Supplemental Experimental Data:

Table S1. ChIP-qPCR and RT-qPCR primer sequences

snR13 gene

TGGCATCTCAAATCGTCTCT (10-F snR13)
ATTTGGCAAAAGCCAAACAG (10-R snR13)
TTGCCAAATCAGTAACGGTG (153-F snR13)
CAAGCCAAACCCAACGTACT (153-R snR13)
GGAAGCGACAGAAAGACAGG (434-F snR13)
TGTTGTTGAAGCTTCGCTTG (434-R snR13)
AGTACTTCGTTTGCGGCATT (765-F snR13)
TCAAACCTTCCCTGTCAAGC (765-R snR13)
TGATTTTTCGTTACCCACGA (1458-F snR13)
AACGTTGAAAAGCCGACAGT (1458-R snR13)

snR45 gene

AAAAGCGAAACACTCGGTACA (1-F snR45)
GATCGCTCCGAGAAGAATTG (1-R snR45
CGCAACCCATTGATCTTGTT (203-F snR45)
AACTGTTTCGGCACGGTTTA (203-R snR45
GGGAAACTTGCCGTTAACAA (393-F snR45)
AGACAGACCCCAGGCGTATT (393-R snR45)

snR33 gene

ATGCCCTCTTTGTACGATGG (99-F snR33)
TGCAAATCGATTGTCCACAC (99-R snR33)
TTTGAGTCGGTTCCTTCGTT (212-F snR33)
GGTCGCTTTTAAAGAAAACGATAA (212-R snR33)
TAACCAGAAGCGAACAGTGG (427-F snR33)
TTGGTAAAATGACCCCACTCA (427-R snR33)

CYC1 gene

ACGTGTTGTGGGACGAAAAT (264-F CYC1)
AAGGGGCCTGTTTACTCACA (264-R CYC1)
CGCTCGAAGGCTTTAATTTG (665-F CYC1)
TGAGGCGAACAAAGTCAGAA (665-R CYC1)

NRD1 gene

AGTACCCTACGGCAATGCAC (1651-F NRD1)
GAGCAGTGGGGTCAAATTGT (1651-R NRD1)

Supplemental Experimental Procedures:

Yeast strains and plasmids

The parent strain yBC1 (*RPB2* shuffle strain) and the yRpb2-TAP plasmid (Domecq et al., 2009) were kind gifts of Benoit Coulombe (Institut de Recherches Cliniques de Montréal). To regenerate the strain yBC25 (Domecq et al., 2009), yBC1 was transformed with yRpb2-TAP plasmid and cured of the *URA3* plasmid-borne copy of *RPB2*. The Rpb2 residues 922-938 were deleted in the yRpb2-TAP plasmid to generate the delta-flap (Δ-flap) rpb2 mutant. The strain yBC1 was transformed with the Δ-flap plasmid, with subsequent plasmid shuffling, to generate strain yEP6. The Rat1-HA strains were constructed in the wild-type (wt) Rpb2 (yEP12) and Δ-flap rpb2 (yEP13) backgrounds using the Pringle method to integrate a C-terminal HA tag at the *RAT1* locus (Longtine, et al., 1998). The tagging was confirmed by Western blotting. Strains yBC25 and yEP6 were transformed with the plasmid pUGCYC1 (generous gift of Nick Proudfoot) and were employed in a subset of ChIP experiments (see below) to examine Pol II occupancy on the *CYC1* gene. All strains were grown in YPAD (YPD rich medium supplemented with adenine) at 30°C, unless otherwise indicated.

Phenotype plating assays

Overnight yBC25 (*RPB2*) and yEP6 *(Δ-flap rpb2*) cultures were diluted to an OD 600 of 0.5 in 10 mM Tris, 1 mM EDTA, pH 7.5 (TE). Five-fold serial dilutions were spotted onto YPD, YPGal and YPD + 1.5% formamide and incubated at 30°C, 34°C and 37°C for 2-3 days.

Yeast extract preparation

To generate extract for the *in vitro* transcription assays, yeast strains were grown to an OD600 of 2.0-5.0. The cells were harvested and resuspended in one volume of AGK buffer (20 mM

HEPES-KOH, pH 7.9; 200 mM KCl; 1.5 mM Mg_2Cl; 10% glycerol; 0.5 mM DTT) supplemented with EDTA-free protease inhibitor cocktail (Roche). Cells were frozen in droplets in liquid nitrogen and lysed with cryo-grinding. The thawed lysate was cleared with ultracentrifugation first at 31,000 rpm in the TLA 100.3 rotor for 30 minutes and then at 90,000 rpm in the same rotor for 30 minutes. Proteins in the cleared lysate were precipitated with 0.35 mg/ml finely ground ammonium sulfate (55% saturation) with stirring on ice for 30 minutes. The ammonium sulfate pellet was collected with ultracentrifugation at 31,000 rpm in the TLA 100.3 rotor for 20 minutes and was carefully resuspended in 40-80 μl of D-alternative buffer (20 mM HEPES, pH 7.9; 75 mM potassium acetate; 1.5 mM magnesium acetate; 20% glycerol; 1 mM DTT) per ml of sample prior to ammonium sulfate precipitation. The resuspension was dialyzed three times against 600 ml of D-alternative buffer, for one hour each time, and cleared with centrifugation for 2 minutes at 15,000 rpm in a tabletop microcentrifuge. The extracts were flash-frozen in liquid nitrogen and stored at -80°C. Addition of ammonium sulfate (final concentration of 0.5 M) to the freshly lysed cells and incubation at 4°C, with rocking, prior to centrifugation resulted in transcriptionally inactive extract.

For preparation of crude whole cell extracts for determination of total protein levels and immunoprecipitations, 50 ml cultures were grown to an OD600 of 0.6-0.8, shifted to 37°C for 30 minutes, harvested, and resuspended in 300 μl extraction buffer (100 mM Tris-Cl, pH 7.9; 250 mM ammonium sulfate; 1 mM EDTA; 10 % glycerol; 0.5 mM DTT) supplemented with EDTA-free protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (1 mM sodium fluoride; 0.5 mM sodium vanadate; 8 mM glycerol-2-phosphate). The resuspension was added to 300 μl glass beads pre-chilled in a 1.5 ml Eppendorf tube, and the cells were lysed with five repetitions of vortexing for 1 minute at 4°C and resting on ice for 1 minute. The lysate was cleared with centrifugation at maximum speed for 1 minute to remove the beads and then again

for 15 minutes to remove precipitated material. The extracts were flash-frozen in liquid nitrogen and stored at -80°C.

In vitro transcription assay

Transcription reactions were performed as described previously (Mariconti et al., 2010), except that 100 μg extract and 0.5 μg plasmid DNA were used. The transcription-template plasmids pKS708, pKS709 and pKS710 were kind gifts of Bernhard Dichtl (Universität Zürich). RNA products were digested with T1 RNase, and the fragments resolved on a 6% polyacrylamide/7 M urea gel. After Phosphorimager detection, the radioactive intensities of each band were measured using ImageQuant software and were normalized to the 100 nt G-less cassette band to calculate the termination efficiency at each downstream G-less cassette. Averages were generated from three independent experiments.

Chromatin Immunopreciptation (ChIP), quantitative PCR (qPCR) and RT-qPCR

Strains (50 ml) were grown to OD600 0.6-0.8, shifted to 37°C for 30 minutes, fixed with 1.035% formaldehyde for 20 minutes and neutralized with 0.135 M glycine for 5 minutes. Washed cells were lysed in FA-lysis buffer (50 mM HEPES-KOH, pH 7.9; 150 mM NaCl; 1% TritonX-100; 1 mM EDTA; 0.1% sodium deoxycholate; 0.1% SDS) with grinding in liquid nitrogen. Crosslinked chromatin was sheared in a final volume of 500 μl FA-lysis buffer in a 2 ml microcentrifuge tube using a Branson water bath sonicator at 4°C for 8 minutes. Two hundred microliters of pre-cleared sheared chromatin were immunoprecipitated with 15 μl protein A beads pre-equilibrated with the respective antibody, as indicated in the figure legends. Antibodies used in the ChIP analysis include the anti-pan CTD mouse monoclonal antibody, 4H8 (Santa Cruz); the anti-Rna15 rabbit polyclonal antibody (generous gift of Horst Domdey); the anti-Nrd1 rabbit polyclonal antibody (generous gift of David Brow); the anti-HA mouse monoclonal antibody, F7

(Santa Cruz); the 3E8 anti-Ser5P antibody (kind gift of Mike Hampsey); the H5 anti-Ser2P antibody (Active Motif); and the 3D12 anti-Tyr1P antibody (Active Motif). Samples were rotated 4 hours to overnight at 4°C. The beads were washed once with each of the following buffers: FA-lysis buffer + 275 mM NaCl; FA-lysis buffer + 500 mM NaCl; LiCl buffer (10 mM Tris-Cl, 1 mM EDTA, 0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate); TE. Upon washing, chromatin was eluted with TE + 1% SDS with incubation at 65°C for 20 minutes. Samples were treated with Proteinase K for 1 hour at 42°C and de-crosslinked at 65°C for 5 hours to overnight. DNA was purified with phenol-chloroform extraction and ethanol precipitation and was resuspended in 200 μl qPCR-grade water.

In a subset of ChIP experiments to examine recruitment of termination factors to the transcription complexes, cells were fixed for 5 minutes with formaldehyde and the extracted chromatin was sheared for 2 minutes at 4°C.

Quantitative PCR (qPCR) was conducted in 20 μl reaction volumes consisting of SYBR Green PCR master mix, 0.5 mM primers (Table S1) and 2 μl of IP, mock or input samples. Up to 40 cycles were used for each experiment. The relative occupancy was calculated as a percentage of input using the equation: $\Delta Ct = 2^{-(IP_{Ct} - input_{Ct})}$. Average relative occupancy values are presented, and the error bars represent the standard deviation from these average values generated from two to four independent experiments. For mRNA genes, the calculated occupancy at each primer pair location was normalized to the value obtained at the 5′ end of the ORF in the Wt strain. For snoRNA genes, the calculated occupancy was normalized to the value obtained at the snoRNA ORF in the Wt strain.

Total RNA was extracted from mid-log cells shifted to 37°C for 30 minutes using the standard hot phenol method. cDNA was synthesized using the iScript kit from BioRad. qPCR was performed as described above.

In vitro co-immunoprecipitation experiments

Wt and Δ-flap Pol II were immunoprecipitated by incubation of 500 μg yBC25 or yEP6 transcription extract with 15 μl pre-equilibrated IgG-coupled sepharose beads (GE) in D-alternative buffer for 2 hours with rotation at 4°C. Beads were washed twice in D-alternative buffer and twice in transcription buffer (50 mM HEPES-KOH, pH 7.6; 100 mM potassium glutamate; 15 mM magnesium acetate; 5 mM EGTA) and resuspended in transcription buffer. One to three micrograms of purified recombinant CF IA, Pcf11/Clp1, Pcf11, or Rna14/Rna15 (CF IA, Pcf11/Clp1 and Rna14/Rna15 were kind gifts of Andrew Bohm, James Gordon, and Celia Harrison, Gordon et al., 2011) were added to the resuspended beads and incubated with rotation for 1 hour at 4°C. Upon washing three times in transcription buffer, the beads were resuspended in 20 μl transcription buffer mixed with 1x SDS-load buffer, incubated at 95°C for 5 minutes, loaded onto an 8% SDS-PAGE and transferred to PVDF membrane for Western blotting.

For the pull-downs with the flap peptide, biotinylated flap peptide was bound to 20 μL high capacity streptavidin-coated agarose beads in PBS for 1 hour at ambient temperature. As a control, biotinylated-BSA was incubated with 20 μL beads. The beads were washed three times in PBS (+ 0.1% BSA) prior to the addition of recombinant protein. The immobilized flap peptide (or the immobilized BSA control) and the recombinant proteins were allowed to incubate in transcription buffer for three hours at 4°C. Upon washing three times in transcription buffer, the beads were resuspended in 20 μl transcription buffer mixed with 1x SDS-load buffer, incubated at 95°C for 5 minutes, loaded onto an 8% SDS-PAGE and transferred to PVDF membrane for Western blotting. For the co-immunoprecipitation of Pcf11 with Rna14/Rna15, three micrograms of both Pcf11 and Rna14/Rna15 were pre-incubated in transcription buffer for 1 hour at 4°C before being added to rabbit anti-Rna15 antibody (generous gifts of Horst Domdey) pre-bound to protein A agarose beads. The mixture was allowed to incubate for 1

hour at 4°C before washing in transcription buffer and elution in SDS-load buffer. For the pull-downs in crude extract, 1.5 mg of yBC25 and yEP6 crude extract were diluted to 1 ml in IP-50 buffer (20 mM Tris-Cl, pH 7.9; 50 mM NaCl; 1mM EDTA; 5% glycerol; 0.005% NP-40; 0.5 mM DTT), with protease and phosphatase inhibitors (as described in previous sections), and incubated with 15 μl pre-equilibrated IgG-sepharose beads, as described above. The beads were washed twice briefly in IP-50 buffer, and treated with or without 10 μg/ml RNase A/T1 and 20 U/ml DNase in IP-50 buffer supplemented with 5 mM $MgCl_2$ and 2 mM $CaCl_2$ for 30 minutes at 30°C (Johnson et al., 2009). The beads were washed three times in either IP-50 buffer or IP-300 (ie. 300 mM NaCl) buffer. Immunoprecipitated proteins were eluted in 2x SDS-load buffer and analyzed as described above. To examine *in vivo* protein levels, crude extract from the equivalent of 1.0 OD600 unit of cells was resolved by an 8% SDS-PAGE and transferred to a PVDF membrane for Western blotting. Antibodies used in the Western blots include the anti-His_6 rabbit polyclonal antibody, H15 (Santa Cruz); the anti-HA mouse monoclonal antibody, F7 (Santa Cruz); the anti-Pcf11 rabbit polyclonal antibody (kind gift of David Bentley); the anti-Rna15 rabbit polyclonal antibody (kind gift of Horst Domdey); the anti-Clp1 rabbit polyclonal antibody (Z. Zhang, Moore Lab); the anti-Nrd1 rabbit polyclonal antibody (generous gift of David Brow); and the pan-CTD mouse monoclonal antibody, 4H8 (Santa Cruz). Incubation with primary antibodies was typically done overnight at 4°C with gentle agitation. Incubation with the Nrd1 antibody was done for one hour at ambient temperature.

SUPPLEMENTAL REFERENCES

Domecq, C., Kireeva, M., Archambault, J., Kashlev, M., and Coulombe, B. (2010). Site-directed mutagenesis, purification and assay of Saccharomyces cerevisiae RNA polymerase II. Protein Expr. Purif. *69*, 83-90.

Gordon, J.M., Shikov, S., Kuehner, J.N., Liriano, M., Lee, E., Stafford, W., Poulsen, M.B., Harrison, C., Moore, C., and Bohm, A. (2011). Reconstitution of CF IA from overexpressed subunits reveals stoichiometry and provides insights into molecular topology. Biochemistry *50*, 10203-10214.

Johnson, S.A., Cubberley, G., and Bentley, D.A. (2009) Cotranscriptional Recruitment of the mRNA export factor Yra1 by direct interaction with the 3′ end processing factor Pcf11. Mol. Cell *33*, 215-226.

Longtine, M.S., McKenzie, A 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast *14*, 953-961.

Mariconti, L., Loll, B., Schlinkmann, K., Wengi, A., Meinhart, A., and Dichtl, B. (2010). Coupled RNA polymerase II transcription and 3′ end formation in yeast whole-cell extracts. RNA *16*, 2205-2217.