30°C, an aliquot was removed for the β-galactosidase assay and the remaining cells were cross-linked with formaldehyde for ChIP assays. The antibody against Rpb3 was obtained from Neoclone Biotechnology. Unpublished primers used for PCR in this study are designated by the name of the gene followed by the position of its 5′ end relative to the translation initiation codon, as follows: Gal1-lacZ$_{-340}$ (CGAACAATAAAGATTCTACAATACTAGC); Gal1-lacZ$_{-70}$ (CATTTGAATAAGAAGTAATACAAACCGA); Gal1-lacZ$_{715}$ (TGTACT GGAGGCTGAAGTTCAGAT); Gal1-lacZ$_{1025}$ (CAGACCATTTTCAATCCG CACCTC); Gal1-lacZ$_{1567}$ (AATGGCTTTCGCTACCTGGAGAGA); Gal1-lacZ$_{1827}$ (CAAAGACCAGACCGTTCATACAGA); Gal1-lacZ$_{2247}$ (ATCGAG CTGGGTAATAAGCGTTGG); Gal1-lacZ$_{2465}$ (GTATCTGCCGTGCACTGC AACAAC); Gal1-lacZ$_{3094}$ (AATGGCGATTACCGTTGATGTTGAA); Gal1-lacZ$_{3414}$ (GTTTCCATCAGTTGCTGTTGACTGT).

Purification and analysis of Set2. TAP-tagged Set2 was purified on immunoglobulin G (IgG) and calmodulin columns from extracts of yeast cells (3 liters) grown in yeast extract-peptone-dextrose medium to an OD_{600} of 1.0 to 1.5. The cell pellets (7 to 10 g) were frozen in liquid nitrogen and lysed by grinding with dry ice in a Krups coffee grinder (model no. 203-70). An equal volume of buffer (250 mM KCl, 100 mM HEPES-KOH [pH 7.9], 1 mM EDTA, 2.5 mM dithiothreitol [DTT]) was added, and following centrifugation in a Beckman Ti70 rotor at 4°C for 2 h at 34,000 rpm, the supernatant was dialyzed against IPP buffer (10 mM Tris-Cl [pH 7.9], 0.1% Triton X-100, 0.5 mM DTT, 0.2 mM EDTA, 20% glycerol, 100 mM NaCl). After dialysis, the extract was again centrifuged in a Ti70 rotor at 4°C for 30 min at 34,000 rpm and the supernatant was mixed for 3 h with 200 μl of IgG-Sepharose (Pharmacia) equilibrated with IPP buffer. Following binding, the IgG-Sepharose was washed with 1 ml of IPP buffer followed by 400 μl of TEV protease cleavage buffer (50 mM Tris-Cl [pH 7.9], 1 mM DTT, 0.1% Triton X-100, 100 mM NaCl). The beads were then incubated overnight at 4°C with 100 U of TEV protease (Life Technologies) in 200 μl of TEV cleavage buffer. The eluate was combined with a 200-μl wash with TEV cleavage buffer. To this were added 200 μl of calmodulin binding buffer (10 mM Tris-Cl [pH 7.9], 10 mM β-mercaptoethanol, 2 mM $CaCl_2$, 0.1% Triton X-100, 100 mM NaCl) and 200 μl of calmodulin beads (Pharmacia) equilibrated with the same buffer. After binding for 1 to 2 h at 4°C, the calmodulin beads were washed with 200 μl of calmodulin binding buffer and 200 μl of calmodulin wash buffer (10 mM Tris-Cl [pH 7.9], 10 mM β-mercaptoethanol, 0.1 mM $CaCl_2$, 0.1% Triton X-100, 100 mM NaCl). The purified protein complexes were eluted from the calmodulin beads with 5 × 100 μl of calmodulin elution buffer (10 mM Tris-Cl [pH 7.9], 10 mM β-mercaptoethanol, 3 mM EGTA [pH 8.0], 0.1% Triton X-100, 100 mM NaCl). The purified proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on gels containing 10% polyacrylamide, and the proteins were visualized by silver staining. Peptide samples were spotted onto a target plate with a matrix of α-cyano-4-hydroxycinnamic acid (Fluka). Matrix-assisted laser desorption ionization–time of flight (mass spectrometry) (MALDI-TOF) analysis was conducted by utilizing a Reflex IV (Bruker Daltonics, Billerica, Mass.) instrument in positive-ion reflectron mode (31, 50). Western blotting was performed by using standard techniques with the monoclonal antibodies 8WG16, H5, and H14 (6, 40, 60).

SGA analysis. Synthetic genetic array (SGA) analysis was carried out as previously described (61).

RESULTS

Interaction of Set2 with RNAPII. In order to further characterize *S. cerevisiae* Set2, we used single-step transformation to place a TAP tag (45) containing a calmodulin-binding peptide and *Staphylococcus aureus* protein A, separated by a TEV protease cleavage site, at the C terminus of Set2. To confirm the success of the tagging procedure, Western blotting was performed on extract derived from the tagged strain by making use of an irrelevant immunoglobulin to recognize the protein A component of the TAP tag. The tagged protein was then purified on IgG and calmodulin columns and analyzed by SDS-PAGE followed by staining with silver. Protein bands absent from a control preparation and corresponding to the tagged protein and any associated proteins were then identified by MALDI-TOF (50). Set2 copurified with substoichiometric amounts of two larger polypeptides identified as Rpb1 and Rpb2, the two largest subunits of RNAPII (Fig. 1A). This physical association was confirmed when Set2 and several elongation factors copurified with RNAPII from a strain harboring a TAP tag on Rpb1 (data not shown). We were not able to identify by mass spectrometry any of the other minor polypeptides that copurified with the Set2-TAP shown in Fig. 1A, other than Rrp5 and fragments of Set2. Tagged Rrp5 did not copurify with Set2 (data not shown). Moreover, the minor polypeptides other than Rpb1 and Rpb2 that copurified with Set2-TAP have not been reproducibly detected in other preparations.

RNAPII phosphorylated on Ser2 on the Rpb1 CTD heptapeptide repeats has been shown to localize to transcribed coding regions and 3′ ends of genes, whereas Ser5 phosphorylation on the CTD is found primarily in 5′ early elongation regions (23). Western blotting analyses performed with monoclonal antibodies H5 and H14 (6, 40), which recognize the Rpb1 CTD repeats phosphorylated on Ser2 and Ser5, respectively, showed that the RNAPII copurifying with Set2 was phosphorylated on both residues (Fig. 1B), suggesting that Set2 might be associated with coding regions during transcription. As a control, Western blotting with H5 and H14 did not detect Rpb1 when a parallel purification was done by using an extract from an untagged strain. The Rpb1 that copurified with Set2 from a Set2-TAP strain was also recognized weakly by the

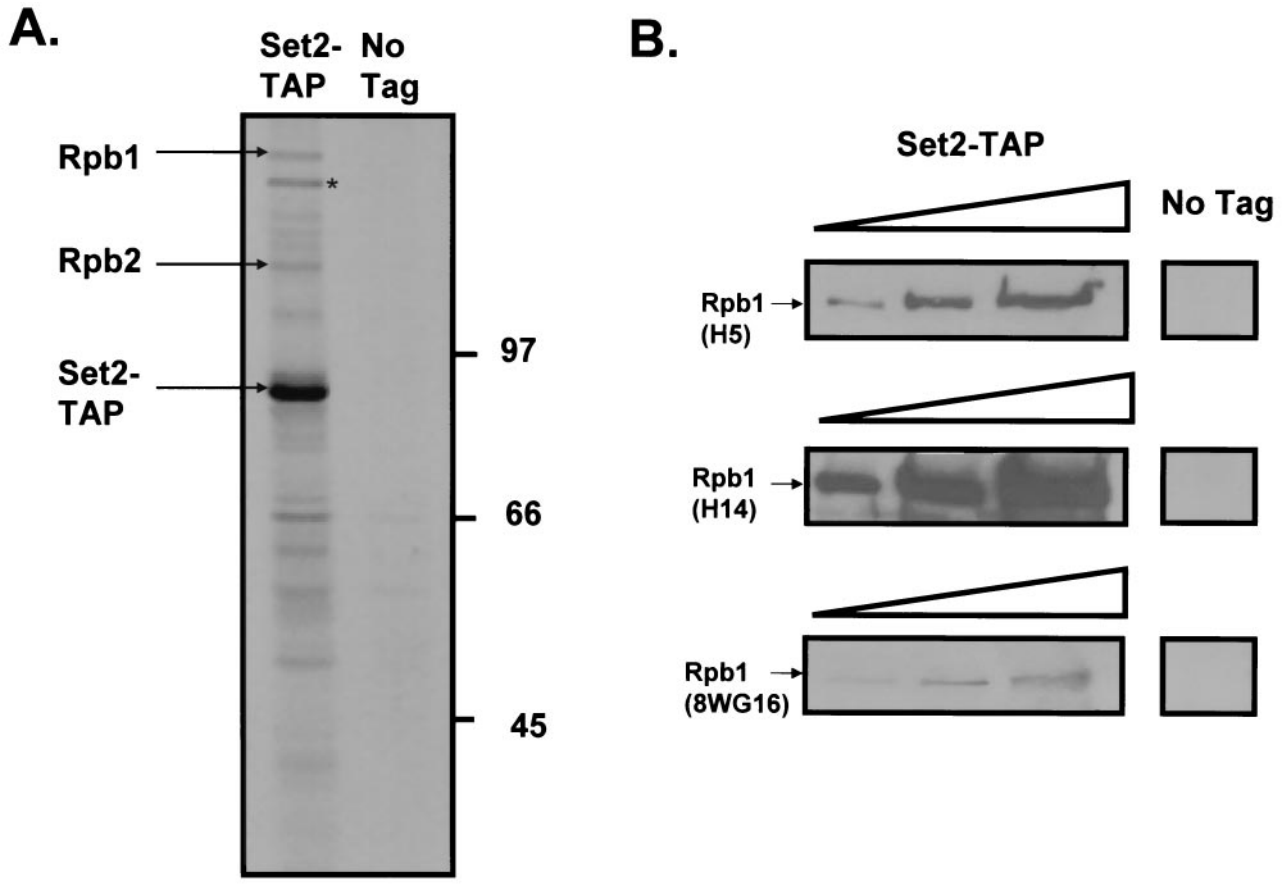


FIG. 1. TAP of Set2. (A) Purification of Set2 was carried out with strains containing either no tagged protein or a TAP-tagged version of Set2. The protein complex was purified in the presence of 100 mM NaCl as described in the text and was then analyzed by SDS-PAGE and silver staining. Set2 and subunits of RNAPII were identified by trypsin digestion and MALDI-TOF. The asterisk indicates another polypeptide, Rrp5, which was also identified in this preparation and is most likely a contaminant. (B) Immunoprecipitation with IgG followed by Western blot analysis using the monoclonal antibodies H5 and H14 (6, 40) demonstrated that Set2 copurified with RNAPII phosphorylated on both Ser2 and Ser5 in the CTD repeats of Rpb1. The RNAPII that copurified with Set2 also reacted with the antibody 8WG16 (60), which recognizes both partially phosphorylated and unphosphorylated Rpb1. Western blotting analyses with immunoprecipitates using extracts from an untagged strain were also performed as controls with all three antibodies. Successive lanes were loaded with 0.5, 1.5, and 2.5 μg of extract protein.

monoclonal antibody 8WG16 (60), which recognizes both unphosphorylated and partially phosphorylated Rpb1 (Fig. 1B). These results suggested that Set2 might be associated with elongating RNAPII.

Localization of Set2 and histone H3 Lys36 methylation to the coding regions of transcribed genes. ChIP assays in which proteins were cross-linked to DNA in vivo by using formaldehyde were employed to analyze the in vivo distribution of Set2 and histone H3 Lys36 methylation along various transcribed genes (23). Following isolation and shearing of chromatin, Set2-TAP or Lys36-methylated histone H3 was immunoprecipitated with either rabbit IgG agarose (directed against protein A on the TAP tag) or antibody directed against methylated histone H3 Lys36 (Upstate Biotechnology). After reversal of the cross-links, PCR analyses were performed on the coprecipitated DNA. Primer pairs directed against promoter regions, coding regions, and 3′ untranslated regions were used to analyze the *ADH1*, *PYK1*, and *PMA1* genes, encoding alcohol dehydrogenase, pyruvate kinase, and plasma membrane ATPase, respectively (Fig. 2A). *Set2-TAP*, of all three genes that were tested, was found to cross-link most strongly to the coding regions and not to the promoter or 3′ untranslated regions. Similarly, when ChIP experiments were carried out with antibody directed against Lys36-methylated histone H3, the same pattern was observed: an enrichment of cross-linked DNA corresponding primarily to the coding regions of the genes that were studied (Fig. 2B).

To directly test whether the presence of Set2 is correlated with active transcription, ChIP was performed on the galactose-inducible gene, *GAL1*, which encodes galactokinase. In the absence of galactose, virtually no Set2 cross-linked to any part of the *GAL1* gene. Upon induction, however, both Set2-TAP and methylated histone H3 Lys36 were detected primarily in the coding region of *GAL1* (Fig. 2C), providing further evidence that the presence of Set2 and its histone-methylating activity require elongation by RNAPII.

Influence of Set2 on transcription by RNAPII. The presence of Set2 in transcribed regions and its interaction with RNAPII suggested that Set2 might influence transcription by RNAPII. To determine whether yeast Set2 influences transcription elon-

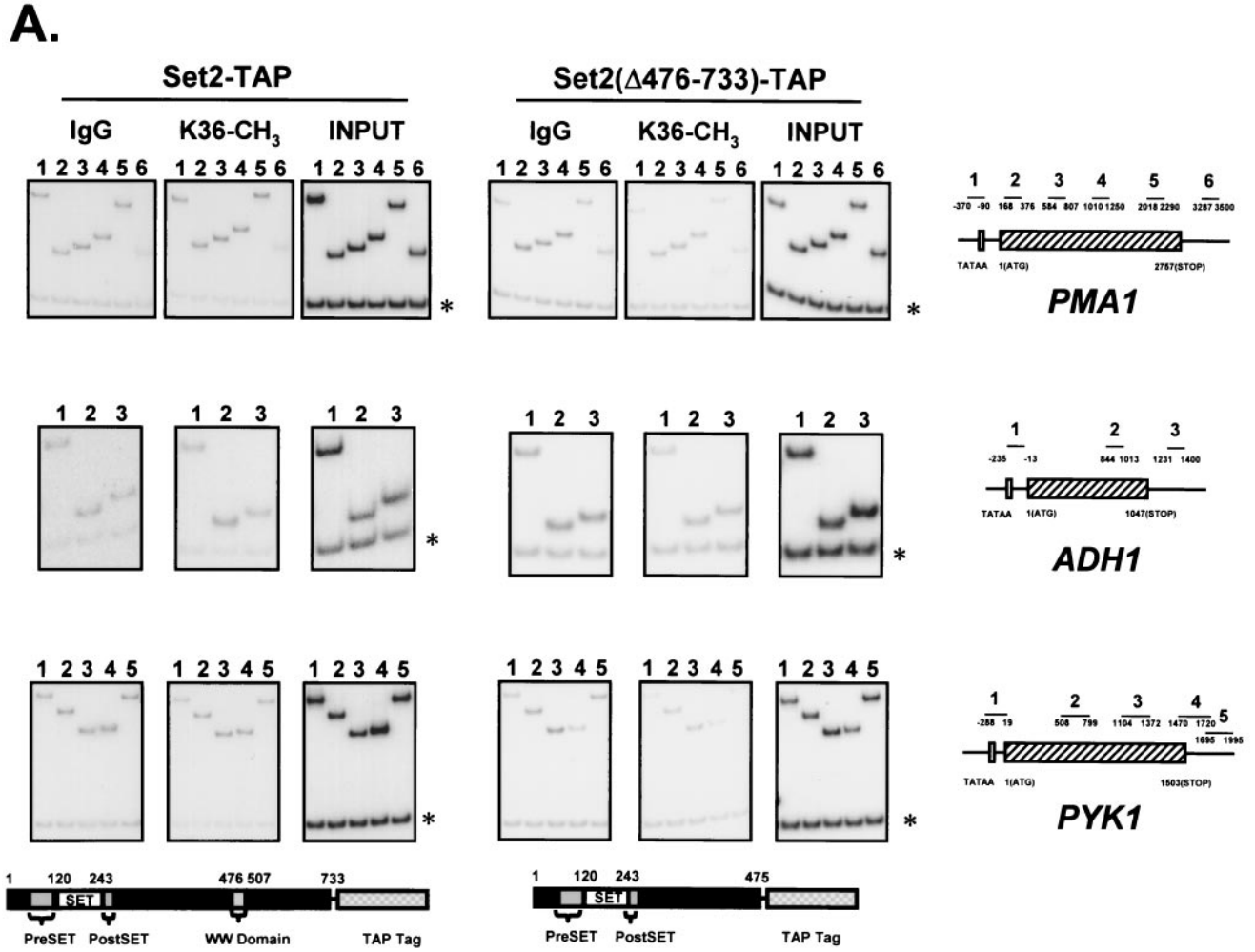
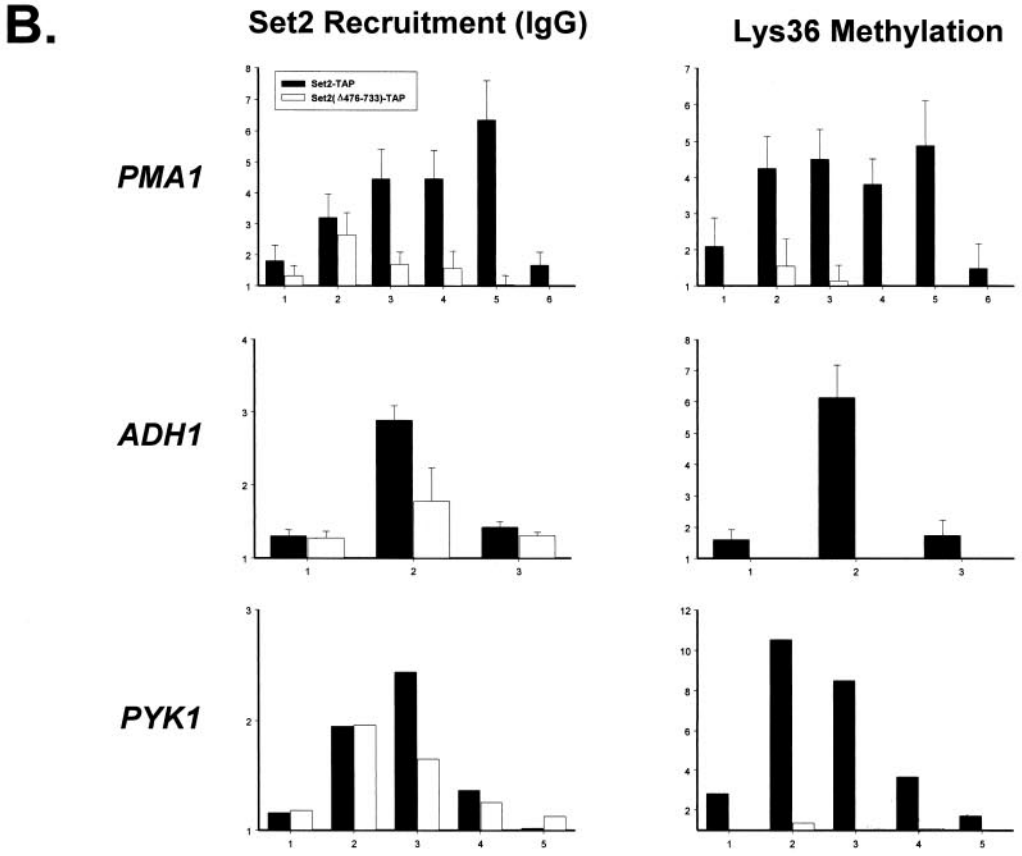


FIG. 2. Set 2 and histone H3 methylation on Lys36 localize to transcribed regions. (A) ChIP was performed to monitor the presence of either the Set2 protein or Lys36 methylation on histone H3 along the *PMA1*, *ADH1*, and *PYK1* genes. Chromatin was immunoprecipitated either with rabbit IgG-agarose from strains containing TAP-tagged versions of Set2 or with antibody against Lys36 methylated histone H3 (Upstate Biotechnology) from a strain with no tag. The Set2(Δ476-733)-TAP strain was constructed by recombining in the TAP tag so as to remove the last 258 amino acids of Set2. PCR amplification was carried out by using primer pairs recognizing promoter (lane 1), coding (lanes 2, 3, 4, and 5), and 3′ untranslated (lane 6) regions for *PMA1*; promoter (lane 1), coding (lane 2), and 3′ untranslated (lane 3) regions for *ADH1*; and promoter (lane 1), coding (lanes 2, 3, and 4), and 3′ untranslated regions for *PYK1*. Each PCR contained a second primer pair that amplified a region of chromosome V devoid of open reading frames (marked by asterisks), thus providing an internal control for background. Input, signal from chromatin before immunoprecipitation. Primer pairs used are as follows: for PMA1, $PMA1_{-370}$ and $PMA1_{-47}$ (lanes 1), $PMA1_{168}$ and $PMA1_{376}$ (lanes 2), $PMA1_{584}$ and $PMA1_{807}$ (lane 3), $PMA1_{1010}$ and $PMA1_{1250}$ (lanes 4), $PMA1_{2018}$ and $PMA1_{2290}$ (lanes 5), and $PMA1_{3287}$ and $PMA1_{3500}$ (lanes 6); for ADH1, $ADH1_{-235}$ and $ADH1_{-13}$ (lanes 1), $ADH1_{844}$ and $ADH1_{1013}$ (lanes 2), and $ADH1_{1231}$ and $ADH1_{1400}$ (lanes 3); for PYK1, $PYK1_{-288}$ and $PYK1_{19}$ (lanes 1), $PYK1_{508}$ and $PYK1_{799}$ (lanes 2), $PYK1_{1104}$ and $PYK1_{1372}$ (lanes 3), $PYK1_{1470}$ and $PYK1_{1720}$ (lanes 4), and $PYK1_{1695}$ and $PYK1_{1995}$ (lanes 5); for the nontranscribed region, Intergenic V-1 and Intergenic V-2. (B) Quantitation of the ChIP assays. Each value is calculated by dividing the ratio of the ChIP signal to the input signal for the experimental PCR product by the ratio of the ChIP signal to the input signal for the control PCR product. (C) Set2 is recruited to active genes. Cells grown overnight in medium containing 2% glucose were harvested, washed twice with sterile water, and inoculated at an OD_{595} of ≈0.02 into 300 ml of medium containing either 2% glucose or 2% galactose and 1% raffinose. Cells were incubated at 30°C until the OD_{595} reached 0.6 and treated with 1% formaldehyde for ChIP assays. Primer pairs used for PCR are as follows: $GAL1_{-190}$ and GAL_{154} (lanes 1), $GAL1_{427}$ and $GAL1_{726}$ (lanes 2), $GAL1_{1039}$ and $GAL1_{1331}$ (lanes 3), $GAL1_{1764}$ and $GAL1_{2079}$ (lanes 4), and $GAL1_{1921}$ and $GAL1_{2153}$ (lanes 5); for the nontranscribed region, Intergenic V-1 and Intergenic V-2.

gation by RNAPII in vivo, we initially examined a *set2*Δ strain for sensitivity to the pyrimidine analog 6-AU. Treatment with 6-AU leads to reduction of the UTP and GTP concentrations in yeast cells and impairs elongation by RNAPII (14). Studies using 6-AU have helped to show that genes like *DST1*, which encodes TFIIS, encode factors having a role in transcriptional elongation (3). Deletion of *SET2* resulted in slight sensitivity to 6-AU, suggesting that Set2 might stimulate elongation by RNAPII (Fig. 3A). Recently, similar observations have been made by two independent groups (28, 29).

To substantiate this conclusion, we examined whether a *set2* deletion would reduce *lacZ* expression from the plasmid, p416*GAL1-lacZ*, which contains the *Escherichia coli lacZ* gene fused to a *GAL1* promoter (9). Based on results obtained by



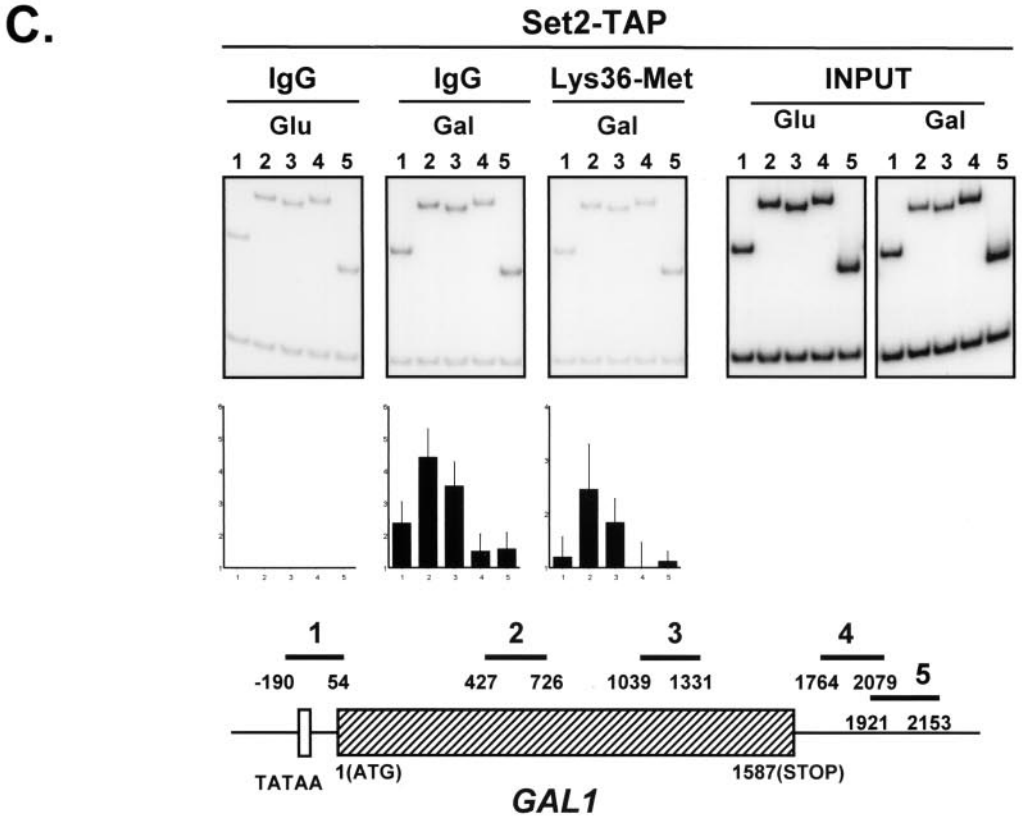


FIG. 2—*Continued*.

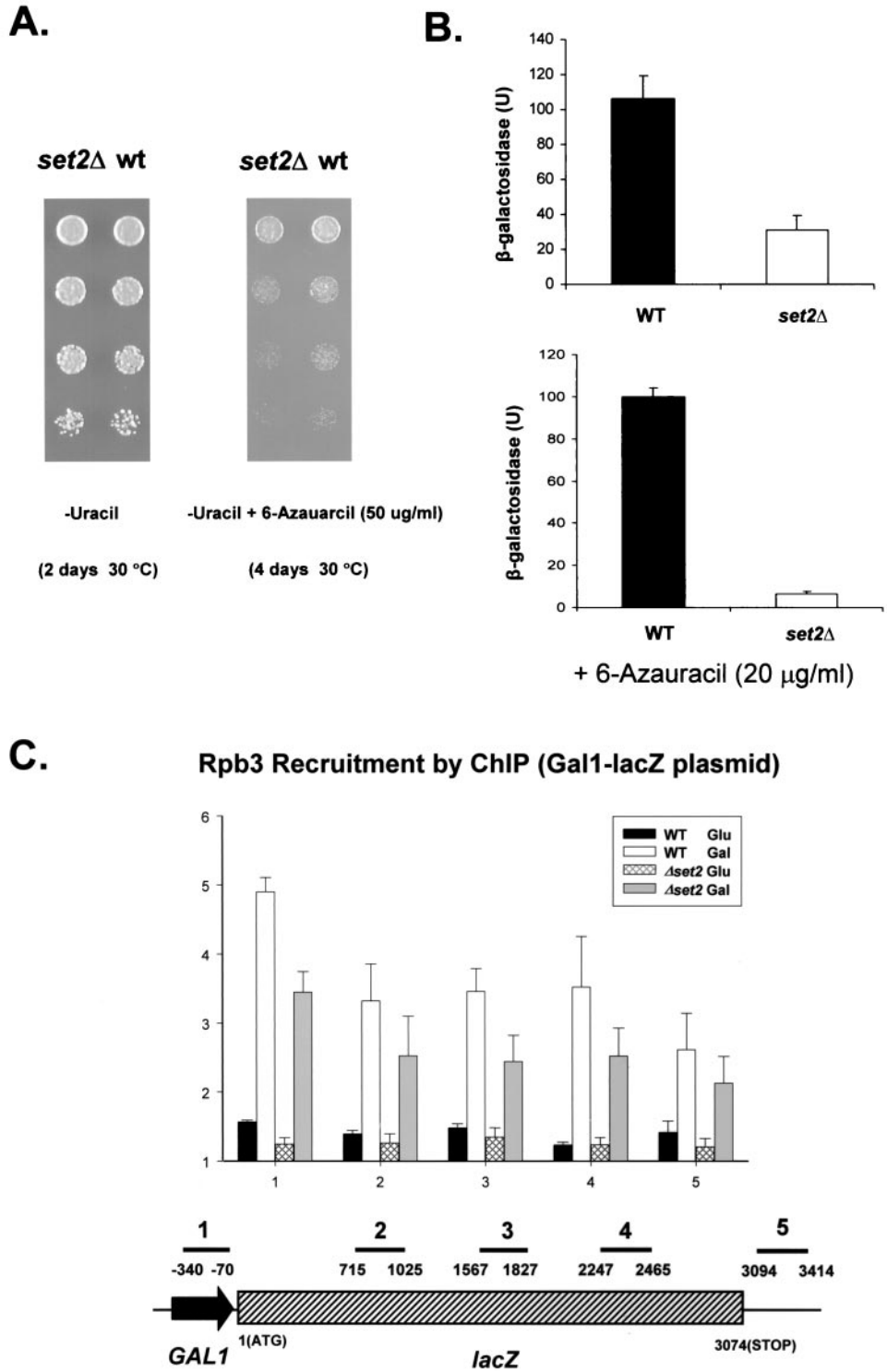


FIG. 3. Set2 influences transcription by RNAPII. (A) Sensitivity of the *set2* deletion strain to 6-AU. Strains containing either wild-type *SET2* or a *set2*Δ allele, as well as the plasmid pRS316 (51), were plated on synthetic dextrose-uracil medium with or without 6-AU (50 μg/ml) and were grown at 30°C for 2 to 4 days. WT, wild type. (B) Expression of β-galactosidase from the *lacZ* fusion plasmid p416*GAL1-lacZ* (9) in WT and *set2*Δ cells. Cells were grown in medium containing 2% glucose until an OD_{595} of ≈1 was reached and washed three times in distilled water; then, medium containing 2% galactose was added with or without 20 μg of 6-AU/ml and β-galactosidase activities were assayed after 4 h of growth at 30°C. (C) Density of RNAPII along the *lacZ* gene on the *GAL1-lacZ* plasmid. By employing an antibody that recognizes the Rpb3 subunit of RNAPII (Neoclone Biotechnology), ChIP was used to monitor the approximate relative concentrations of RNAPII at various positions across the *lacZ* gene. Wild-type and *set2*Δ strains harboring p416*GAL1-lacZ* were incubated in medium containing 2% glucose until an OD_{595} of 1 was reached. Cells were harvested, washed three times with water, and reinoculated into media containing either 2% glucose or 2% galactose. After 4 h of incubation at 30°C, cells were cross-linked with formaldehyde for the ChIP assays. PCR amplification was carried out with primer pairs recognizing promoter (labeled 1), coding (labeled 2, 3, and 4) and 3′ untranslated (labeled 5) regions for *GAL1-lacZ*. Primer pairs used for PCR were as follows: 1, GAL1-lacZ$_{-340}$ and GAL-lacZ$_{-70}$; 2, GAL1-lacZ$_{715}$ and GAL1-lacZ$_{1025}$; 3, GAL1-lacZ$_{1567}$ and GAL1-lacZ$_{1827}$; 4, GAL1-lacZ$_{2247}$ and GAL1-lacZ$_{2465}$; and 5, GAL1-lacZ$_{3094}$ and GAL1-lacZ$_{3414}$; for the nontranscribed region, Intergenic V-1 and Intergenic V-2.

deleting genes encoding TFIIS or components of the TREX complex, it has been argued that a decrease in β-galactosidase production with this plasmid correlates with a defect in transcriptional elongation (9, 42, 56; our unpublished data). After 4 h of galactose induction, β-galactosidase synthesis was reduced about threefold in a *set2*Δ strain compared to that of a strain with wild-type *SET2* (Fig. 3B). The addition of 20 μg of 6-AU/ml to a *set2* deletion strain harboring the *lacZ* reporter plasmid resulted in an approximately 20-fold reduction of β-galactosidase compared to that of a wild-type strain (Fig. 3B). These results, combined with the slight sensitivity to 6-AU of a *set2*Δ strain, suggested that Set2 is a positively acting transcription factor, perhaps an elongation factor. To further study the effect that a *set2* deletion had on the amount of β-galactosidase produced from the reporter plasmid, ChIP was performed to monitor the approximate concentration of Rpb3, the third largest subunit of RNAPII, at various positions across the *lacZ* gene (Fig. 3C). As has been previously observed, there was an apparent decline in the density of RNAPII from the 5′ end to the 3′ end of the gene even in a wild-type strain (23), and there was a similar 5′-to-3′ decline in a *set2*Δ strain. However, the apparent concentration of RNAPII in the promoter-proximal region was lower in a *set2*Δ strain than in a wild-type strain, suggesting that transcriptional initiation might be defective when *SET2* is deleted. The uniform decline of RNAPII across the *lacZ* gene in a *set2*Δ strain suggests either that Set2 does not affect elongation or else that Set2 uniformly accelerates elongation without preventing the release of RNAPII at particular sites on the *lacZ* gene.

Evidence for cotranscriptional methylation of histone H3 Lys36 by Set2. In order to test whether Set2 functions during transcriptional elongation, the recruitment of Set2, as well as the presence of Lys36 H3 methylation, was monitored on the *PMA1* gene when several known elongation factors were deleted. The Paf1 complex, which contains five subunits and associates with RNAPII, is thought to be an RNAPII elongation factor on the basis of both biochemical and genetic criteria (25, 34, 52). In particular, it colocalizes with RNAPII in the coding regions of various genes (25, 43). Interestingly, deletion of genes encoding either of two components of the Paf1 complex, Rtf1 or Cdc73, resulted in a marked decrease in the recruitment of Set2 across *PMA1* (Fig. 4A) and abolished Lys36 H3 methylation (Fig. 4B), demonstrating that the Paf1 complex is important for the recruitment of Set2 and essential for its methylation activity.

The RNAPII CTD kinase Ctk1 (27) phosphorylates Ser2 on the CTD heptapeptide repeats during transcriptional elongation (10). Deletion of the gene encoding Ctk1 also nearly eliminated the recruitment of Set2 and its histone H3 Lys36 methylation activity on the *PMA1* gene (Fig. 4A and B). The RNAPII CTD consists of proline-rich heptapeptide repeats (2). Set2 contains a WW domain in its C-terminal region (Fig. 2A), and WW domains are known to recognize proline-rich peptides (30). Consistent with this, we found that deleting the C-terminal portion of Set2, including its WW domain, significantly reduced the recruitment of Set2 to the *PMA1*, *ADH1*, and *PYK1* genes, especially toward their 3′ ends and virtually eliminated histone H3 Lys36 methylation even though the catalytic SET domain of Set2 was still intact (Fig. 2A and B). These data suggest that an interaction between the WW domain or C-terminal region of Set2 and the Ser2-phosphorylated CTD might stabilize the association of Set2 with elongating RNAPII and trigger Lys36 methylation of histone H3.

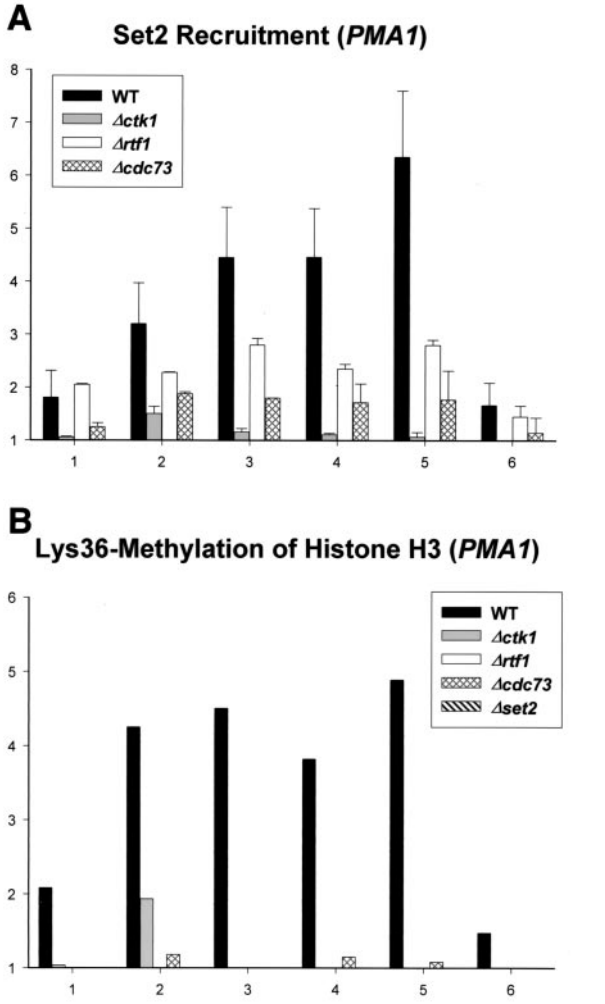


FIG. 4. Effects of deleting genes encoding elongation factors on Set2 recruitment and histone H3 Lys36 methylation. (A) Recruitment of Set2 to an actively transcribed gene in strains containing deletions of genes encoding elongation factors. ChIP was used in strains containing TAP tags on Set2 to monitor the presence of Set2 along the *PMA1* gene. Recruitment of Set2 is severely compromised when genes encoding subunits of the Paf1 complex, Rtf1 and Cdc73, or the RNAPII CTD kinase, Ctk1, are deleted. (B) The presence of Lys36 methylation on histone H3 in strains containing deletions of genes encoding elongation factors. Deletions of *CDC73*, *RTF1*, and *CTK1*, as well as *SET2*, eliminate Lys36 methylation on the *PMA1* gene.

Genetic evidence relating Set2 function to elongation by RNAPII. To further evaluate the function of Set2, we used a method for systematic construction of double mutants, termed SGA analysis (61), in which the *set2*Δ mutation was crossed to an array of ~4,700 mutant strains, each carrying a unique gene deletion. Inviable or slow-growing double-mutant meiotic progeny identify functional relationships between genes. The *set2* deletion was first introduced into a haploid starting strain of mating type *MAT*α and then crossed to the array of gene deletion mutants of the opposite mating type, *MAT***a**. Sporulation of the resulting diploid cells led to the formation of

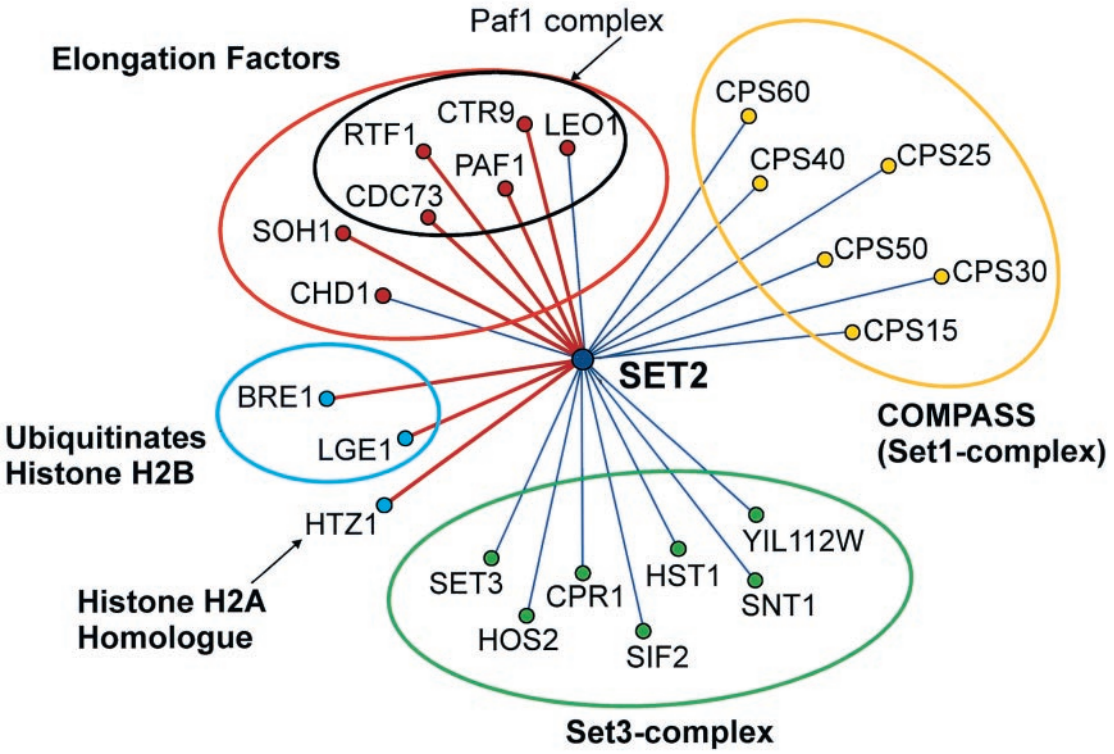


FIG. 5. Genetic interaction network representing the synthetic growth defects identified by SGA analysis. Genes are represented by nodes, and interactions are represented by lines that connect the nodes. All of the interactions were confirmed by either tetrad analysis (for synthetic lethals) or random sporulation (for synthetic growth defects). Synthetic lethal interaction are shown as red lines, and synthetic growth defects are shown as blue lines. There were approximately 40 other genetic interactions with *SET2* that were identified in the screen and are not shown in this diagram.

double-mutant meiotic progeny. This resulted in an ordered array of double-mutant haploid strains whose growth rate was monitored by visual inspection and image analysis of colony size. Putative genetic interactions were then confirmed by either tetrad dissection or random sporulation.

There were approximately 60 double-deletion combinations that resulted in synthetic growth defects. Of these, seven are currently thought to function in transcriptional elongation, namely *RTF1*, *CDC73*, *LEO1*, *CTR9*, *PAF1*, *SOH1*, and *CHD1* (Fig. 5). Rtf1, Cdc73, Leo1, Paf1, and Ctr9 are components of the Paf1 complex, which is associated with RNAPII and the RNAPII elongation factor Spt16/Pob3 (25, 34, 52). *SOH1* was originally identified as a suppressor of *HPR1* (15), a component of the transcription elongation complex, TREX (56). Soh1 is also thought to associate with the Mediator initiation complex in higher eukaryotic cells (18). Chd1 is a member of the chromodomain-helicase-DNA-binding family. It has been shown to be involved in ATP-dependent nucleosome remodeling (62) and has also recently been implicated in transcriptional elongation (11, 25). These genetic interactions with known elongation factors implied that Set2 functions during transcriptional elongation.

Synthetic growth defects were also detected between *set2*Δ and all seven components of the Set3 complex, namely *CPR1*, *HOS2*, *HST1*, *SIF2*, *SNT1*, *YIL112w*, and *SET3* (Fig. 5) (41). The Set3 complex contains two known histone deacetylases, Hos2 and Hst1, but no specific methylation activity has yet been attributed to the complex. Similarly, deletions of six of the eight subunits of the Set1-containing complex, COMPASS (*CPS60*, *CPS40*, *CPS35*, *CPS30*, *CPS25*, and *CPS15*) (7, 24, 33, 36, 46), were found to be synthetically sick with *set2*Δ (Fig. 5). The remaining two components of COMPASS were not tested, since the *CPS35* gene is essential and no *set1*Δ strain was present in the original deletion array. These results suggested that the function of Set2 is similar in some way to the functioning of COMPASS and the Set3 complex.

A *set2* deletion also generated synthetic growth defects when combined with a deletion of the gene encoding either of two components of the histone H2B ubiquitination complex, Bre1 or Lge1 (Fig. 5) (19, 67; our unpublished data), consistent with observations that histone H2B ubiquitination is essential for histone H3 methylation by Set1 (13, 58). Interestingly, a *set2*Δ *htz1*Δ double mutant also has a synthetic growth defect. Htz1 is a histone H2A variant (20), and this result suggested that Htz1 might have a role in histone methylation or transcriptional elongation or their consequences.

The specificity of the synthetic genetic interactions uncovered by the SGA screen with *SET2* was quite striking. In 64 other full-scale SGA screens, synthetic genetic interactions were only rarely detected with genes encoding subunits of COMPASS, the Set3 complex, or the Paf1 complex (e.g., for the Set3 complex: 1 of 64 were detected for *SET3*, 2 of 64 for *YIL112W*, 2 of 64 for *HOS2*, 0 of 64 for *HST1*, 5 of 64 for *SIF2*, 3 of 64 for *SNT1*, 2 of 64 for *CPR1*) and then only when screens

were done with genes that are likely to be involved in transcriptional elongation (A. Tong and C. Boone, unpublished data). In total, there were 45 interactions with subunits of COMPASS, the Set3 complex, or the Paf1 complex in 64 screens, averaging less than one hit per screen. Of the 37 other synthetic genetic interactions with *set2*Δ that were detected in our screen (data not shown), 17 were open reading frames of unknown function and none are known to be involved in transcription, although further study may indeed reveal that some are involved in transcriptional elongation and/or histone methylation.

DISCUSSION

Histone H3 methylation on Lys36 was recently attributed to the SET domain-containing protein Set2 in *S. cerevisiae* (54). In an effort to further characterize this protein, we used single-step transformation to place a TAP tag on the C terminus of Set2. Following purification and analyses by SDS-PAGE and mass spectrometry, we discovered that a substoichiometric amount of RNA polymerase II (RNAPII) copurified with Set2.

This physical interaction suggested that methylation of histone H3 Lys36 might be intimately linked to transcription. ChIP analyses demonstrated that Set2 localizes primarily to the coding regions of three genes that were tested, and this correlated well with the locations of histone H3 Lys36 methylation. Consistent with the notion that the recruitment of Set2 is linked to elongation by RNAPII, the presence of Set2 was found to correlate with active transcription when ChIP was performed on the *GAL1* gene following induction with galactose. Strahl et al. tethered Set2 to a heterologous promoter and demonstrated that, under these conditions, Set2 represses transcription (54). However, we found little or no Set2 or histone H3 Lys36 methylation in various promoter regions, suggesting that Set2 activity is not normally directed towards this region. Deletion of the *SET2* gene caused slight sensitivity to 6-AU and a large reduction in β-galactosidase produced by a reporter gene. Both the sensitivity to 6-AU and the reduced β-galactosidase synthesis from the reporter gene that we used in our experiments have been used as indicators that a gene is involved in enhancing elongation by RNAPII (3, 9, 25, 42, 52). A similar suggestion has been made based on the enhanced sensitivity to 6-AU generated when a *SET2* deletion is combined with a deletion of *DST1*, which encodes TFIIS (28). Follow-up ChIP experiments showed that the concentration of RNAPII is lower across the *lacZ* gene in a *set2*Δ background than in a wild-type strain. Therefore, we propose that wild-type Set2 normally has a positive role in transcription. This experiment did not, however, prove that Set2 specifically stimulates elongation by RNAPII.

Phosphorylation and dephosphorylation of the CTD of the largest subunit of RNAPII, Rpb1, are required for gene regulation (22, 63). Phosphorylation on Ser5 of the CTD heptapeptide repeats, YSPTSPS, by the Kin28 subunit of the general transcription factor TFIIH is associated with early transcriptional elongation, whereas Ser2 phosphorylation by Ctk1 is found at later elongation steps (10, 23). Our Western blot analyses demonstrated that both types of RNAPII copurify with Set2, again implying that the recruitment of Set2 to a transcription unit and histone H3 methylation on Lys36 might be cotranscriptional. It is striking that the distribution of Set2 along various genes, strong in the coding region and weak in the promoter-proximal and 3′ untranslated regions, is virtually identical to the distribution of Ser2-phosphorylated CTD (10, 23) and the Ser2 kinase, Ctk1 (M. Kim, S. H. Ahn, and S. Buratowski, unpublished data). Moreover, deletion of *CTK1* results in substantially less recruitment of Set2 to the *PMA1* gene and virtually eliminates histone H3 Lys36 methylation, indicating that Lys36 methylation depends on Ser2 phosphorylation of the CTD during transcriptional elongation.

The WW domains of Ess1/Pin1 and Rsp5 are known to interact with the proline-rich CTD repeats of RNAPII (8, 35, 57). Since our ChIP experiments showed that deletion of the C-terminal region of Set2, including its WW domain, reduced the recruitment of Set2 and essentially eliminated its methylation activity, we propose that there is a physical interaction between the WW domain of Set2 and the Ser2-phosphorylated CTD of RNAPII that stabilizes the association of Set2 with RNAPII and triggers the histone H3 methylation activity of Set2. Consistent with this conclusion, Li et al. recently also found that Set2 copurifies with RNAPII and showed that Set2 can bind to high concentrations of a Ser2-phosphorylated CTD peptide (29). Although this study found that deletion of the WW domain of Set2 eliminates the association between RNAPII and Set2, we have observed that Set2 (Δ476-733)-TAP, which lacks the WW domain, still coimmunoprecipitates with some RNAPII (N. J. Krogan and J. Greenblatt, unpublished data) and is still recruited, albeit less efficiently, to actively transcribed genes (Fig. 2A and B). Xiao et al. (68) have found that a C-terminal portion of Set2 that is distal to its WW domain is sufficient to bind RNAPII. Therefore, a site on RNAPII other than the Ser2-phosphorylated CTD is also likely to mediate an interaction with Set2.

To further pursue Set2's role in transcriptional elongation, the presence of histone H3 Lys36 methylation and the recruitment of Set2 were examined on the *PMA1* gene when genes encoding subunits of the Paf1 complex were deleted. The Paf1 complex contains five subunits (Paf1, Cdc73, Rtf1, Leo1, and Ctr9) and has recently been implicated, both biochemically and genetically, in the process of transcriptional elongation (11, 25, 34, 52). Specifically, deletions of genes encoding components of the Paf1 complex cause sensitivity to 6-AU and genetically interact with mutations in genes encoding other known elongation factors. Furthermore, the Paf1 complex interacts physically with both RNAPII and Spt16/Pob3, the yeast homologue of the human elongation factor FACT (39). ChIP experiments have also shown that the Paf1 complex colocalizes with RNAPII in the coding regions of various genes (25, 43) and, like Set2, declines in the region beyond the poly(A) signal (Kim et al., unpublished). We have shown here that when genes encoding two components of the Paf1 elongation complex, Cdc73 and Rtf1, are deleted, Set2 recruitment is significantly reduced and histone H3 methylation is virtually eliminated.

Consistent with a functional connection between Set2 and the Paf1 complex, synthetic growth defects were observed when a *set2* deletion was individually combined with deletions of genes encoding all five subunits of the Paf1 complex. The *SET2* gene also interacts with the genes encoding two other putative elongation factors, Soh1 and Chd1. Mutations in

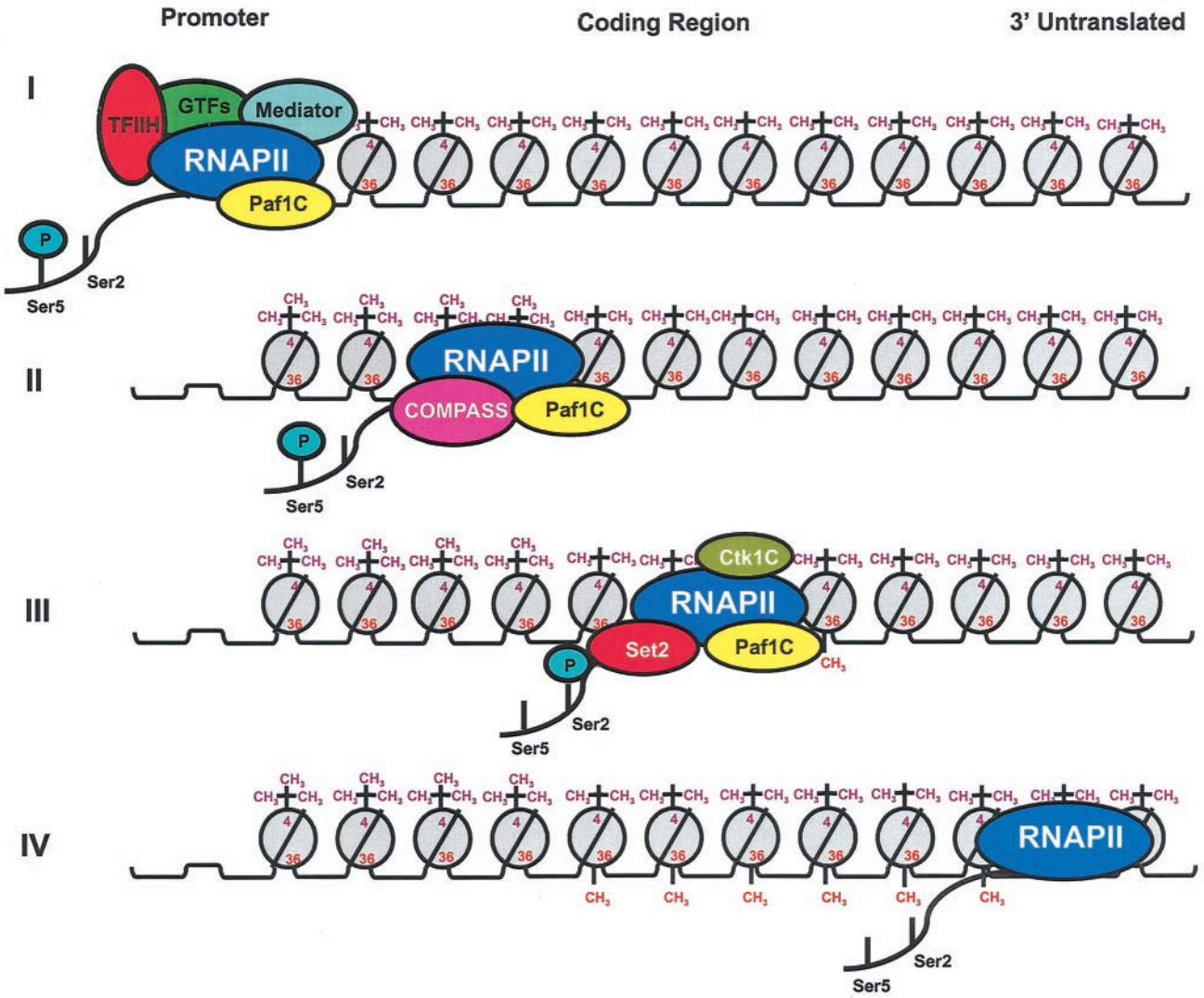


FIG. 6. Model for the coupling of histone methylation to transcriptional elongation and the phosphorylation of RNAPII in *S. cerevisiae*. See the text for details.

SOH1 were originally identified as suppressors of *HPR1* (15), a component of the transcription elongation complex TREX (56). We have recently found that a *soh1* deletion also interacts genetically with genes encoding a number of other known elongation factors, including *TFIIS*, and causes significant sensitivity to 6-AU and a marked decrease in expression of the *lacZ* gene on the plasmid p416*GAL1-lacZ* (Krogan and Greenblatt, unpublished). The *CHD1* gene interacts genetically with a number of genes encoding elongation factors (11; Krogan and Greenblatt, unpublished) and was recently implicated in termination by RNAPII (1). Moreover, we recently found that Chd1 is associated with casein kinase II and Spt16/Pob3 (25) and can be effectively cross-linked to the coding regions of a number of genes (Kim et al., unpublished). The dependence of Set2 function on the Paf1 complex and the CTD kinase Ctk1, as well as the many genetic interactions between the *SET2* gene and genes encoding known positive elongation factors, strengthens our conclusion that Set2 function is cotranscriptional and that Set2 may act as an activator of elongation.

Surprisingly, synthetic growth defects were obtained when a *set2* deletion was combined with deletions of genes encoding all seven components of the Set3 complex (41). The Set3 complex has a negative effect on the expression of certain meiosis-specific genes, perhaps because the Set3 complex contains two histone deacetylases, Hos2 and Hst1, as well as Set3. The synthetic phenotypes generated when a *set2* deletion is combined with deletions of genes encoding components of the Set3 complex suggest, however, that the Set3 complex may also have a positive role in transcription by RNAPII. This idea is supported by the recent observation that at least two components of the Set3 complex, Set3 and, surprisingly, Hos2, are recruited to actively transcribed genes (65).

A *set2* deletion also generated synthetic growth defects when combined with deletions of genes encoding components of the Set1 complex, COMPASS. COMPASS methylates Lys4 of histone H3, a modification that has been shown to be needed for effective silencing at telomeres and ribosomal DNA loci (7, 24, 33, 36, 46). Interestingly, components of COMPASS as well as

histone H3 Lys4 methylation, like Set2 and components of the Paf1 complex, localize to the transcribed regions of various genes and interact genetically with a number of known or suspected elongation factors (5, 48; our unpublished data). Moreover, methylation of Lys4 by COMPASS, like methylation of Lys36 by Set2, also requires components of the Paf1 complex, which associates with COMPASS (26, 37). Genetic interactions among components of three different Set protein-containing complexes imply that these complexes are functionally redundant and may all associate directly or indirectly with RNAPII and function during transcriptional elongation.

Our data and other published studies on COMPASS and Set2 have uncovered a remarkable coordination of RNAPII phosphorylation with histone H3 methylation during transcriptional elongation, as illustrated in Fig. 6. COMPASS is recruited specifically to the early transcribed region (26, 37), and this depends on both the Paf1 complex and phosphorylation of the RNAPII CTD on Ser5 by Kin28. The consequence of COMPASS recruitment is histone H3 trimethylation on Lys4 in the same region (37). Upon further elongation by RNAPII, Ser5 phosphorylation declines and is replaced by Ser2 phosphorylation mediated by Ctk1 (10, 23). We found that Set2 is recruited to this region, where it methylates Lys36 of histone H3, and the recruitment of Set2 depends on both the Paf1 complex and phosphorylation of RNAPII by Ctk1 on Ser2, as has also been observed by Li et al. (28) and Xiao et al. (68). Finally, in the region beyond the poly(A) signal, Set2 disappears and histone H3 Lys36 declines in concert with dephosphorylation of RNAPII on Ser2 (10, 23) and the disappearance of the Paf1 complex (Kim et al., unpublished).

Finally, *set2*Δ *bre1*Δ and *set2*Δ *lge1*Δ double mutants also have synthetic growth defects. Bre1, an E3 ubiquitin ligase, is associated with Rad6 and Lge1, and all three are required for ubiquitination of histone H2B, a modification which is necessary for methylation of histone H3 on Lys4 by COMPASS (13, 19, 58, 67; our unpublished data). Therefore, disruption of Lys4 methylation by deleting genes encoding components of COMPASS or the Bre1/Lge1 complex results in a growth defect when *SET2* is also deleted. This further confirms the functional redundancy between the Lys36 and Lys4 methylations on histone H3. Interestingly, *SET2* also genetically interacts with *HTZ1*, which encodes a variant histone, H2A (20). This genetic interaction may predict a role for Htz1 in histone ubiquitination, histone methylation, and/or transcriptional elongation.

ACKNOWLEDGMENTS

We are grateful to A. Aguilera for the gift of the plasmid p416*GAL1-lacZ* and to B. Strahl and K. Struhl for sharing unpublished data.

N.J.K. was supported by a PGS-B Scholarship Award from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a Doctoral Fellowship from the Canadian Institute of Health Research (CIHR). This research was supported by grants to J.F.G. from the Canadian Institutes of Health Research, the Ontario Genomics Institute, and the National Cancer Institute of Canada with funds from the Canadian Cancer Society.

N.J.K. and M.K. contributed equally to this report.

REFERENCES

1. **Allen, C., N. A. Kent, H. S. Jones, J. O'Sullivan, A. Aranda, and N. J. Proudfoot.** 2002. A role for chromatin remodeling in transcriptional termination by RNA polymerase II. Mol. Cell **10:**1442–1452.
2. **Allison, L. A., M. Moyle, M. Shales, and C. J. Ingles.** 1985. Extensive homology among the largest subunits of eukaryotic and prokaryotic RNA polymerases. Cell **42:**599–610.
3. **Archambault, J., F. Lacroute, A. Ruet, and J. D. Friesen.** 1992. Genetic interaction between transcription elongation factor TFIIS and RNA polymerase II. Mol. Cell. Biol. **12:**4142–4152.
4. **Bähler, J., J. Wu, M. S. Longtine, N. G. Shah, A. McKenzie III, A. B. Steever, A. Wach, P. Philippsen, and J. R. Pringle.** 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe.* Yeast **14:**943–951.
5. **Bernstein, B. E., E. L. Humphrey, R. L. Erlich, R. Schneider, P. Bouman, J. S. Liu, T. Kouzarides, and S. L. Schreiber.** 2002. Methylation of histone H3 Lys4 in coding regions of active genes. Proc. Natl. Acad. Sci. USA **99:**8695–8700.
6. **Bregman, D. B., L. Du, S. van der Zee, and S. L. Warren.** 1995. Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J. Cell Biol. **129:**287–298.
7. **Briggs, S. D., M. Bryk, B. D. Strahl, W. L. Cheung, J. K. Davie, S. Y. Dent, F. Winston, and C. D. Allis.** 2001. Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. Genes Dev. **15:**3286–3295.
8. **Chang, A., S. Cheang, X. Espanel, and M. Sudol.** 2000. Rsp5 WW domains interact directly with the carboxyl-terminal domain of RNA polymerase II. J. Biol. Chem. **275:**20562–20571.
9. **Chavez, S., and A. Aguilera.** 1997. The yeast *HPR1* gene has a functional role in transcriptional elongation that uncovers a novel source of genome instability. Genes Dev. **11:**3459–3470.
10. **Cho, E. J., M. S. Kobor, M. Kim, J. Greenblatt, and S. Buratowski.** 2001. Opposing effects of Ctk1 kinase and Fcp1 phosphatase at Ser2 of the RNA polymerase II C-terminal domain. Genes Dev. **15:**3319–3329.
11. **Costa, P. J., and K. M. Arndt.** 2000. Synthetic lethal interactions suggest a role for Saccharomyces cerevisiae Rtf1 protein in transcription elongation. Genetics **156:**535–547.
12. **DiMartino, J. F., T. Miller, P. M. Ayton, T. Landewe, J. L. Hess, M. L. Cleary, and A. Shilatifard.** 2000. A carboxy-terminal domain of ELL is required and sufficient for immortalization of myeloid progenitors by MLL-ELL. Blood **96:**3887–3893.
13. **Dover, J., J. Schneider, M. A. Tawiah-Boateng, A. Wood, K. Dean, M. Johnston, and A. Shilatifard.** 2002. Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. J. Biol. Chem. **277:**28368–28371.
14. **Exinger, F., and F. Lacroute.** 1992. 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr. Genet. **22:**9–11.
15. **Fan, H. Y., and H. L. Klein.** 1994. Characterization of mutations that suppress the temperature-sensitive growth of the *hpr1*Δ mutant of *Saccharomyces cerevisiae*. Genetics **137:**945–956.
16. **Flanagan, P. M., R. J. Kelleher, M. H. Sayre, H. Tschochner, and R. D. Kornberg.** 1991. A mediator required for activation of RNA polymerase II transcription *in vitro*. Nature **350:**436–438.
17. **Gietz, R. D., and R. H. Schiestl.** 1995. Transforming yeast with DNA. Methods Mol. Cell. Biol. **5:**255–269.
18. **Gu, W., S. Malik, M. Ito, C. X. Yuan, J. D. Fondell, X. Zhang, E. Martinez, J. Qin, and R. G. Roeder.** 1999. A novel human SRB/MED-containing co-factor complex, SMCC, involved in transcription regulation. Mol. Cell **1:**97–108.
19. **Hwang, W. W., S. Venkatasubrahmanyam, A. G. Ianculescu, A. Tong, C. Boone, and H. D. Madhani.** 2003. A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. Mol. Cell **11:**261–266.
20. **Jackson, J. D., and M. A. Gorovsky.** 2000. Histone H2A.Z has a conserved function that is distinct from that of the major H2A sequence variants. Nucleic Acids Res. **28:**3811–3816.
21. **Jaju, R. J., C. Fidler, O. A. Hass, A. J. Stirckson, F. Watkins, K. Clark, N. C. Cross, J. F. Cheng, P. D. Aplan, L. Kearney, J. Boultwood, and J. S. Wainscoat.** 2001. A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in *de novo* childhood acute myeloid leukemia. Blood **98:**1264–1267.
22. **Kobor, M. S., and J. Greenblatt.** 2002. Regulation of transcription elongation by phosphorylation. Biochim. Biophys. Acta **1577:**261–275.
23. **Komarnitsky, P., E. J. Cho, and S. Buratowski.** 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. **14:**2452–2460.
24. **Krogan, N. J., J. Dover, S. Khorrami, J. F. Greenblatt, J. Schneider, M. Johnston, and A. Shilatifard.** 2002. COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression. J. Biol. Chem. **277:**10753–10755.
25. **Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, and J. F. Greenblatt.** 2002. RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. Mol. Cell. Biol. **22:**6979–6992.
26. **Krogan, N. J., J. Dover, A. Wood, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, O. W. Ryan, A. Golshani, M. Johnston, J. F. Greenblatt, and A. Shilatifard.** 2003. The Paf1 complex is required for histone H3 methylation

by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol. Cell **11:**721–729.
27. **Lee, J. M., and A. L. Greenleaf.** 1991. CTD kinase large subunit is encoded by CTK1, a gene required for normal growth of *Saccharomyces cerevisiae.* Gene Expr. **1:**149–167.
28. **Li, B., L. Howe, S. Anderson, J. R. Yates, and J. L. Workman.** 2003. The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. J. Biol. Chem. **278:**8897–8903.
29. **Li, J., D. Moazed, and S. P. Gygi.** 2002. Association of the histone methyltransferase Set2 with RNA polymerase II plays a role in transcription elongation. J. Biol. Chem. **277:**49383–49388.
30. **Macias, M. J., S. Wiesner, and M. Sudol.** 2002. WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. FEBS Lett. **513:**30–37.
31. **Mann, M., P. Hojrup, and P. Roepstorff.** 1993. Use of mass spectrometry molecular weight information to identify proteins in sequence databases. Biol. Mass Spectrom. **22:**338–345.
32. **McCracken, S., N. Fong, E. Rosonina, K. Yankulov, G. Brothers, D. Siderovski, A. Hessel, S. Foster, Amgen EST Program, S. Shurman, and D. L. Bentley.** 1997. 5′-capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. Genes Dev. **11:**3306–3318.
33. **Miller, T., N. J. Krogan, J. Dover, H. Erdjument-Bromage, P. Tempst, M. Johnston, J. F. Greenblatt, and A. Shilatifard.** 2001. COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. Proc. Natl. Acad. Sci. USA **98:**12902–12907.
34. **Mueller, C. L., and J. A. Jaehning.** 2002. Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Mol. Cell. Biol. **22:**1970–1980.
35. **Myers, J. K., D. P. Morris, A. L. Greenleaf, and T. G. Oas.** 2001. Phosphorylation of RNA polymerase II CTD fragments results in tight binding to the WW domain from the yeast prolyl isomerase Ess1. Biochemistry **40:**8479–8486.
36. **Nagy, P. L., J. Griesenbeck, R. D. Kornberg, and M. L. Cleary.** 2002. A trithorax-group complex purified from *Saccharomyces cerevisiae* is required for methylation of histone H3. Proc. Natl. Acad. Sci. USA **99:**90–94.
37. **Ng, H. N., F. Robert, R. A. Young, and K. Struhl.** 2003. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol. Cell **11:**709–719.
38. **Orphanides, G., and D. Reinberg.** 2002. A unified theory of gene expression. Cell **108:**439–451.
39. **Orphanides, G., W. H. Wu, W. S. Lane, M. Hampsey, and D. Reinberg.** 1999. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature **400:**284–288.
40. **Patturajan, M., R. J. Schulte, B. M. Sefton, R. Berenzney, M. Vincent, O. Bensaude, S. L. Warren, and J. L. Corden.** 1998. Growth-related changes in phosphorylation of yeast RNA polymerase II. J. Biol. Chem. **273:**4689–4694.
41. **Pijnappel, W. W., D. Schaft, A. Roguev, A. Shevchenko, H. Tekotte, M. Wilm, G. Rigaut, B. Seraphin, R. Aasland, and A. F. Stewart.** 2001. The S. cerevisiae SET3 complex includes two histone deacetylases, Hos2 and Hst1, and is a meiotic-specific repressor of the sporulation gene program. Genes Dev. **15:**2991–3004.
42. **Piruat, J. I., and A. Aguilera.** 1998. A novel yeast gene, *THO2*, is involved in RNA polII transcription and provides new evidence for transcriptional elongation-associated recombination. EMBO J. **17:**4859–4872.
43. **Pokholok, D. K., N. M. Hannett, and R. A. Young.** 2002. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell **9:**799–809.
44. **Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z. W. Sun, M. Schmidt, S. Opravil, K. Mechtler, C. P. Ponting, C. D. Allis, and T. Jenuwein.** 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature **406:**593–899.
45. **Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann, and B. Séraphin.** 1999. A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotech. **17:**1030–1032.
46. **Roguev, A., D. Schaft, A. Shevchenko, W. W. Pijnappel, M. Wilm, R. Aasland, and A. F. Stewart.** 2001. The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. EMBO J. **20:**7137–7148.
47. **Rosati, R., R. La Starza, A. Veronese, A. Aventin, C. Schwienbacher, T. Vallespi, M. Negrini, M. F. Martelli, and C. Mecucci.** 2002. NUP98 is fused to the NSD3 gene in acute myeloid leukemia associated with t(8;11)(p11.2; p15). Blood **99:**3857–3860.
48. **Santos-Rosa, H., R. Schneider, A. J. Bannister, J. Sherriff, B. E. Bernstein, N. C. Emre, S. L. Schreiber, J. Mellor, and T. Kouzarides.** 2002. Active genes are tri-methylated at K4 of histone H3. Nature **419:**407–411.
49. **Schroeder, S. C., B. Schwer, S. Shuman, and D. Bentley.** 2000. Dynamic association of capping enzymes with transcribing RNA polymerase II. Genes Dev. **14:**2435–2440.
50. **Shevchenko, A., M. Wilm, O. Vorm, and M. Mann.** 1996. Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. Anal. Chem. **68:**850–858.
51. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
52. **Squazzo, S. L., P. J. Costa, D. L. Lindstrom, K. E. Kumer, R. Simic, J. L. Jennings, A. J. Link, K. M. Arndt, and G. A. Hartzog.** 2002. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J. **21:**1764–1774.
53. **Stec, I., T. J. Wright, G. J. van Ommen, P. A. de Boer, A. van Haeringen, A. F. Moorman, M. R. Altherr, and J. T. den Dunnen.** 1998. WHSC1, a 90 kb SET domain-containing gene, expressed in early development and homologous to a *Drosophila* dysmorphy gene maps in the Wolf-Hirschhorn syndrome critical region and is fused to IgH in t(4;14) multiple myeloma. Hum. Mol. Genet. **7:**1071–1082.
54. **Strahl, B. D., P. A. Grant, S. D. Briggs, Z. W. Sun, J. R. Bone, J. A. Caldwell, S. Mollah, R. G. Cook, J. Shabanowitz, D. F. Hunt, and C. D. Allis.** 2002. Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol. Cell. Biol. **22:**1298–1306.
55. **Strahl, B. D., R. Ohba, R. G. Cook, and C. D. Allis.** 1999. Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in *Tetrahymena*. Proc. Natl. Acad. Sci. USA **96:**14967–14972.
56. **Strasser, K., S. Masuda, P. Mason, J. Pfannstiel, M. Oppizzi, S. Rodriguez-Navarro, A. G. Rondon, A. Aguilera, K. Struhl, R. Reed, and E. Hurt.** 2002. TREX is a conserved complex coupling transcription with messenger RNA export. Nature **417:**304–308.
57. **Sudol, M., K. Sliwa, and T. Russo.** 2001. Functions of WW domains in the nucleus. FEBS Lett. **490:**190–195.
58. **Sun, Z. W., and C. D. Allis.** 2002. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature **418:**104–108.
59. **Thompson, C. M., A. J. Koleske, D. M. Chao, and R. A. Young.** 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding in yeast. Cell **73:**1361–1375.
60. **Thompson, N. E., D. Aronson, and R. R. Burgess.** 1990. Purification of eukaryotic RNA polymerase II by immunoaffinity chromatography: elution of active enzyme with protein stabilizing agents from a polyol-responsive monoclonal antibody. J. Biol. Chem. **265:**7069–7077.
61. **Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader, N. Page, M. Robinson, S. Raghibizadeh, C. W. Hogue, H. Bussey, B. Andrews, M. Tyers, and C. Boone.** 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:**2364–2368.
62. **Tran, H. G., D. J. Steger, V. R. Iyer, and A. D. Johnson.** 2000. The chromo domain protein Chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. EMBO J. **19:**2323–2331.
63. **Valay, J. G., M. Simon, M. F. Dubois, O. Bensaude, C. Facca, and G. Faye.** 1995. The *KIN28* gene is required both for RNA polymerase II mediated transcription and phosphorylation of the Rpb1p CTD. J. Mol. Biol. **249:**535–544.
64. **Van Holde, K. E.** 1989. Histone modifications, p. 111–148. Springer, New York, N.Y.
65. **Wang, A., S. K. Kurdistani, and M. Grunstein.** 2002. Requirement of Hos2 histone deacetylase for gene activity in yeast. Science **298:**1412–1414.
66. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387–391.
67. **Wood, A., N. J. Krogan, J. Dover, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, Y. Zhang, J. F. Greenblatt, M. Johnston, and A. Shilatifard.** 2003. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol. Cell **11:**267–274.
68. **Xiao, T., H. Hall, K. O. Kizer, Y. Shibata, M. C. Hall, C. H. Borchers, and B. D. Strahl.** 2003. Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. Genes Dev. **17:**654–663.
69. **Yue, Z., E. Maldonado, R. Pillutla, H. Cho, D. Reinberg, and A. J. Shatkin.** 1997. Mammalian capping enzyme complements mutant *Saccharomyces cerevisiae* lacking mRNA guanylyltransferase and selectively binds the elongating form of RNA polymerase II. Proc. Natl. Acad. Sci. USA **94:**12898–12903.