The Yeast Cap Binding Complex Modulates Transcription Factor Recruitment and Establishes Proper Histone H3K36 Trimethylation during Active Transcription

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Recent studies have revealed a close relationship between transcription, histone modification, and RNA processing. In fact, genome-wide analyses that correlate histone marks with RNA processing signals raise the possibility that specific RNA processing factors may modulate transcription and help to “write” chromatin marks. Here we show that the nuclear cap binding complex (CBC) directs recruitment of transcription elongation factors and establishes proper histone marks during active transcription. A directed genetic screen revealed that deletion of either subunit of the CBC confers a synthetic growth defect when combined with deletion of genes encoding either Ctk2 or Bur2, a component of the *Saccharomyces cerevisiae* ortholog of P-TEFb. The CBC physically associates with these complexes to recruit them during transcription and mediates phosphorylation at Ser-2 of the C-terminal domain (CTD) of RNA polymerase II. To understand how these interactions influence downstream events, histone H3K36me3 was examined, and we demonstrate that *CBCΔ* affects proper Set2-dependent H3K36me3. Consistent with this, the CBC and Set2 have similar effects on the ability to rapidly induce and sustain activated gene expression, and these effects are distinct from other histone methyltransferases. This work provides evidence for an emerging model that RNA processing factors can modulate the recruitment of transcription factors and influence histone modification during elongation.

mRNA synthesis and processing are achieved through a tightly coordinated and highly regulated set of reactions. As the first few nucleotides are transcribed, the 5' end of the RNA is capped, and the RNA is cotranscriptionally spliced and processed at its 3' end. In recent years, there has been a growing appreciation that some of the proteins involved in RNA synthesis play roles in RNA processing and vice versa. Thus, in order to understand how each reaction is regulated, it is crucial to identify and characterize these multifunctional proteins.

The highly conserved *Saccharomyces cerevisiae* cap binding complex (CBC), comprised of a 100-kDa and a 20-kDa subunit, appears to be one of these critical, multifunctional complexes. It is cotranscriptionally recruited to genes (1, 2), is present in the coding region from the 5' to the 3' end of the gene (3–5), facilitates cotranscriptional assembly of the spliceosome (6), participates in transcription termination and 3' end formation (3–5, 7), and accompanies the mature mRNA as it is exported from the nucleus (1, 4, 7–9). However, the activity of the CBC during transcription elongation has been poorly understood. The yeast CBC has a number of phenotypes that implicate it in transcription elongation. For example, deletion of the CBC confers 6-azauracil and mycophenolic acid sensitivity, phenotypes associated with transcription elongation defects (5, 10). Furthermore, the complex copurifies with a number of transcription elongation factors (11) and shows genetic interactions with factors implicated in transcription elongation, such as the THO complex component Hpr1 (12).

One of the central reactions leading to tight regulation of transcription is the reversible phosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (Pol II) (RNAPII) (reviewed in reference 13). After preinitiation complex (PIC) formation, the CTD of RNA polymerase is phosphorylated by TFIIH on serine 5 and the phosphorylation level remains high

as the polymerase synthesizes the first few hundred nucleotides of the RNA. Further downstream, as the Ser-5 phosphorylation levels decrease (through the activity of a Ser-5 phosphatase) (14), Ser-2 phosphorylation is observed. In mammals, a significant contributor to Ser-2 phosphorylation is the cyclin-dependent kinase Cdk9. Furthermore, it has recently been shown that the *Drosophila* and human CDK12 proteins also catalyze Ser-2 phosphorylation. The yeast orthologs of CDK9 and CDK12 appear to be the cyclin-dependent kinases Bur1 and Ctk1, respectively (15).

While Ctk1 carries out the bulk of the Ser-2 phosphorylation, particularly near the middle and 3' regions of genes (16, 17), it has also been demonstrated that Bur1 phosphorylates the CTD at Ser-2 in the 5' region of the gene (17). Consistent with the location within genes where each complex targets its CTD kinase activity, the Bur complex is found near the 5' region of genes, while Ctk is found in downstream regions (18). Hence, the combined activities of the Bur and Ctk complexes establish CTD Ser-2 phosphorylation. Intriguingly, a recent report indicates that the mammalian CBC physically interacts with Cdk9 and its cyclin partner cyclin T1 and can affect CTD phosphorylation (19), reminiscent of earlier reports from genome-wide yeast studies that identified interactions between Bur1/2 and the CBC (11). Interactions be-

Received 14 July 2012 Returned for modification 20 August 2012

Accepted 4 December 2012

Published ahead of print 10 December 2012

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doi:10.1128/MCB.00947-12

tween either CDK12 or its yeast ortholog Ctk1 and the CBC have not been reported.

CTD phosphorylation influences transcription primarily by mediating its interactions with other factors that promote transcription elongation. For example, CTD phosphorylation dynamics are critical for proper histone modification. While Ser-5 phosphorylation stimulates histone H3K4me3 (20), Ser-2 phosphorylation is required for histone H3K36me3, both of which are active transcription marks. Set2, the histone methyltransferase that is responsible for H3K36 methylation, interacts directly with the Ser-2 phosphorylated CTD (21–23). Deletion of components of the Ctk complex abrogates recruitment of Set2 and leads to a dramatic decrease in K36me3 within the coding region (24, 25). Furthermore, more recent data have demonstrated that deletion of the gene encoding Bur2 also leads to a decrease in H3K36me3 (17, 26). *BUR2* deletion specifically decreases H3K36me3 in the 5' region of the gene, closely correlated with its occupancy (26). The Bur1/2 complex is also required for recruitment of the RNA polymerase II-associated factor (PAF) complex to the coding region of the gene (27) and the PAF complex has also been directly implicated in H3K36me3 (26, 27). Moreover, histone H3K36 is regulated by cross-histone marks. H2B ubiquitination acts as a barrier to prevent Ctk1 recruitment and activity (28). This histone mark must be removed by the ubiquitin protease Ubp8 and its associated proteins Sus1, Sgf11, and Sgf73 before Set2 recruitment can occur (28).

Despite a growing appreciation for the role of histone modification in gene expression and the importance of the CTD of RNA polymerase in establishing histone marks, little is known about the way in which RNA processing events, which are spatially and temporally linked to transcription, and the specific proteins associated with RNA processing affect polymerase movement through a chromatin template. Furthermore, it is unknown how such a collaboration between RNA processing and chromatin modification allows cells to mount the appropriate gene expression response. Recent work from our lab has shown that the CBC can affect histone H2B ubiquitination and, as a consequence, genome-wide gene expression through its effects on splicing of a component of the H2B ubiquitin protease machinery *SUS1* (29). However, direct roles for processing factors in histone modification remain to be elucidated.

Here we show that the CBC has genetic interactions with the Bur complex, the Ctk complex, and the CTD of RNA polymerase. Furthermore, we observe physical interactions between the CTD kinase complexes and the CBC, and in the absence of the CBC, cotranscriptional recruitment of both the Bur and the Ctk complexes is dramatically reduced, leading to abrogated Ser-2 phosphorylation. In light of the close relationship between CTD phosphorylation at serine 2 and active chromatin marks, particularly H3K36me3, we analyzed the CBC's role in establishing this mark and find that the CBC facilitates proper histone H3K36me3 modification. To elucidate how these interactions influence biological function, a detailed analysis was carried out to determine how Set2-dependent H3K36 methylation and the CBC affect the cell's ability to initiate and sustain expression of an activated gene. Remarkably, although RNA expression eventually reaches near-wild-type (WT) levels in both the CBC- and Set2-deleted cells, activation is delayed and cannot be sustained, and while Set2 and CBC mutant cells have very similar effects, they are distinct from other histone methyltransferases. Moreover, the kinetics of Set2- and CBC-mediated expression corresponds to the proteins' effects

on Pol II occupancy, reinforcing a critical role for the CBC and its mediation of H3K36me3 during active transcription.

MATERIALS AND METHODS

Yeast strains and plasmids. All *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Strains were generated by standard genetic and molecular biology techniques. The plasmid pRS315-BUR1-HA₃ was a gift from S. Buratowski (30). All *RPB1* plasmids were obtained from R. Young except for pRP001, the *RPB1-LEU* plasmid, generated by digesting pRP112 and the pRP114 with SphI and PvuII and swapping the pRP112 insert into the pRP114 backbone (Table 2). The expression plasmid pRS315-CTK1-HA₃ was made by cloning ~2.1 kb of the *CTK1* promoter and open reading frame (ORF) into the SacI-BamHI site of pRS315-HA3-SSN6 vector (30). The plasmids Npl3-HA₃ and Cbp20-HA₃ were generated by cloning ~1.5 kb *NPL3* and 970 bp *CBP20*, respectively, into the vector pRS315-HA3-SSN6.

Western blot analysis. For Western blot analysis of immunoprecipitation (IP), 20 µl of the IP sample and 20 µl of input were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. Both Cbp80 and Cbp20 proteins included tandem affinity purification tags. To detect Cbp80-TAP and Cbp20-TAP, the blots were probed with horseradish peroxidase (HRP)-conjugated anti-TAP antibody (Upstate) at a 1:3,000 dilution; for Bur1-HA, the blot was probed with anti-hemagglutinin (anti-HA) antibody (12CA5) (Roche) at a 1:3,000 dilution. For Pgk1 detection, anti-Pgk1 antibody (Molecular Probes) was used at a 1:1,000 dilution. The blots were processed, and the signal was detected using ECL Super Signal (Amersham Biosciences) according to the manufacturer's instructions.

To determine the level of TAP-tagged protein in WT, *cbp80Δ*, and *cbp20Δ* strains, equal amounts of precleared lysates from the chromatin immunoprecipitation (ChIP) study were separated by 10% SDS-PAGE and transferred to the PVDF membrane. The membrane was probed with anti-TAP (Upstate) and anti-Pgk1 (Molecular Probes) antibodies as described above. For Rpb3 detection, lysates from WT, *cbp80Δ*, and *cbp20Δ* strains were separated by 10% SDS-PAGE and transferred to a PVDF membrane. The membrane was then probed with anti-Rpb3 antibody (Neoclone) at a 1:1,000 dilution and also with anti-Pgk1 antibody as mentioned above.

Coimmunoprecipitation (co-IP). Strains were grown in 150 ml of media to an optical density at 600 nm (OD₆₀₀) of 0.6 to 0.8, cross-linked with 1% formaldehyde for 15 min at room temperature, and quenched with 8.5 ml of 2.5 M glycine for 5 min. The cells were harvested, washed with 1× phosphate-buffered saline (PBS), and quickly frozen in liquid nitrogen. Cells were lysed by bead beating in lysis buffer (50 mM HEPES-KOH, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate) supplemented with protease inhibitors (1 mM PMSF, 1 µg/ml pepstatin, 1 µg/ml aprotinin, 1 µg/ml leupeptin) for 40 min with acid-extracted glass beads at 4°C. The glass beads were removed, and the lysates were sonicated. Sonicated lysates were clarified by centrifuging at 18,000 × g for 20 min at 4°C. A 700-µl portion of clarified supernatant was precleared with 100 µl (50% slurry) of Sepharose CL-4B beads (Amersham Biosciences) for 1 h with rotation. The precleared lysates were incubated with 30 µl of IgG-Sepharose 6 Fast Flow bead (50% slurry) (Amersham Biosciences) and rotated for 2 h at 4°C. The beads were then washed as follows: three times in FA-1 buffer, once in FA-2 buffer (50 mM HEPES-KOH, pH 7.5, 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate), once in FA-2 (700 mM NaCl) buffer, once in FA-3 buffer (20 mM Tris, pH 8.0, 250 mM LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% sodium deoxycholate), and twice in TE (10 mM Tris, pH 8.0, 1 mM EDTA). All the washes were performed at 4°C for 5 min, and the buffers were supplemented with fresh protease inhibitors. Proteins were extracted by boiling the beads at 95°C with 1× SDS-PAGE loading buffer.

For RNase experiments, 700 µl of clarified lysates was incubated with 7.5 units of RNase A and 300 units of RNase T1 (RNase A/T1 mixture;

TABLE 1 Yeast strains used in this study^a

Yeast strain description	Genotype	Source or reference
WT	<i>MATa LYS2⁺ met15Δ</i>	Open Biosystems
<i>cbp20Δ</i>	<i>MATα lys2Δ MET15⁺ cbp20::KAN</i>	Open Biosystems
<i>cbp80Δ</i>	<i>MATα lys2Δ MET15⁺ cbp80::KAN</i>	Open Biosystems
<i>rpb1Δ</i>	<i>MATα uraΔ0 leu2Δ0 lys2Δ0 his3Δ1 rpb1::URA3(pRP112)</i>	Open Biosystems
<i>bur2Δ</i>	<i>MATa LYS2⁺ met15Δ bur2::KAN</i>	Open Biosystems
<i>set2Δ</i>	<i>MATa LYS2⁺ met15Δ set2::KAN</i>	Open Biosystems
<i>ctk2Δ</i>	<i>MATa LYS2⁺ met15Δ ctk2::KAN</i>	Open Biosystems
<i>ctk3Δ</i>	<i>MATa LYS2⁺ met15Δ ctk3::KAN</i>	Open Biosystems
<i>paf1Δ</i>	<i>MATa LYS2⁺ met15Δ paf1::KAN</i>	Open Biosystems
<i>cdc73Δ</i>	<i>MATa LYS2⁺ met15Δ cdc73::KAN</i>	Open Biosystems
<i>rtf1Δ</i>	<i>MATa LYS2⁺ met15Δ rtf1::KAN</i>	Open Biosystems
<i>leo1Δ</i>	<i>MATa LYS2⁺ met15Δ leo1::KAN</i>	Open Biosystems
CBP80-TAP	<i>MATα CBP20-TAP-HIS3 lys2Δ MET15⁺</i>	Open Biosystems
CBP20-TAP	<i>MATa CBP20-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
BUR2-TAP	<i>MATa BUR2-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
CTK2-TAP	<i>MATa CTK2-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
PAF1-TAP	<i>MATa PAF1-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
CDC73-TAP	<i>MATa CDC73-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
BUR1-TAP	<i>MATa BUR1-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
CTK1-TAP	<i>MATa CTK1-TAP-HIS3 LYS2⁺ met15Δ</i>	Open Biosystems
<i>cbp20Δ BUR2-TAP</i>	<i>MATα lys2Δ MET15⁺ cbp20::KAN BUR2-TAP-HIS3</i>	This study
<i>cbp20Δ CTK2-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp20::KAN CTK2-TAP-HIS3</i>	This study
<i>cbp20Δ PAF1-TAP</i>	<i>MATα lys2Δ MET15⁺ cbp20::KAN PAF1-TAP-HIS3</i>	This study
<i>cbp20Δ CDC73-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp20::KAN CDC73-TAP-HIS3</i>	This study
<i>cbp20Δ CBP80-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp20::KAN CBP80-TAP-HIS3</i>	This study
<i>cbp80Δ BUR2-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp80::KAN BUR2-TAP-HIS3</i>	This study
<i>cbp80Δ CTK2-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp80::KAN CTK2-TAP-HIS3</i>	This study
<i>cbp80Δ PAF1-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp80::KAN PAF1-TAP-HIS3</i>	This study
<i>cbp80Δ CDC73-TAP</i>	<i>MATa LYS2⁺ met15Δ cbp80::KAN CDC73-TAP-HIS3</i>	This study
BUR1-HA	<i>MATa LYS2⁺ met15Δ bur1::KAN(pRS315-BUR1-HA)</i>	30
CBP80-TAP BUR1-HA	<i>MATα CBP20-TAP-HIS3 lys2Δ MET15⁺(pRS315-BUR1-HA)</i>	This study
CBP80-TAP CTK1-HA	<i>MATα CBP20-TAP-HIS3 lys2Δ MET15⁺(pRS315-CTK1-HA)</i>	This study
CTK1-HA	<i>MATa LYS2⁺ met15Δ ctk1::KAN(pRS315-CTK1-HA)</i>	This study
CBP20-TAP BUR1-HA	<i>MATa CBP20-TAP-HIS3 LYS2⁺ met15Δ(pRS315-BUR1-HA)</i>	This study
CBP20-TAP CTK1-HA	<i>MATa CBP20-TAP-HIS3 LYS2⁺ met15Δ(pRS315-CTK1-HA)</i>	This study
<i>cbp20Δ CBP80-TAP BUR1-HA</i>	<i>MATa LYS2⁺ met15Δcbp20::KAN CBP80-TAP-HIS3(pRS315-BUR1-HA)</i>	This study
<i>cbp20Δ CBP80-TAP CTK1-HA</i>	<i>MATa LYS2⁺ met15Δ cbp20::KAN CBP80-TAP-HIS3(pRS315-CTK1-HA)</i>	This study

^a All *S. cerevisiae* strains are isogenic with BY4743 (*his3Δ leu2Δ ura3Δ*) from Open Biosystems.

Ambion) at room temperature for 40 min on a nutator before preclearing and processing as described above. As a control, an equal amount of lysates was incubated with RNase storage buffer at room temperature for 40 min.

Western blot analysis of CTD phosphorylation and H3K36me3. Whole-cell extracts were prepared according to the protocol described previously (31). To determine the level of CTD phosphorylation, 10 μl of lysates from each strain was separated by 8% SDS-PAGE and then transferred to a PVDF membrane. One blot was probed with antibody that specifically recognizes Ser-2 phosphorylation of the CTD (Bethyl Laboratories). Another blot was probed with antibody that specifically recognizes

Ser-5 phosphorylation (Bethyl Laboratories). Both the antibodies were used at a 1:5,000 dilution in 5% milk. To determine the level of total RNA Pol II, blots were then stripped using the Restore Western blot stripping buffer (Thermo Scientific) and probed with 8WG16 antibody (Covance) at a 1:500 dilution in 2% milk.

To analyze the H3K36me3, 15 μl of lysates from each strain was separated by 15% SDS-PAGE and then transferred to a PVDF membrane. Blots were probed with an antibody that specifically recognizes histone H3 trimethylated at lysine 36 (H3K36me3) (Abcam) at 1:1,000 in 1% milk. To determine the H3 levels, 5 μl of the same lysates was separated by 15% SDS-PAGE and probed with anti-histone H3, CT, pan antibody (Upstate)

TABLE 2 List of RPB1 plasmids

Plasmid	Alias(es)	Genotype	Source
pRP112	Z26, N247, WT	<i>URA3 RPB1</i> YCp50-based centromere plasmid	R. Young
pRP1-101	C1, N259	<i>LEU2 rpb1Δ101</i> pRP114-based centromere plasmid	R. Young
pRP1-103	C3, N261	<i>LEU2 rpb1Δ103</i> pRP114-based centromere plasmid	R. Young
pRP1-110	V5, N327	<i>LEU2 rpb1Δ110</i> pRP114-based centromere plasmid	R. Young
pRP001	WT-Leu	<i>LEU2 RPB1</i> pRP112- and pRP114-based centromere plasmid	This work

TABLE 3 Primers used for real-time PCR ChIP analysis

Primer name	Sequence	Region (bp)
GAL1 TATA 1	5'-TTTGTAGCCTTATTTCTGGGGTAA-3'	-210 to -185
GAL1 TATA 2	5'-TTAAAGTGGTTATGCAGCTTTTC-3'	-140 to -114
GAL1 5'-ORF1	5'-AGACCATTGGCCGAAAAGTG-3'	+56 to +76
GAL1 5'-ORF2	5'-ACTCTACCAGGCGATCTAGCAAC-3'	+138 to +161
GAL1 M-ORF1	5'-GCAGTTGAAGGCTACTCCGTTT-3'	+607 to +629
GAL1 M-ORF2	5'-ATAGTTGGTTGGGGCGGTTT-3'	+702 to +722
GAL1 3'-ORF1	5'-AGCCCTTGCCAATGAGTTCTAC-3'	+1469 to +1494
GAL1 3'-ORF2	5'-GCCCAATGCTGGTTTAGAGAC-3'	+1545 to +1570
Chr.V1	5'-GTCAGAATATGGGGCCGTAG-3'	+9720 to +9740
Chr.V2	5'-CCTCGGGTCAAACACTACACA-3'	+9786 to +9801
PMA1 TATA 1	5'-GATGGTGGGTACCGCTTATG-3'	-378 to -359
PMA1 TATA 2	5'-TTCAAATGTCCTATCATTATCGTCT-3'	-308 to -283
PMA1 5'-ORF1	5'-TGACGCTGCATCTGAATCTT-3'	+92 to +121
PMA1 5'-ORF2	5'-TCGTCGACACCGTGATTAGA-3'	+154 to +174
PMA1 M-ORF1	5'-AACAAGTTGTCCTTGCACGA-3'	+1155 to +1175
PMA1 M-ORF2	5'-CTTCTGGAAGCAGCCAAAC-3'	+1225 to +1245
PMA1 3'-ORF1	5'-TGTCACCTTCTGAA GCCTTTG-3'	+2627 to +2647
PMA1 3'-ORF2	5'-CAGCCATGAAGTCTTCGACA-3'	+2696 to +2716
ADH1 TATA 1	5'-AACGGTATACGGCCTTCTCT-3'	-198 to -188
ADH1 TATA 2	5'-AGGGAACGAGAACAATGACG-3'	-90 to -70
ADH1 5'-ORF1	5'-CTTCTACGAATCCCACGGTAAG-3'	+33 to +53
ADH1 5'-ORF2	5'-GTGCAAGTCAGTGTGACAGACA-3'	+126 to +146
ADH1 M-ORF1	5'-CTGCTGGTGGTCTAGGTTCTTT-3'	+536 to +567
ADH1 M-ORF2	5'-TACCTTCACCACCGTCAATACC-3'	+597 to +619
ADH1 3'-ORF1	5'-CTTACGTCGGTAACAGAGCTGA-3'	+880 to +902
ADH1 3'-ORF2	5'-CAAGTAGACAAGCCGACAAC-3'	+958 to +978

at 1:3,000 dilution in 5% milk. All the blots were processed and developed using SuperSignal WestPico chemiluminescent substrate (Thermo Scientific).

ChIP. To analyze the *PMA1* and *ADH1* genes, cells were grown in 150 ml of yeast extract-peptone-dextrose (YPD) to an OD₆₀₀ of 0.6 to 0.8. For *GAL1*, the culture was grown in 75 ml of YPR (yeast extract, peptone, and 2% raffinose) to an OD₆₀₀ of 0.5 and then precipitated, washed with 10 ml YPG (yeast extract, peptone, and 2% galactose), and suspended in 75 ml of YPG. Cells grown for 45 min were processed as described previously (32–34).

To analyze the H3K36me3, one aliquot of 400 µl lysates was incubated with 2.5 µl of anti-H3 antibody (Abcam) for 2 h followed by 1 h of incubation with 80 µl of Gammabind G Sepharose beads (GE Healthcare). An aliquot of 400 µl from the same lysate was incubated separately with 8 µl of anti-H3K36me3 antibody (Abcam) for 16 to 18 h at 4°C followed by 1 h of incubation with 90 µl of protein A-agarose (Upstate). For CTD phosphorylation ChIP, 400 µl of lysate was incubated with 1 µl of anti-Ser-2 CTD antibody (Bethyl Laboratories) and another 400-µl aliquot was incubated with 2 µl of anti-Ser-5 CTD antibody (Bethyl Laboratories) for 2 h at 4°C, followed by 1 h of incubation with 80 µl of Gammabind G Sepharose beads (GE Healthcare). For immunoprecipitating RNA Pol II, 400 µl of precleared lysates from the same strain were incubated with 2.5 µl of anti-Rpb3 antibody (Neoclone) for 2 h at 4°C on a rotator followed by 1 h of incubation with 80 µl of Gammabind G Sepharose beads.

PCR and quantitation. Primers used for real-time PCR are described in Table 3. All primers were designed using Primer3 software (35). Each primer set was individually optimized to determine the optimal concentration. A standard curve was generated using serially diluted genomic DNA, and the efficiency of each primer set was determined. RT-PCR was performed using the ABI Prism 7000 (Applied Biosystems) or Mastercycler ep realplex (Eppendorf) real-time PCR system, and amplicons were detected using SYBR green. The nontranscribed region (NTR) in chromosome V was used as a “no ORF” control. The occupancy of protein in a specific region of the ORF was determined using the following equation:

occupancy of tagged protein = (IP value for ORF/IP value for NTR)/(input value for ORF/input value for NTR). The background occupancy of each primer pair was also determined in an isogenic nontagged strain using the same equation, and fold occupancy was determined by normalizing the occupancy value determined for tagged protein with occupancy for an isogenic nontagged strain.

Growing yeast cells to study *GAL1* gene expression. Cells were grown overnight in YPR and transferred into 150 ml of YPR to adjust the starting OD₆₀₀ to 0.1. Cells were grown at 30°C to an OD₆₀₀ of between 0.5 and 0.55, collected by centrifugation, resuspended in 150 ml of YPG (yeast extract, peptone, and 2% galactose), and allowed to grow at 30°C. A 5-ml sample of cells was taken at each indicated time point from 0 h to 9 h.

Northern blot analysis. RNA was isolated from cell pellets using a hot phenol extraction method and dissolved in diethyl pyrocarbonate (DEPC)-treated water, and total RNA was estimated by spectrophotometry. The RNA was processed and hybridization was performed as described previously (31) using probes for *GAL1* and *SCR1* (loading control). To generate the *GAL1* probe, the *GAL1* gene was PCR amplified using the primers 5'-AGACCATTGGCCGAAAAGTG-3' and 5'-GCCCAATGCTGGTTTAGAGAC-3' from the genomic DNA. The PCR product was then digested with HindIII restriction enzyme, and the products were run on an agarose gel. A 270-bp fragment of the 3' end of *GAL1* was gel purified, and 50 ng of DNA was radiolabeled and used as a probe. For *SCR1*, the probe DNA was PCR amplified using the primers 5'-AGGCTGTAATGGCTTTCTGGTGG-3' and 5'-ATGGTTTCAGGACACACTCCATC-3'. The PCR product was then gel purified and used as the template for probe preparation. Quantitation of three independent experiments is shown.

RESULTS

The cap binding complex has functional interactions with the Bur and the Ctk complexes. In order to identify functional interactions between the cap binding complex and factors involved in

FIG 1 The yeast cap binding complex has genetic interactions with the CDK complexes. (A) Tetrad dissection of *cbp80Δ* and *cbp20Δ* strains crossed with *bur2Δ* shows synthetic lethality of the double mutant. Following dissection, the genotype of each spore was confirmed by PCR and is represented below each image. The left side of the slash represents the first gene named in the cross, and the right side of the slash represents the second gene. Minus signs represent deletion of the corresponding gene, and plus signs represent a wild-type copy of the gene. Circles represent nonviable spores and, based upon the tetrad designation, are presumed to be double deletions. Tetrads are designated either TT (tetatype) or NPD (nonparental ditype), based upon the confirmed genotypes of the surviving spores. (B) Tetrad dissection of *cbp80Δ* and *cbp20Δ* strains crossed with *ctk2Δ* shows synthetic lethality of the double mutant. Genotypes of the spores are indicated below each figure as described for panel A. (C) Synthetic growth defect of *cbp80Δ* combined with CTD truncation. *rpb1Δ* (top of each pair) or *cbp80Δ* *rpb1Δ* (bottom of each pair) strains carrying the indicated plasmids were assayed by 10-fold serial dilutions on 5-FOA-Leu plates to select against the URA plasmid carrying wild-type *RPB1*. Plates were incubated at 30°C for 3 days prior to photographing. (D) *cbp20Δ* *rpb1Δ* strains carrying the indicated plasmids were assayed as described above. Plates were incubated at 30°C for 3 days prior to photographing. This set of dilution series was all grown on the same plate.

transcription, a genetic screen was performed to identify deletions in nonessential transcription components that, when combined with deletion of either *CBP80* or *CBP20*, lead to synthetic lethality. *cbp80Δ* and *cbp20Δ* were crossed with cells deleted of the gene encoding the nonessential component of the Bur complex *BUR2* (30). The resulting double-mutant cells were synthetically lethal; i.e., in the absence of a functional CBC, the cells became dependent on an intact Bur complex for viability (Fig. 1A), suggesting a functional relationship between the two complexes.

Next, *CTK2* deletion was analyzed in combination with *CBC* deletion. Similar to what we observed with the *BUR2* deletion, when cells were also deleted of the *CBC*, they became dependent on a functional Ctk complex for viability. The *cbp80Δ ctk2Δ* double mutant was inviable, and *cbp20Δ ctk2Δ* cells displayed a severe synthetic growth defect (Fig. 1B). Similar results were observed with cells deleted of *CTK3* (data not shown).

Since both the Bur and Ctk complexes target the CTD of RNA polymerase II, we considered the possibility that the Bur and Ctk genetic interactions with CBC involved their roles in regulating CTD activity. Therefore, we next analyzed genetic interactions between the CBC and the CTD of RNA polymerase II. Although complete deletion of the CTD is lethal to cells (36), RNAPII mutants with a truncated CTD are viable; hence, genetic interactions between the truncated CTD and the CBC were examined. Both *cbp80Δ* and *cbp20Δ* yeast strains had an increasingly severe growth defect as the CTD was truncated (Fig. 1C and D). In fact, while truncation of the CTD to as few as 10 repeats had little effect on viability under normal growth conditions, when combined with *cbp80Δ* these cells were dead, and a truncation to 13 repeats revealed a severe synthetic growth defect (Fig. 1C). *cbp20Δ* strains were affected by CTD truncation in a similar manner; when the CTD was truncated to 10 repeats, cells lacking *CBP20* were nonviable (Fig. 1D). These data demonstrate that when the CTD

length is suboptimal, cells become dependent on a fully functional cap binding complex. The difference seen in the genetic interactions between the two subunits of the CBC with the CTD truncations and with the Ctk complex suggest that the two subunits may not have completely overlapping roles, which is consistent with microarray expression data indicating that the *CBP80* and *CBP20* deletions have slightly different expression profiles (37).

The cap binding complex coimmunoprecipitates with the Bur and Ctk complexes. The combination of our genetic analyses suggested that the CBC may contribute to activities of the Bur complex, the Ctk complex, and the CTD, perhaps by targeting Bur complex and Ctk complex activities toward the CTD. Such a function would suggest physical interactions between the CTD kinase complexes and the CBC.

To examine this, we analyzed interactions between the complexes by coimmunoprecipitation (co-IP). Each component of the CBC was TAP tagged, and Bur1 or Ctk1 was tagged with HA. To be sure that the observed co-IPs represented *in vivo* association, we performed co-IP after cross-linking the cells, as these conditions preserve native, weak, and transient interactions between the CBC and the transcription machineries (38). Moreover, there are reports that some interactions between proteins inferred from co-IP may actually occur after cell lysis and may not represent *in vivo* association (39). First, upon immunoprecipitation of the tagged components of the CBC complex, both Cbp80 and Cbp20 coprecipitated with Bur1 (Fig. 2A, lanes 2 and 6). No IP was observed with untagged controls (Fig. 2B). Although interactions were detected between Bur1 and either Cbp80 or Cbp20, no co-IP between Cbp80 and Bur1 was observed in the absence of Cbp20, indicating that an intact CBC complex was required for the interaction (Fig. 2A, lane 4). Since in *cbp80Δ* cells the stability of Cbp20 decreases significantly, it was not possible to determine if the in-

FIG 2 The yeast cap binding complex coimmunoprecipitates with the CDK complexes and the interaction between CBC and CDK complexes persists in the presence of RNase. (A) Bur1 coimmunoprecipitates with Cbp80 (lane 2) and with Cbp20 (lane 6). Results of Western blot analysis of cross-linked immunoprecipitation using a strain in which Cbp80 (lanes 1 to 4) or Cbp20 (lanes 5 and 6) was TAP tagged and Bur1 was HA tagged are shown. The TAP-tagged CBC was immunoprecipitated from the lysates using IgG beads, and the sample was analyzed by Western blot to detect Bur1. The co-IPs in lanes 3 and 4 were performed using strains in which Cbp20 was deleted. Input was 1/40 of the total lysate used for immunoprecipitation. The upper part of the blot was probed with the anti-TAP antibody, the middle part was probed with anti-HA, and the lower part was probed with anti-Pgk1 antibody. (B) Western blots from untagged samples treated as in Fig. 2A show that there was no nonspecific interaction between the IgG beads and either untagged proteins in the extract (lane 2) or HA (lane 4), while IgG beads specifically immunoprecipitated TAP-tagged proteins Cbp80-TAP (lane 6) and Cbp20-TAP (lane 8). (C) Ctk1 coimmunoprecipitates with Cbp80 (lane 2) and Cbp20 (lane 6). The experiments were carried out as described for panel A. (D) Western blot of extracts with untagged proteins show that there is no nonspecific interaction between IgG beads and Ctk1-HA (lane 2), whereas they specifically recognize Cbp80-TAP (lane 4) and Cbp20-TAP (lane 6). (E) Cbp20 coimmunoprecipitates with Bur1 (lane 2) and Ctk1 (lane 4). Extracts were made from a strain in which Bur1 was TAP tagged and Cbp20 was HA tagged (lanes 1 and 2) and Bur1 was immunoprecipitated with IgG beads. Similarly, Ctk1 was immunoprecipitated with IgG, and Cbp20-HA was tagged (lanes 3 and 4). The experiments were carried out as described for panel A. (F) Cbp80 coimmunoprecipitates with Bur1 (lanes 3 and 4) and Ctk1 (lanes 7 and 8). The interactions were analyzed using a strain in which Bur1 (lanes 1 to 4) or Ctk1 (lanes 5 to 8) was TAP tagged and Cbp80 was tagged with HA (lanes 1 to 8). Experiments were carried out as described for panel E. (G) The Bur complex does not interact with Npl3 but interacts with Cbp20 under the same RNase/IP conditions. Coimmunoprecipitation of Bur1 was carried out using the strain in which Bur1 was TAP tagged and both Npl3 and Cbp20 were tagged with HA (lane 1). The blot was probed with a mixture of anti-TAP (1:3,000) and anti-HA (1:2,000) antibodies. Lane 3 shows the immunoprecipitation in which the lysate was treated with RNase A/T1. (H) co-IP of Cbp80 or Cbp20 and Bur1 persists in the presence of RNase treatment. Experiments were carried out as described in Fig. 2A, except the lysates (lanes 2 and 4) were treated with RNase A/T1 (7.5 units of RNase A and 300 units of RNase T1). The upper and lower blots were probed with anti-TAP antibody, and the middle blot was probed with anti-HA antibody. (I) co-IPs of Cbp80 or Cbp20 and Ctk1 persist in the presence of RNase. Experiments were carried out as described for panel A. Lanes 2 and 4 show the co-IPs in which lysates were treated with RNase A/T1. (J) Representative example of RNase treatment of the total RNA present in the lysate from cross-linked cells. Cells were treated as described for panels H and I. Samples shown in lanes 4 and 7 were treated with RNase A/T1 (7.5 units of RNase A and 300 units of RNase T1), while samples in lanes 2, 3, 5, and 6 were incubated with RNase storage buffer.

teration between Cbp20 and the Bur complex occurs in the absence of Cbp80.

Interactions between the CBC and the Ctk complex were analyzed next. We also observed the co-IP of Ctk1 when either Cbp80

or Cbp20 was immunoprecipitated (Fig. 2C, lanes 2 and 6), although the interaction is stronger between Cbp20 and Ctk1, suggesting that the interactions between the CBC and Ctk1 may be primarily mediated through Cbp20. Similar to the results seen

with Bur1, deletion of Cbp20 disrupts the interaction between Cbp80 and Ctk1, confirming that an intact CBC complex is required for interactions with Ctk1 (Fig. 2C, lane 4). Moreover, untagged controls confirm the specificity of these interactions (Fig. 2D). The interaction between Bur or Ctk and the CBC was also observed when the tags were reversed (Fig. 2E and F). Interestingly, we consistently observed more efficient pulldown of Cbp80 with Bur1 than Ctk1 (Fig. 2F; compare Fig. 2A and C). The co-IP results described here between either of the CTD kinase complexes and the CBC were also observed in the absence of cross-linking. However, cross-reactivity between the IgG and HA-tagged protein could be eliminated only by high-salt washes, which interfered with binding of the complexes. Under the conditions described here, no cross-reactivity between IgG and Bur1-HA or Ctk1-HA (Fig. 2B, lane 4, and D, lane 2) was observed. Together these data indicate that, while the co-IP results cannot exclude the possibility that the interactions are stabilized by other proteins, it is clear that an intact CBC has *in vivo* interactions with both cyclin-dependent kinase complexes.

Next, we wanted to address the possibility that the CBC associations with the Bur and Ctk complexes were mediated through their binding to nascent RNA. If this were the case, one would predict that in addition to the CBC, another cotranscriptionally recruited RNA binding protein would also be coimmunoprecipitated by the Bur or Ctk complex under the same conditions. To test this, co-IPs were carried out in a strain in which BUR1 was TAP tagged and both Cbp20 and Npl3 were tagged with HA. Npl3, like the CBC, binds cotranscriptionally to the nascent RNA (40). Moreover, Npl3 has been shown to interact with the CBC (9). Npl3 showed no interaction with Bur1 under the exact conditions under which the CBC-Bur1 interaction was detected (Fig. 2G, lanes 2 and 3), even when the blot was overexposed (data not shown), indicating that the interactions are unique and that we did not simply capture RNA-mediated association between RNA binding proteins and transcription elongation complexes. Moreover, when the immunoprecipitated lysates were treated with RNase, interactions between the CBC and the cyclin-dependent kinase complexes persisted (Fig. 2G, lane 2, and H and I, lanes 2 and 4). This was true despite the fact that the RNase concentrations used were sufficient to degrade endogenous RNA normally found in RNPs under cross-linking conditions (Fig. 2J). These results indicate that the interactions we observe are at least partially mediated through protein-protein interactions. Furthermore, although Npl3 and the CBC have overlapping interactions with proteins involved in export and termination/polyadenylation, the fact that Npl3 was not detected in our CBC-Bur1 immunoprecipitation suggests that the CBC-Bur association was not mediated through Npl3 or an Npl3-containing complex. Intriguingly, the mammalian ortholog of the Bur complex P-TEFb co-IPs with the mammalian cap binding complex (19), indicating that this interaction is evolutionarily conserved. Our finding that Ctk1, the mammalian CDK12 ortholog, also immunoprecipitated the CBC suggests a broader role for the CBC in interactions that underlie CTD Ser-2 phosphorylation.

The CBC facilitates cotranscriptional recruitment of the Bur and Ctk complexes. In light of our data showing functional and physical interactions between the CBC and the CTD kinases, we next wanted to determine if the CBC interaction with the Bur and Ctk complexes affects their cotranscriptional recruitment to genes. Therefore, we analyzed Bur2 occupancy in the constitu-

tively expressed *PMA1* and *ADH1* genes (Fig. 3A and B). In WT cells, Bur2 showed the highest recruitment at the 5' end and gradually decreased toward the 3' end of both *PMA1* and *ADH1* genes (Fig. 3C and D), a result consistent with observations by others (18, 30, 41, 42). The higher levels of Bur2 observed in the middle (M-ORF) and 3' ORF of *ADH1* compared to those in *PMA1* likely reflect decreased resolution of these amplicons, as *ADH1* is half the length of *PMA1*. When either *CBP80* or *CBP20* was deleted, recruitment of Bur2 was significantly decreased, particularly at the 5' end of the *PMA1* gene (Fig. 3C), while the CBC had little effect on Bur2 association within the M-ORF or the 3' end of the *PMA1* gene. A similar pattern was observed for the *ADH1* gene (Fig. 3D). Importantly, the steady-state level of Bur2 protein (or Bur1 protein; data not shown) in the CBC-deleted cells remains unchanged (Fig. 3E).

Ctk2 occupancy was then analyzed along the *PMA1* and *ADH1* genes in Cbp20- or Cbp80-deleted cells. Deletion of either subunit of the CBC significantly decreased Ctk2 occupancy in the middle and 3' end of the *PMA1* gene (Fig. 3F). Similarly when Ctk2 occupancy on *ADH1* was analyzed in WT cells, Ctk2 was particularly enriched in the middle and 3' end of *ADH1*. Upon deletion of either component of the CBC, the Ctk2 recruitment was decreased in these regions, while CBC deletion had only a modest effect in the 5' region of the gene (Fig. 3G). However, the level of Ctk2 protein remained unchanged in *CBCΔ* cells (Fig. 3H).

These results demonstrate that the CBC plays a role in recruiting the Bur and Ctk complexes to the constitutively expressed *PMA1* and *ADH1* genes, consistent with our physical and genetic interactions. Moreover, the CBC appears to play differential roles throughout the gene, the interactions with the Bur complex being particularly important near the 5' end of the gene and interactions with the Ctk complex being particularly important further downstream. In light of our results that the Ctk and Bur complexes both interact with the CBC complex but have different affinities for Cbp80 versus Cbp20, it is possible that these differential interactions contribute to the sequential association of the Bur and the Ctk complexes during transcription.

The CBC facilitates proper serine 2 phosphorylation of the CTD of RNA Pol II. We predicted that since the CBC establishes proper association of the Bur and Ctk complexes with the constitutively expressed *PMA1* and *ADH1* genes, we would also observe CBC-dependent phosphorylation of the CTD, indicative of a role for the CBC in transcription elongation. To test this, chromatin immunoprecipitation experiments were performed using specific antibodies that recognize the Ser-2 and Ser-5 phosphorylation states of the CTD, which we confirm in Fig. 4A. The experiment was also performed using the same lysates to analyze the occupancy of Rpb3 on these genes and normalize Ser-2 and Ser-5 phosphorylation to total Pol II. Deletion of the CBC led to a 2- to 3-fold decrease in Ser-2 phosphorylation on both *PMA1* and *ADH1* (Fig. 4B and C), while there was no significant decrease in Ser-5 phosphorylation on either gene (Fig. 4D and E) or total polymerase (Fig. 4F and G). The CBC effect on Ser-2 phosphorylation was evident throughout the gene, suggesting that the CBC's role in recruiting each of the CTD kinase complexes, which target RNA polymerase differentially at the 5' and 3' ends of genes, is important for proper CTD phosphorylation. Interestingly, we observed a modest but consistent decrease in total Ser-2-phosphorylated RNAP II in the CBC-deleted strains (Fig. 4I), despite the fact that neither *CBP80* nor *CBP20* deletion had any effect on the levels of

FIG 3 The CBC is required for proper cotranscriptional recruitment of the CDK complexes. (A and B) Schematic representations of the *PMA1* and *ADH1* genes, respectively. The black bars indicate the region of the quantitative PCR amplicons analyzed by ChIP. The coding regions of the genes are indicated by the shaded boxes. (C) Bur2 recruitment to *PMA1* is CBC dependent. Bur2 occupancy is represented as fold occupancy over the NTR following normalization relative to a nontagged isogenic control. Primers used represent regions of *PMA1* within the promoter (TATA), the 5' ORF, the middle of the ORF, and the 3' end of the ORF. The data represent the averages from three independent experiments with standard errors of the means (SEM). (D) Bur2 recruitment to the *ADH1* gene. Experiments were performed as described for panel C. The data represent the averages from three independent experiments with SEM. (E) Western blot analyses showing equal levels of Bur2-TAP in WT, *cbp80Δ*, and *cbp20Δ* strains when cells were grown in YPD. Each lane was loaded with ~50 μ g of total protein from each strain. Pgk1 was included as an internal loading control. (F) Ctk2 recruitment to the *PMA1* gene. Experiments were performed as described for panel A. The data represent the result of three independent experiments. (G) Ctk2 recruitment to *ADH1*. Experiments were performed as described for panel A. The data represent the result of three independent experiments. (H) Western blot depicting equal levels of Ctk2-TAP protein in WT, *cbp80Δ*, and *cbp20Δ* strains when cells were grown in YPD. Each lane was loaded with ~40 μ g of total protein from each strain. Pgk1 served as an internal loading control.

the proteins that catalyze this modification (Fig. 3E and H). This result is similar to the observed effect of mammalian CBC on total Ser-2 phosphorylation (19) and is not surprising in light of the fact that the CBC interacts with Ser-2 CTD kinase complexes and can target their activities to active polymerase. Consistent with this, on every gene that we have analyzed (Fig. 3 and 5 and data not shown), recruitment of the Bur and Ctk complexes is diminished severalfold.

The CBC and its effect on CTD Ser-2 phosphorylation are crucial for establishing proper H3K36me3. One of the ways in

which CTD phosphorylation has significant effects on transcription is that it serves as a platform for other critical regulatory factors, including factors that catalyze posttranslational modifications on histone proteins such as the histone methyltransferases (21, 22, 43). Furthermore, both the Bur and Ctk complexes have been shown to be required for proper H3K36me3: Bur1/2 at the 5' end of the gene and Ctk at the 3' end. As *CBCΔ* was defective in cotranscriptional recruitment of the Bur and Ctk complexes and CTD Ser-2 phosphorylation, we analyzed H3K36me3 on both *PMA1* and *ADH1* genes (Fig. 4J and K) using an antibody that we

FIG 4 The CBC regulates the Ser-2 phosphorylation of RNAPII and H3K36me3 on *PMA1* and *ADH1* genes. (A) Western blot analysis showing that the CTD Ser-2 phosphorylation antibody (Bethyl Laboratories) specifically recognizes Ser-2 and not Ser-5 CTD phosphorylation. Lysates from WT and *ctk2Δ* strains were analyzed to determine the specificity of the CTD Ser-2 antibody. The upper panel of the Western blot was probed with Ser-2 phosphorylation-specific antibody, and the middle panel was probed with Ser-5 phosphorylation-specific antibody (both from Bethyl Laboratories). The lower panel shows the RNA Pol II (RNAPII) in the same lysates probed with 8WG16 antibody (Covance). (B) ChIP profile depicting the altered distribution of the Ser-2-phosphorylated CTD of RNAPII within the *PMA1* coding region in *cbcΔ* cells compared to WT cells. The phosphorylated Ser-2 of the CTD was immunoprecipitated using Ser-2-specific antibody (Bethyl Laboratories). Fold occupancy of Ser-2-phosphorylated Pol II was normalized to the nontranscribed control (NTR) following normalization to Rpb3 occupancy. The data represent the averages from at least three independent experiments with standard errors of the means (SEM). (C) Distribution of RNAPII phosphorylated at Ser-2 within the *ADH1* coding region in *cbcΔ* cells compared to WT cells. Experiments were performed as described for panel B. (D) ChIP profile depicting the Ser-5 phosphorylation of the CTD of RNAPII on the *PMA1* coding region in WT and *cbcΔ* cells. Ser-5-phosphorylated CTD was immunoprecipitated using the Ser-5-specific antibody (Bethyl Laboratories). Fold occupancy of Ser-5-phosphorylated CTD of RNA Pol II is represented relative to an NTR following normalization to Rpb3. The data represent the averages from three independent experiments with SEM. (E) ChIP analysis depicting the Ser-5-phosphorylated CTD of RNA Pol II on the *ADH1* gene. The experiments and normalization were carried out as described for panel D. (F and G) Recruitment of RNA Pol II in the coding regions of *PMA1* and *ADH1*, respectively. RNA Pol II was immunoprecipitated using an anti-Rpb3 antibody (Neoclone), and fold occupancy was measured relative to NTR. The data represent the averages from three independent experiments with SEM. (H) Total Rpb3 protein levels remain unchanged in the *cbcΔ* cells. Western blot shows the protein levels of Rpb3 in WT, *cbp80Δ*, and *cbp20Δ* cells. Each lane was loaded with an equal amount (~50 μg) of total protein from each strain. The upper blot was probed with Rpb3 antibody (Neoclone). Pgk1 served as the loading control for each strain. (I) Western blot showing the effect of CBC deletion on total CTD Ser-2 and Ser-5 phosphorylation levels. Whole-cell lysates from WT, *cbp80Δ*, and *cbp20Δ* strains were analyzed by Western blot. The upper panel was probed with antibody specific to CTD Ser-2 phosphorylation (Bethyl Laboratories), and the middle panel was probed with antibody specific to CTD Ser-5 phosphorylation (Bethyl Laboratories). The lower panel was probed with 8WG16 antibody (Covance) to detect RNA Pol II. (J) ChIP analyses showing the distribution of H3K36me3 at the 5' region, middle, and 3' end of the *PMA1* gene in WT and *CBCΔ* cells. Immunoprecipitation was performed using the antibody specific for H3K36me3 (Abcam), and then an aliquot of lysate from the same strain was used to immunoprecipitate histone H3 using an anti-histone H3 antibody (Abcam). H3K36me3 is represented as fold enrichment over the NTR following normalization to H3. The data represent the averages from three independent experiments, and the error bars represent the SEM. (K) H3K36me3 within the coding region of *ADH1* in WT and *CBCΔ* cells. The experiments were performed as described for panel J. The data represent the averages from three independent experiments with SEM.

FIG 5 CBC regulates the recruitment of the CDK complexes and affects the Ser-2 CTD phosphorylation and histone H3K36me3 on the inducible *GAL1* gene. (A) Schematic representation of the *GAL1* gene. The amplicons are indicated by black bars, and the shaded box represents the coding region. (B) Bur2 recruitment to the induced *GAL1* gene is CBC dependent. Bur2 occupancy is represented as fold occupancy over the NTR following normalization relative to a nontagged isogenic control. Primers used represent regions of *GAL1* within the promoter (TATA), the 5' ORF, the middle of the ORF, and the 3' end of the ORF. The data represent the averages from three independent experiments with SEM. (C) ChIP profile of Ctk2 recruitment to the *GAL1* gene. Experiments were performed as described for panel B. The data represent the results of three independent experiments with SEM. (D) Deletion of the CBC leads to impaired cotranscriptional recruitment of the PAF complex component, Paf1, on the *GAL1* gene. ChIP depicts the decreased recruitment of Paf1 on the *GAL1* gene in *cbp80Δ* cells compared to WT cells. Data represent the averages from four independent experiments with SEM. A Western blot detecting the Paf1 protein levels in WT and CBC-deleted cells is shown on the right. (E). The double mutants *cbp80Δ paf1Δ* and *cbp20Δ paf1Δ* are synthetically lethal. Tetrad dissection of *cbp80Δ* and *cbp20Δ* strains with *paf1Δ* is shown. The genotype of the each spore is indicated below each figure as described for Fig. 1A. (F) ChIP profile depicting distribution of phosphorylated Ser-2 of RNAPII within the *GAL1* coding region in *cbp80Δ* and WT cells. Phosphorylated Ser-2 of CTD was immunoprecipitated using the specific antibody (Bethyl Laboratories). Ser-2 phosphorylation is normalized to the NTR following normalization to Rpb3. The data represent the averages from three independent experiments with SEM. (G) ChIP profile depicting phosphorylated Ser-5 RNA Pol II distribution within the *GAL1* coding region in *cbp80Δ* and WT cells. Phosphorylated Ser-5 of the CTD was immunoprecipitated using Ser-5-specific antibody (Bethyl Laboratories). Ser-5 phosphorylation is normalized to the NTR following normalization to Rpb3. The data represent the averages from three independent experiments with SEM. (H) ChIP depicting the occupancy of RNA Pol II within the induced *GAL1* gene of WT, *cbp80Δ*, and *cbp20Δ* cells. RNA Pol II was immunoprecipitated using the antibody that recognizes Rpb3 (Neodclone), and fold occupancy was normalized to the NTR. The data represent the averages from three independent experiments with SEM. (I) ChIP analyses depicting H3K36me3 at the 5' end, middle, and 3' end of the *GAL1* open reading frame in WT and *cbp80Δ* cells. Histone H3K36me3 was immunoprecipitated using an antibody targeting H3K36me3 (Abcam), and histone H3 was immunoprecipitated from an aliquot of the same lysate using antibody against histone H3 (Abcam). H3K36me3 is represented as fold enrichment over the NTR following normalization to H3. The data represent the averages from three independent

confirmed was specific for Set2-dependent K36 trimethylation (data not shown). Deletion of either *CBP80* or *CBP20* led to a decrease in H3K36me3 throughout the coding region of the *PMA1* and *ADH1* genes (Fig. 4J and K), suggesting that the ability of the CBC to mediate exchange of factors in the transition from the 5' to the 3' end of the gene affects H3K36me3.

The CBC affects cotranscriptional recruitment of the Bur and Ctk complexes to the inducible *GAL1* gene. In order to get a more detailed understanding of the CBC's effect on transcription, we analyzed the inducible *GAL1* gene (Fig. 5A). When WT yeast cells are grown in galactose, the *GAL1* gene is rapidly induced and maintained at high levels over multiple generations. Yeast cells require a precise functional interplay between the transcription elongation machineries Bur and Ctk in order to rapidly induce and maintain optimal *GAL1* gene expression (28, 30, 44), so we wanted to determine how deletion of the CBC affects the association and function of these proteins on the actively transcribed *GAL1* gene. First, we analyzed the recruitment of Bur2 to *GAL1*. In the presence of the CBC, Bur2 was recruited to *GAL1* upon induction with galactose, with the highest recruitment at the 5' end of the gene and a gradual decrease toward the 3' end of the gene (Fig. 5B). When either *CBP80* or *CBP20* was deleted, recruitment of Bur2 was decreased, particularly at the 5' end of the gene (Fig. 5B), while the CBC had no effect on Bur2 occupancy in the middle or the 3' end of the *GAL1* gene.

A similar experiment was performed to examine the Ctk complex. When Ctk occupancy was analyzed in Cbp20- or Cbp80-deleted cells, Ctk2 was significantly decreased, particularly in the middle and 3' end of the *GAL1* gene, but had no effect in the 5' ORF (Fig. 5C). The Bur and Ctk complex protein levels remained unchanged in CBC-deleted cells grown under these conditions (data not shown).

In light of the CBC's role in cotranscriptional recruitment of the CTD kinase complexes, we predicted that it would also be important for optimal recruitment of complexes downstream of Bur or Ctk such as PAF, which requires the Bur complex for association with the coding region of *GAL1* (41, 42). To this end, we found that when the CBC was deleted, PAF complex recruitment was abrogated, as indicated by a decrease in Paf1 occupancy in the *GAL1* gene (Fig. 5D); the level of Paf1 remained unchanged when the CBC was deleted (Fig. 5D). Similar results were observed with other PAF components such as Cdc73 (data not shown). Moreover, the CBC and PAF complex showed functional interactions, as deletion of either subunit of the CBC was synthetically lethal when combined with deletion of either *PAF1* or *CDC73* (Fig. 5E and data not shown).

The CBC affects CTD Ser-2 phosphorylation and histone H3K36me3 within the inducible *GAL1* gene. Next, we wanted to analyze the role of the CBC in CTD phosphorylation and H3K36me3 during transcription of the inducible *GAL1* gene. As with the constitutively expressed *PMA1* and *ADH1* genes, a 2- to 3-fold decrease in Ser-2-phosphorylated RNA Pol II was observed on the *GAL1* gene in the *CBCΔ* cells relative to Rpb3 (Fig. 5F) while the Ser-5-phosphorylated RNA Pol II remained relatively unchanged (Fig. 5G).

Additionally, H3K36me3 decreased on the inducible *GAL1* gene when either subunit of the CBC was deleted (Fig. 5I). Consistent with this decrease in H3K36me3, we also observed decreased occupancy of Set2, the H3K36 methyltransferase, in the *GAL1* coding region, while total Set2 protein levels were not affected by CBC deletion (Fig. 5J).

The CBC and Set2 have similar effects on the ability to induce and sustain *GAL1* expression. The ability of cells to respond effectively to a changing environment is determined by several important parameters such as the abilities to rapidly induce expression, to reach optimal expression levels, and to sustain expression over multiple generations. The activities of transcription factors and modifications of chromatin help to achieve this tight regulation. In light of these observations that the CBC is important to direct the activity of transcription complexes and mediate H3K36me3, a mark of active transcription, we assessed how H3K36me3 and, more specifically, deletion of the CBC affect induction and sustenance of *GAL1* expression in the presence of galactose over several hours in *set2Δ* and *cbcΔ* cells.

A probe designed to detect the full-length *GAL1* transcript by Northern blot was used to assess *GAL1* RNA levels upon exposure of the cells to galactose. Cells deleted of the genes encoding either Set2 or the CBC (Cbp20 or Cbp80) showed a similar pattern of expression over time: an initial delay in induction (e.g., at 15 min, all three strains have an approximately 10-fold decrease in *GAL1* relative to WT cells) and a steep increase in expression to nearly WT levels, followed by a rapid drop in *GAL1* RNA levels (Fig. 6A). On the other hand, WT cells reached maximum expression at 3 h, and the levels remained nearly unchanged for up to 8 h (Fig. 6). The similarity in the profiles of *set2Δ* and *cbcΔ* is more apparent when compared to the effects that two other histone methyltransferases have on *GAL1* expression (Fig. 6B). Deletion of the gene encoding Dot1, which catalyzes H3K79 methylation, had no significant effect on *GAL1* expression. Deletion of the H3K4 methyltransferase Set1 led to more rapid *GAL1* induction than WT, a result reported previously (45). However, after about an hour, when *GAL1* expression in the WT cells is continuing to rise, expression in the *set1Δ* strain began to drop precipitously and remained low.

Interestingly, while the general profiles of *GAL1* expression were similar in the CBC- or Set2-deleted strains, and in all strains maximum expression was reached between 2 and 4 h, the CBC-deleted strains consistently only achieved 80 to 85% of the WT or *set2Δ* RNA level. However, this was not surprising, since the CBC's effect on CTD phosphorylation may have additional effects on RNA synthesis, independent of Set2 recruitment (Fig. 5F). Additionally, it has also been reported that the CBC can promote recruitment of the transcription factor Mot1 to stimulate preinitiation complex (PIC) formation (46). To address the effects of CBC and Set2 on Pol II transcription directly, we analyzed Pol II occupancy on *GAL1* at several key time points during galactose exposure.

Deletion of the CBC alters Pol II occupancy upon induction in a manner similar to Set2. In order to determine how deletion of the CBC affects polymerase association with the *GAL1* gene, we

experiments with SEM. (J) CBC affects the recruitment of the histone H3K36me3 methyltransferase, Set2, on the *GAL1* gene. ChIP analyses depict the CBC-dependent Set2 recruitment at the 5' end, middle, and 3' end of the *GAL1*. The occupancy of Set2 is represented as fold enrichment over the nontranscribed region following normalization relative to an untagged isogenic control. The data represent the averages from three independent experiments with SEM. A Western blot showing Set2 protein levels in WT and CBC-deleted cells is shown; Pgk1 serves as a loading control.

FIG 6 CBC and Set2 are required to activate and sustain maximal expression of *GAL1* gene. (A) *GAL1* activation is delayed (between 0 h to 1 h) and decreased (between 6 h to 8 h) in *set2Δ*, *cbp80Δ*, and *cbp20Δ* cells. The line graph shows the expression pattern of the *GAL1* gene over time in WT, *set2Δ*, *cbp80Δ*, and *cbp20Δ* strains. *GAL1* gene expression was analyzed by Northern blotting and quantitated by phosphorimaging (GE Healthcare). The expression of *GAL1* transcripts at each time point was normalized to a loading control (*SCR1*). Each point indicates the average from at least three independent experiments, and the error bars indicate the standard deviation. (B) *set1Δ* and *dot1Δ* strains show different patterns of *GAL1* expression over time compared to *set2Δ* or *cbcΔ* expression in galactose. Quantitation was carried out as described for panel A. The line graph shows the averages from two independent experiments with standard deviations. (C, D, and E) Recruitment of Rpb3 on *GAL1* at the promoter (TATA), 5' region (5' ORF), middle region (M-ORF), and 3' region (3' ORF) when WT, *cbp20Δ*, and *set2Δ* strains were grown in galactose for 0.25 h, 4 h, and 7 h, respectively. The bar graph represents the averages from three different experiments with standard errors of the means.

analyzed Pol II occupancy in a *cbp20Δ* strain by ChIP. As previously shown, low (but above background) levels of Rpb3 were present at the promoter in all strains, although no RNA was detected (data not shown). When WT cells were shifted to galactose, recruitment of Rpb3 increased in the promoter region and Rpb3 was observed across the gene. In the absence of Set2 or Cbp20, we observed a decrease in Pol II association (Fig. 6C), consistent with the delayed activation that we observed by Northern analysis (Fig. 6A). Over time (4 h), we observed that RNA polymerase occupancy increased and, in fact, *set2Δ* achieved equal levels of Rpb3 compared to WT at the promoter and throughout the 5' and middle regions of the gene. Cbp20 also neared WT levels but not to the same extent as *set2Δ*, consistent with the expression data (Fig. 6D). Notably, while Rpb3 occupancy was higher in the 3' ORF at 4 h compared to 0.25 h (15 min) in the WT cells, neither *set2Δ* nor *cbp20Δ* could sustain Pol II occupancy throughout the gene to the same extent as WT (Fig. 6D). At later time points (7 h), the effect of Set2 on Rpb3's ability to reach the 3' end of the gene, which was already apparent at 4 h, was greater, and deletion of Cbp20 had similar effects on Pol II occupancy at the 3' end of the gene, consistent with the observation that *cbp20Δ* and *set2Δ* showed com-

parably low levels of *GAL1* mRNA (Fig. 6E and A). While *cbp20Δ* and *set2Δ* showed similar effects on Pol II occupancy throughout *GAL1* at 15 min and 4 h, it is notable that at 7 h occupancy in the 5' ORF and the middle of the ORF was lower in *cbp20Δ* than *set2Δ* (Fig. 6E). This likely explains the generally more pronounced decrease in RNA levels in the *cbp20Δ* mutant, perhaps because the *cbcΔ* effect on Ser-2 phosphorylation could contribute to elongation defects on top of the effect on Set2 recruitment. Nonetheless, it is clear that, like Set2, the protein that the CBC helps recruit to *GAL1*, the CBC affects transcription elongation—most significantly early in induction and during sustained exposure to galactose. We cannot exclude the possibility that the overall lower levels of Rpb3 compared to *set2Δ* also reflect the effects of the CBC on PIC formation as well as effects on CTD phosphorylation that may be independent of Set2. However, it is notable that the overall patterns of Pol II association across *GAL1* are strikingly similar between *set2Δ* and *cbp20Δ* at these time points.

DISCUSSION

A growing number of studies demonstrate that RNA processing is tightly linked, both temporally and spatially, to transcription (23).

The studies described here provide evidence of functional and physical (Fig. 1 and 2) communication between the yeast cap binding complex and the transcription elongation machinery. As a result of this communication, the CBC ensures proper recruitment of the Bur1/2 and the Ctk1/2/3 complexes (Fig. 3 and 5) to maintain proper CTD phosphorylation and H3K36me3 (Fig. 4 and 5) throughout the coding region of both constitutively expressed and inducible genes. These data also demonstrate that through these interactions, the CBC contributes to proper Pol II elongation and the ability to induce and maintain optimal expression of the inducible gene *GAL1* (Fig. 6). These results illustrate a novel role for an RNA processing factor in histone modification.

Dynamic recruitment of transcription elongation factors and proper histone modification are mediated by the CBC. The yeast CBC has long been speculated to affect transcription elongation; however, evidence for such a function has been lacking. Our published observations demonstrated that, genome-wide, the CBC had dramatic effects on gene expression, particularly on intronless genes. We went on to show that the CBC's role in splicing is critical for expression of the H2B deubiquitinase machinery (29, 31). However, restoring proper H2B ubiquitination was insufficient for establishing proper cotranscriptional K36me3 in the cells deleted of the genes encoding the CBC (data not shown), suggesting that the CBC acted downstream and perhaps more directly on proteins involved in K36me3. Hence, our observations described here, that deletion of *CBC* decreases the recruitment of the Bur complex in the 5' region and the Ctk complex in the middle and 3' region of the genes (Fig. 3 and 5), demonstrate a splicing-independent mechanism by which the CBC affects transcription and histone modification. Since our data also demonstrate that the intact CBC complex physically associates with both complexes, we propose that the CBC, through its interaction with the 5' end of the RNA, may "measure" the progression of transcription elongation and, through protein-protein interactions, reinforce recruitment of factors to the correct regions of the gene for optimal gene expression (Fig. 7). Together, these observations demonstrate that interaction of the CBC with the transcription machinery is required for dynamic recruitment of transcription factors within the coding region of the gene.

Although the CBC-Bur1/2 and the CBC-Ctk1/2/3 interactions do not require RNA, it is known that optimal CBC recruitment to actively transcribed genes is facilitated by a 5' cap (5). Hence we envision a model whereby the CBC-bound nascent RNA adopts a conformation that allows the CBC to simultaneously interact with the transcription machinery and the 5' end of the RNA such as RNA looping (Fig. 7). Cbp80 has a number of protein interaction domains (such as HEAT domains) that resemble protein-protein interaction domains in transcription factors (J. M. Claggett and T. L. Johnson, unpublished data). These may facilitate the CBC's interactions with transcription elongation factors near the 5' end of the gene versus the middle and 3' end. Moreover, the CBC has also been shown to interact with Mot1 (46) to affect PIC formation and can also interact with the 3' end processing factors to regulate the cotranscriptional recruitment of transcription termination factors (5). Hence, in addition to the role we report here for the CBC in histone modification by affecting the recruitment of factors during transcription elongation, the CBC appears to play roles in transcription throughout the transcription cycle. An RNA looping model helps to explain how the CBC can be present at the 5' end of the RNA while it simultaneously interacts with proteins

FIG 7 Proposed model of how the CBC affects dynamic recruitment of transcription factors and histone H3K36me3. (I) The CBC is cotranscriptionally recruited to the 5' cap. (II) Near the 5' end of the gene, CBC interacts with the Bur complex, which phosphorylates the CTD at Ser-2. This phosphorylation leads to Set2 and the PAF complex recruitment. (III) As RNAPII elongates the RNA, CBC interacts with the Ctk complex (probably via Cbp20) to recruit the Ctk complex to RNAPII and facilitate CTD phosphorylation in the middle and 3' end of the gene with concomitant H3K36me3. Although none are shown, the model does not preclude additional protein interactions with the CBC and the Bur/Ctk complexes.

downstream. Moreover, in its capacity as a "molecular ruler" the CBC is well positioned to play a critical role in ensuring that proteins important at each stage of transcription are properly recruited at the right time to the correct location.

Further evidence of the importance of the interactions that we describe here is that they appear to be well conserved. A recent report describes interactions between the mammalian P-TEFb complex and the mammalian cap binding complex that influence patterns of alternative splicing. Here we extend these studies to show that both the P-TEFb ortholog Bur1/2 and the yeast Ctk complex interact with the CBC, and both are recruited to genes in a manner that is stimulated by the CBC. It remains to be explored whether the CBC affects splicing via its effects on histone modification; however, ongoing studies will address this directly.

One of the interesting challenges raised by these and other studies has been to understand the biological significance of the CBC's role in H3K36me3. Our data support an important role for H3K36me3 in the expression of an inducible gene. Specifically, the mark influences the ability of the cell to induce and sustain expression over time and over multiple generations. Both Set2 and CBC play roles in this important biological response to environmental changes.

RNA processing in general may play a critical role in histone modification. While RNA processing and transcription through a chromatin template are spatially and temporally linked, there is little mechanistic detail about the relationship between these re-

actions, particularly how RNA processing factors influence establishment of histone marks. For example, several recent mammalian studies report that histone H3K36 methylation is mediated through splicing signals (47, 48). However, the proteins involved in splicing that broker this relationship have yet to be identified. Our studies raise the intriguing possibility that the cap binding complex is involved in this type of coordination. Furthermore, the effects of RNA processing factors such as the CBC in histone modification could contribute to their roles in cotranscriptional RNA processing. Histone marks as well as chromatin-modifying enzymes have been shown to affect recruitment of RNA processing enzymes (for example, see references 49 and 50). Hence, RNA processing factors that stimulate histone modification could provide positive feedback that ensures proper cotranscriptional RNA processing. It will be interesting to determine how changes in K36me3 affect RNA processing in yeast and in metazoans and to identify other RNA processing factors that contribute to establishing H3K36me3 and other histone marks.

ACKNOWLEDGMENTS

We thank Stephen Buratowski for the pRS315 Bur1-HA₃ expression plasmid and Richard Young for the CTD truncation plasmids. We also thank Imre Barta for his technical expertise in the early stages of the work.

This work was supported by the NSF (CAREER award to T.L.J.; MCB-0448010 and MCB-1051921) and the NIGMS (GM085474 and GM085764).

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