Synthetic Lethal Interactions Suggest a Role for the *Saccharomyces cerevisiae* Rtf1 Protein in Transcription Elongation

Patrick J. Costa and Karen M. Arndt

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Manuscript received April 21, 2000
Accepted for publication June 14, 2000

ABSTRACT

Strong evidence indicates that transcription elongation by RNA polymerase II (pol II) is a highly regulated process. Here we present genetic results that indicate a role for the *Saccharomyces cerevisiae* Rtf1 protein in transcription elongation. A screen for synthetic lethal mutations was carried out with an *rtf1* deletion mutation to identify factors that interact with Rtf1 or regulate the same process as Rtf1. The screen uncovered mutations in *SRB5*, *CTK1*, *FCP1*, and *POB3*. These genes encode an Srb/mediator component, a CTD kinase, a CTD phosphatase, and a protein involved in the regulation of transcription by chromatin structure, respectively. All of these gene products have been directly or indirectly implicated in transcription elongation, indicating that Rtf1 may also regulate this process. In support of this view, we show that *RTF1* functionally interacts with genes that encode known elongation factors, including *SPT4*, *SPT5*, *SPT16*, and *PPR2*. We also show that a deletion of *RTF1* causes sensitivity to 6-azauracil and mycophenolic acid, phenotypes correlated with a transcription elongation defect. Collectively, our results suggest that Rtf1 may function as a novel transcription elongation factor in yeast.

TRANSCRIPTION of mRNA by RNA polymerase (pol) II involves multiple steps, which include initiation, promoter clearance, elongation, and termination. Transcription regulatory factors could potentially target any of these steps to determine the level of transcript production. Recent evidence indicates that the transition from initiation to elongation is a highly regulated event in the transcription cycle. An important participant in this transition is the essential carboxyl-terminal domain (CTD) of the largest subunit of RNA pol II. The CTD contains highly conserved tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser (Dahmus 1996). Yeast RNA pol II contains 26 or 27 repeats, while the mammalian enzyme contains 52 repeats. Phosphorylation of the CTD accompanies the transition from transcription initiation to elongation (Dahmus 1996). The hypophosphorylated form of RNA pol II preferentially enters the preinitiation complex (PIC), which assembles at the promoter (Lu *et al.* 1991; Chesnut *et al.* 1992). Subsequently, the CTD is extensively phosphorylated. Several CTD kinases have been described. In yeast, these include CTDK-I, Srb10, and the essential TFIIH-associated kinase, Kin28 (Dahmus 1996). The coordinate regulation of these kinases is not well understood. However, as a component of the PIC, Kin28 appears to play a pivotal role in phosphorylating the CTD early in the transcription process (Dahmus 1996; Hampsey 1998). Transcription elongation is then executed by hyperphosphorylated RNA pol II (Cadena and Dahmus 1987; Payne *et al.* 1989; O'Brien *et al.* 1994). Upon completion of the transcript, the CTD must be dephosphorylated to reinitiate the transcription cycle. A CTD phosphatase, whose activity is stimulated by TFIIF, has been identified in human and yeast cells (Chambers *et al.* 1995; Archambault *et al.* 1997; Cho *et al.* 1999; Kobor *et al.* 1999).

Several factors that affect initiation by RNA pol II also have roles in transcription elongation. Chromatin and chromatin remodeling factors are involved in the regulation of both processes, since nucleosomes provide a potent impediment to promoter recognition and mRNA chain elongation (Paranjape *et al.* 1994; Kingston *et al.* 1996; Uptain *et al.* 1997). The general transcription factors (TF) IIF and TFIIH, which are essential for PIC assembly and initiation, also regulate elongation. TFIIF interacts directly with RNA pol II and suppresses transient pausing by the enzyme (Uptain *et al.* 1997). The kinase activity of TFIIH has been shown by several studies to participate in elongation (Yankulov *et al.* 1995, 1996; Parada and Roeder 1996; Cujec *et al.* 1997; García-Martínez *et al.* 1997). Interestingly, phosphorylation of the CTD by TFIIH can be stimulated by the human immunodeficiency virus (HIV)-1 Tat protein, providing one mechanism by which Tat promotes transcription through an elongation block (Parada and Roeder 1996; Cujec *et al.* 1997; García-Martínez *et al.* 1997). Last, certain transcriptional activators facilitate elongation, possibly by recruiting elongation or chromatin remodeling factors to the polymerase (Yankulov *et al.* 1994; Brown *et al.* 1996).

Corresponding author: Karen M. Arndt, Department of Biological Sciences, University of Pittsburgh, 269 Crawford Hall, Fifth and Ruskin Aves., Pittsburgh, PA 15260. E-mail: arndt@pitt.edu

Relative to the initiation step of transcription, much less is known about the factors that expressly control elongation. However, recent work has led to the characterization of several elongation factors, including TFIIS, P-TEFb, ELL, the elongator complex, and the Spt4-Spt5 complex (Marshall and Price 1995; Uptain *et al.* 1997; Hartzog *et al.* 1998; Wada *et al.* 1998; Otero *et al.* 1999; Wittschieben *et al.* 1999). Of these proteins, TFIIS is the best characterized. TFIIS facilitates RNA pol II passage through arrest sites by stimulating an intrinsic ribonuclease activity of RNA pol II and causing cleavage of the nascent transcript near the 3′ end. In essence, this action resets RNA pol II and provides an additional opportunity to progress through an arrest site (Uptain *et al.* 1997). The Spt4 and Spt5 proteins form a complex that binds to RNA pol II and regulates elongation (Hartzog *et al.* 1998; Wada *et al.* 1998). Interestingly, *SPT4*, *SPT5*, and a related gene, *SPT6*, were originally identified in a genetic selection for factors that regulate transcription initiation in yeast (Winston 1992). Considerable evidence suggests that these genes regulate transcription through an effect on chromatin structure (Swanson and Winston 1992; Bortvin and Winston 1996; Hartzog *et al.* 1998). Undoubtedly, the complexity of the RNA pol II transcription circuitry will require the involvement of additional initiation and elongation factors.

In accordance with this prediction, we previously reported the identification of a novel *Saccharomyces cerevisiae* gene, *RTF1* (*R*estores *T*BP *F*unction), whose product affects TATA-binding protein (TBP) function *in vivo*. *RTF1* was uncovered in a genetic selection for extragenic suppressors of a TBP-altered specificity mutant, TBP-L205F (Arndt *et al.* 1994; Stolinski *et al.* 1997). The altered DNA-binding specificity of TBP-L205F causes an Spt$^-$ phenotype (Arndt *et al.* 1994). This phenotype reflects the ability of TBP-L205F to suppress the transcriptional defects caused by the insertion of the retrotransposon Ty or its long terminal repeat (δ) within the promoter of a gene. Because Ty elements contain several transcription signals, including a potent TATA box, their integration within a promoter establishes a competition between *cis*-acting transcription elements (Winston 1992). Mutations that confer an Spt$^-$ phenotype are thought to affect this competition, and we have previously suggested that Rtf1 suppresses the Spt$^-$ phenotype of TBP-L205F by directly or indirectly regulating TATA site selection by TBP (Stolinski *et al.* 1997). Importantly, *rtf1* deletion mutations (*rtf1*Δ) confer an Spt$^-$ phenotype even in the presence of wild-type TBP (Stolinski *et al.* 1997). *RTF1* encodes a nuclear protein with a predicted mass of 65.8 kD (Stolinski *et al.* 1997). The protein is rich in charged amino acids, a feature common to many transcription factors (Karlin 1993; Stolinski *et al.* 1997), but lacks known functional motifs.

To clarify the role of Rtf1 in transcription, we have performed a genetic screen for mutations that cause lethality in combination with an *rtf1* deletion mutation. The results of this screen, together with additional genetic interactions between Rtf1 and known elongation factors, suggest that Rtf1 is important for transcription elongation in yeast.

MATERIALS AND METHODS

Genetic methods and media: Rich (YPD), YPGlycerol (YPG), minimal (SD), synthetic complete (SC), 5-fluoro-orotic acid (5-FOA), and sporulation media were prepared as previously described (Rose *et al.* 1990). Galactose and sucrose media contained YEP (1% yeast extract, 2% Bacto-peptone), 1 μg/ml antimycin A, and either 2% galactose or 2% sucrose, respectively. Formamide, LiCl, and NaCl media contained YEP and the appropriate chemical (3% deionized formamide, 0.3 m LiCl, 1.2 m NaCl, or 1.4 m NaCl). SD media lacking (−Ino) or containing (+Ino) inositol were prepared as previously described (Sherman *et al.* 1981). Hydroxyurea media were prepared by supplementing SC media with 100 mm hydroxyurea (US Biological). 6-azauracil and mycophenolic acid media were prepared by supplementing SC-Ura media with 50 μg/ml 6-azauracil (Aldrich Chemical, Milwaukee) and 20 μg/ml mycophenolic acid (Sigma, St. Louis), respectively. All yeast strains used to test for 6-azauracil and mycophenolic acid sensitivity contained a *URA3*$^+$ allele in the genome. Transformation of yeast cells was performed using the lithium acetate procedure and plasmids were recovered from yeast as described (Arndt *et al.* 1994).

Yeast strains: The *S. cerevisiae* strains used in this study appear in Table 1. Strains were constructed by standard methods (Rose *et al.* 1990). All FY, GHY, GY, and KY strains are isogenic with FY2, a *GAL2*$^+$ derivative of S288C (Winston *et al.* 1995). To introduce the *ade2* and *ade3* mutations into an *rtf1*Δ background, strain PSY137 (Koepp *et al.* 1996) was mated to KY409 (Stolinski *et al.* 1997). This cross generated KA48, the original strain used for the synthetic lethal screen. With the exception of KA49, KA50, KA51, KA52, KA53, KA68, KA72, and KA76, all subsequently numbered KA strains were obtained from genetic crosses with KA48 derived mutants. The *srb5*Δ strain L937 is described in Roberts and Winston (1997).

Plasmids: Standard techniques were used for plasmid construction (Ausubel *et al.* 1998). pPC1, which harbors the *RTF1*, *ADE3*, and *URA3* genes, was constructed by cloning the 3.1-kb *Sal*I fragment from pKA61 (Stolinski *et al.* 1997) into the *Sal*I site of pPS719 (pRS426 + *ADE3*). pPC3, which contains the *RTF1*, *ADE3*, and *TRP1* genes, was created by cloning the same insert into the *Sal*I site of pPS793 (pRS424 + *ADE3*). pLS20, which contains *RTF1* in pRS314, has been described (Stolinski *et al.* 1997).

The following plasmids were created to verify the identity of the genes responsible for synthetic lethality with *rtf1*Δ and to determine linkage of the complementing genes to the synthetic lethal mutations. pPC13 (*SRB5*) and pPC14 (*SRB5*) were created by inserting a 1.9-kb *Bam*HI-*Eco*RI fragment from pCT39 (Thompson *et al.* 1993) into the corresponding sites of pRS314 and pRS304 (Sikorski and Hieter 1989), respectively. pPC20 (*CTK1*) and pPC19 (*CTK1*) were created by inserting a 3.7-kb *Pvu*II insert from pPC15, one of three *CTK1*-containing library isolates, into the *Sma*I site of pRS314 and pRS304, respectively. pPC26 (*FCP1*) and pPC27 (*FCP1*) were constructed by subcloning a 2.7-kb *Sna*BI-*Xho*I insert from pPC25, the original *FCP1*-containing library isolate, into the *Sma*I and *Xho*I sites of pRS314 and pRS304, respectively. pPC29 (*POB3*) and pPC30 (*POB3*) are derived from pPC23. pPC23

TABLE 1

***Saccharomyces cerevisiae* strains**

Strain	Genotype
FY2	*MAT*α *ura3-52*
FY23	*MAT***a** *leu2Δ1 ura3-52 trp1Δ63*
FY69	*MAT***a** *leu2Δ1*
FY91	*MAT*α *ade8*
FY243	*MAT***a** *spt4Δ1::URA3 his4-912δ lys2-128δ leu2Δ1 ura3-52*
FY300	*MAT***a** *spt5-194 his4-912δ lys2-128δ leu2Δ1 ura3-52*
FY348	*MAT***a** *spt16-197 his4-912δ lys2-128δ leu2Δ1 ura3-52*
FY1256	*MAT*α *sin4Δ::TRP1 lys2-173R2 leu2Δ1 ura3-52 trp1Δ63 arg4-12*
FY1257	*MAT*α *gal11Δ::TRP1 lys2-173R2 leu2Δ1 ura3-52 trp1Δ63 arg4-12*
FY1285	*MAT***a** *srb2Δ::HIS3 his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63*
FY1289	*MAT***a** *rgr1Δ2::TRP1 his4-917δ lys2-173R2 leu2Δ1 ura3-52 trp1Δ63*
FY1671	*MAT*α *ppr2Δ::hisG his4-912δ lys2-128δ leu2Δ1 ura3-52*
GHY285	*MAT*α *ppr2Δ::URA3-hisG his4-912δ lys2-128δ leu2Δ1 ura3-52*
GHY364	*MAT***a** *spt6-14 his4-912δ lys2-128δ leu2Δ1*
GHY492	*MAT***a** *rpb2Δ297::HIS3 his3Δ200 lys2-128δ leu2Δ1 ura3-52 trp1Δ63* [pRP2-10(U) = *rpb2-10 URA3 CEN*]
GHY713	*MAT***a** *chd1Δ::URA3 his3Δ200 lys2-128δ leu2Δ1 ura3-52*
GY759	*MAT***a** *srb10Δ::TRP1 his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 suc2ΔUAS(-1900/-390)*
L937	*MAT*α *srb5Δ::URA3-hisG his3Δ200 leu2 ura3-52 trp1Δ63 arg4-12*
KY404	*MAT***a** *rtf1Δ101::LEU2 his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63*
KY405	*MAT*α *rtf1Δ101::LEU2 his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63*
KY409	*MAT*α *rtf1Δ101::LEU2 his4-917δ lys2-173R2 leu2Δ1 ura3-52 trp1Δ63*
KY424	*MAT***a** *rtf1Δ100::URA3 his4-912δ lys2-128δ ura3-52 trp1Δ63 ade8*
KY425	*MAT***a** *rtf1Δ100::URA3 his4-912δ lys2-128δ ura3-52*
KY426	*MAT*α *rtf1Δ100::URA3 his4-912δ lys2-128δ ura3-52 trp1Δ63*
KY459	*MAT*α *rtf1Δ100::URA3 his3Δ200 lys2-173R2 leu2Δ1 ura3-52 ade8*
KY473	*MAT***a** *rtf1Δ101::LEU2 his4-917δ lys2-173R2 leu2Δ1 ura3-52 trp1Δ63*
KY571	*MAT***a** *his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 suc2ΔUAS(-1900/-390)*
KY573	*MAT***a** *his4-912δ lys2-128δ ura3-52 trp1Δ63 suc2ΔUAS(-1900/-390)*
KY607	*MAT*α *rtf1Δ101::LEU2 his3Δ200 lys2-128δ leu2Δ1 ura3-52*
KY608	*MAT*α *rtf1Δ101::LEU2 his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63 suc2ΔUAS(-1900/-390)*
KY610	*MAT*α *rtf1Δ101::LEU2 spt4Δ1::URA3 his4-912δ lys2-128δ leu2Δ1 ura3-52*
KY611	*MAT***a** *rtf1Δ101::LEU2 spt4Δ1::URA3 his4-912δ lys2-128δ leu2Δ1 ura3-52*
KY612	*MAT***a** *rtf1Δ101::LEU2 spt4Δ1::URA3 his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63*
KY613	*MAT***a** *rtf1Δ101::LEU2 spt5-194 his4-912δ lys2-128δ leu2Δ1 ura3-52*
KY614	*MAT*α *rtf1Δ101::LEU2 spt16-197 his4-912δ lys2-128δ leu2Δ1 ura3-52 trp1Δ63*
KY616	*MAT***a** *his4-912δ lys2-128δ leu2Δ1 ura3-52 suc2ΔUAS(-1900/-390)*
KY617	*MAT*α *his4-912δ lys2-128δ leu2Δ1 ura3-52 suc2ΔUAS(-1900/-390)*
KY624	*MAT***a** *ppr2Δ::URA3-hisG ura3-52 trp1Δ63*
KA48	*MAT*α *rtf1Δ101::LEU2 his4-917δ lys2 leu2 ura3 trp1Δ63 ade2 ade3*
KA49	*MAT***a** *rtf1Δ101::LEU2 leu2 ura3 ade2 ade3*
KA50	*MAT*α *rtf1Δ101::LEU2 leu2 ura3 trp1Δ63 ade2 ade3*
KA51	*MAT***a** *rtf1Δ101::LEU2 leu2 ura3 trp1Δ63 ade2 ade3*
KA52	*MAT*α *rtf1Δ101::LEU2 his4-917δ leu2 ura3 trp1Δ63 ade2 ade3*
KA53	*MAT***a** *rtf1Δ101::LEU2 his4-917δ leu2 ura3 trp1Δ63 ade2 ade3*
KA54	*MAT***a** *rtf1Δ101::LEU2 srb5-77 leu2 ura3 trp1Δ63 ade2 ade3* [pPC1]
KA55	*MAT*α *rtf1Δ101::LEU2 ctk1-217 leu2 ura3 trp1Δ63 ade2 ade3* [pPC1]
KA56	*MAT***a** *rtf1Δ101::LEU2 fcp1-110 his4-917δ leu2 ura3 trp1Δ63 ade2 ade3* [pPC1]
KA57	*MAT*α *rtf1Δ101::LEU2 pob3-272 chd1-52 his4-917δ leu2 ura3 trp1Δ63 ade2 ade3* [pPC1]
KA58	*MAT*α *pob3-272 chd1-52 his4-912δ lys2-128δ leu2 ura3 trp1Δ63 ade2 suc2ΔUAS(-1900/-390)*
KA59	*MAT*α *pob3-272 chd1-52 his4-917δ lys2-128δ leu2 ura3 ade2 ade3 suc2ΔUAS(-1900/-390)*
KA60	*MAT*α *pob3-272 chd1-52 his4-912δ lys2-128δ leu2 ura3 suc2ΔUAS(-1900/-390)*
KA61	*MAT***a** *pob3-272 his4-912δ lys2-128δ ura3 trp1Δ63 suc2ΔUAS(-1900/-390)* [pPC21]
KA62	*MAT*α *pob3-272 his4-912δ lys2-128δ leu2 ura3 suc2ΔUAS(-1900/-390)*
KA63	*MAT*α *pob3-272 chd1Δ::URA3 his4-912δ lys2-128δ leu2 ura3 suc2ΔUAS(-1900/-390)*
KA65	*MAT***a** *fcp1-110 leu2Δ1 trp1Δ63 ade3*
KA66	*MAT***a** *srb5-77 his3Δ200 lys2-128δ leu2Δ1*
KA67	*MAT***a** *rtf1Δ101::LEU2 srb5-77 his4-912δ lys2-128δ leu2Δ1 ura3-52*
KA68	*MAT***a** *rtf1Δ101::LEU2 srb5Δ::URA3-hisG his4-912δ leu2 ura3-52 trp1Δ63*
KA72	*MAT***a** *kin28-ts4 his4-912δ lys2-128δ leu2Δ1 trp1Δ63 suc2ΔUAS(-1900/-390)*
KA76	*MAT***a** *kin28-ts3 his4-912δ lys2-128δ leu2Δ1*
KA79	*MAT*α *fcp1-110 leu2Δ1 ura3-52*
PSY137	*MAT***a** *lys2 leu2 ura3 ade2 ade3 can1*

FY, GHY, and GY strains were obtained from Fred Winston, Grant Hartzog, and Greg Prelich, respectively.

was generated by inserting an 8.8-kb *Sal*I-*Sac*I fragment from pPC21, one of two *POB3*-containing library isolates, into the corresponding sites of pRS314. pPC29 and pPC30 were then created by subcloning a 2.4-kb *Sca*I-*Eco*RI fragment from pPC23 into the *Sma*I and *Eco*RI sites of pRS314 and pRS304, respectively.

The cloning of the *pob3-272* and *fcp1-110* mutations from strains KA58 and KA65, respectively, was achieved by gap repair (Orr-Weaver *et al.* 1983). pPC23 was digested with *Bst*EII and *Bgl*II to delete a 4.0-kb fragment containing the *POB3* gene. pPC25 was digested with *Stu*I and *Sph*I to excise a 3.7-kb fragment containing *FCP1*. The resulting vector fragments were transformed into the appropriate yeast strains. Plasmid DNA was recovered from Trp^+ transformants, propagated in *Escherichia coli*, and retransformed into KA58 or KA65 to confirm by phenotypic analysis that the mutations had been cloned. The locations of the mutations were determined by subcloning and sequence analysis.

Synthetic lethal screen with *rtf1*Δ: To identify mutations that are synthetically lethal with *rtf1*Δ, we employed a red/white colony-sectoring assay (Kranz and Holm 1990). In brief, this assay is based on the observations that *ade2* mutant colonies are red and that *ade2 ade3* double mutant colonies are white, because *ade3* mutations are epistatic to *ade2* mutations (Kranz and Holm 1990). An *ade2 ade3* strain carrying the wild-type *ADE3* gene on a plasmid will form solid red colonies only when the plasmid is stably maintained. When grown under nonselective conditions, rapidly dividing cells can lose the *ADE3*-containing plasmid and generate colonies with red and white sectors. Therefore, this colony-sectoring assay can be used to detect mutants that require the *ADE3*-containing plasmid for life and thus appear solid red.

To find mutations that are lethal in combination with *rtf1*Δ, strain KA48 was transformed with plasmid pPC1. The transformed strain was plated on YPD and mutagenized by exposure to 7500 μJ/cm^2 of UV light to ~60% survival. Approximately 45,000 colonies were screened for those that appeared red and nonsectored ($Sect^-$ phenotype). After purification, 235 colonies maintained the $Sect^-$ phenotype. The $Sect^-$ strains were then subjected to a second screen on plates containing 5-FOA, a drug that kills cells with a functional *URA3* gene (Rose *et al.* 1990). Thirty-four $Sect^-$ strains were 5-FOA^S, and these strains were subjected to two additional tests to confirm that the synthetic lethality was specific to *RTF1* and only one other gene. First, the 5-FOA^S $Sect^-$ strains were transformed individually with the centromeric plasmids pPC3 and pLS20 (Stolinski *et al.* 1997). Plasmid pPC3 bears the wild-type *RTF1*, *ADE3*, and *TRP1* genes, while pLS20 harbors only *RTF1* and *TRP1*. If the synthetic lethality is not specific to *URA3* or *ADE3* expression, both pPC3 and pLS20 should confer 5-FOA^R, but only pLS20 should allow the mutant strains to regain a sectored phenotype ($Sect^+$). Second, 5-FOA^S $Sect^-$ strains that passed the above criteria were backcrossed to KA49, to test for dominance/recessivity and for 2:2 segregation of the 5-FOA^S and $Sect^-$ phenotypes. Fourteen mutants exhibited 2:2 segregation of these phenotypes, indicating that the synthetic lethal mutation in these mutants was due to a single gene. To determine if the synthetic lethal mutations conferred Spt^- and/or Bur^- phenotypes, the mutant strains were crossed to either KY616 or KY617.

Identification of synthetic lethal genes: The genes responsible for the synthetic lethality of complementation groups A (*SRB5*), B (*CTK1*), and C (*FCP1*) were cloned from a pRS200 (*TRP1 CEN*)-based *S. cerevisiae* genomic library (American Type Culture Collection, Rockville, MD; Sikorski and Hieter 1989) by complementing the 5-FOA^S and $Sect^-$ phenotypes of strains KA54, KA55, and KA56. Plasmid DNA was purified from 5-FOA^R and $Sect^+$ transformants that had lost plasmid pPC1 and was retransformed into the original $Sect^-$ strain to confirm that the complementing activity was due to the library plasmid. Clones possessing complementing activity were subjected to DNA sequence analysis. In some instances, *RTF1* clones were obtained, as established by restriction endonuclease analysis and/or DNA sequencing. The gene corresponding to complementation group D (*POB3*) was cloned from a YCp50-based *S. cerevisiae* genomic library (Rose *et al.* 1987) by complementing the Spt^- phenotype of strain KA59. Two complementing library plasmids that carried overlapping inserts were obtained. To confirm that a shared ORF, *POB3*, also complemented the 5-FOA^S and $Sect^-$ phenotypes, a *POB3*-containing fragment was inserted into plasmid pRS314 and transformed into strain KA57.

To determine if the cloned genes were allelic to the original synthetic lethal mutations, *TRP1*-marked integrating plasmids containing the cloned genes were transformed into yeast and linkage between *TRP1* and the synthetic lethal mutations was examined. This analysis was performed using the following manipulations: (1) pPC14 was linearized by digestion with *Bst*BI, transformed into KA50, and the resulting integrant was crossed to KA54; (2) pPC19 was linearized with *Nde*I, transformed into KA51, and the resulting integrant was crossed to KA55; (3) pPC27 was linearized with *Msc*I, transformed into KA52, and the resulting integrant was crossed to KA56; and (4) pPC30 was linearized with *Bsm*I, transformed into KA53, and the resulting integrant was crossed to KA57. Following tetrad analyses, all Trp^- segregants exhibited 5-FOA^S and $Sect^-$ phenotypes, demonstrating that the integration constructs were targeted to the genetically identified loci. To further demonstrate that we had cloned the correct gene responsible for complementing the Spt^- and Bur^- phenotypes of complementation group D, plasmid pPC30 was linearized by digestion with *Bsm*I, transformed into KY571, and the resulting integrant was crossed to KA58. Following tetrad analysis, all Trp^- segregants were Spt^- and Bur^-, demonstrating that we had cloned the gene responsible for these phenotypes.

Identification of the *chd1-52* suppressor mutation: A *pob3-272 ura3* strain preferentially maintains a *CEN URA3* plasmid harboring *POB3* and exhibits weak 5-FOA sensitivity. This characteristic was used to clone the gene responsible for suppressing the extreme growth defect caused by the *pob3-272* mutation. To test for dominance/recessivity and for 2:2 segregation of the growth suppression phenotype, strain KA61 was crossed to KA60. The resulting diploid exhibited weak 5-FOA sensitivity, indicating the suppressor mutation was recessive. Following tetrad analysis, the weak 5-FOA sensitivity segregated 2:2, demonstrating that this phenotype was due to a mutation in a single gene.

The gene responsible for suppressing the *pob3-272* growth defect was determined as follows. Strain KA58 was transformed with plasmid pPC21. A Ura^+ transformant was subsequently transformed with a pRS200 (*TRP1 CEN*)-based yeast genomic library. Double transformants that contained a library plasmid that complemented the suppressor mutation would strongly maintain pPC21, because plasmid loss would uncover the *pob3-272* allele in an otherwise wild-type background. Ura^+ Trp^+ transformants that exhibited weak 5-FOA sensitivity (*i.e.*, poor growth on 5-FOA media lacking tryptophan after 2 days at 30°) were identified by replica plating. The 5-FOA^S transformants were mated to the wild-type strain FY23. Library plasmid DNA was obtained from selected diploids after causing the loss of plasmid pPC21 on 5-FOA media lacking tryptophan. Two different library plasmids, one of which contained *CHD1*, elicited a weak 5-FOA^S phenotype upon retransformation into the initial strain used for cloning. To demonstrate that the suppressor mutation was linked to *CHD1*, GHY713 was crossed to KA60. All *pob3-272* segregants from 19 complete

TABLE 2

Mutations identified in the synthetic lethal screen with *rtf1*Δ

Mutation	Phenotypes[a]
srb5-77	Ino$^-$
	Weak Gal$^-$
	Sensitive to 3% formamide
	Weakly sensitive to 6-AU
ctk1-217	Cs$^-$
	Sensitive to 0.3 M LiCl
	Sensitive to 1.2 M NaCl
fcp1-110	Weak Ino$^-$
	Sensitive to 1.4 M NaCl
	Weakly sensitive to 100 mM hydroxyurea
	Weakly sensitive to 6-AU
pob3-272	Spt$^-$
	Bur$^-$

[a] Ino$^-$, inositol auxotrophy; Gal$^-$, inability to use galactose as the sole carbon source; Cs$^-$, cold sensitivity for growth at 15°; Spt$^-$, suppression of Ty solo δ insertion mutations; and Bur$^-$, ability to bypass the UAS requirement of *SUC2*. The 6-AU sensitivity of *srb5-77* and *fcp1-110* strains is most evident at high concentrations of 6-AU (*e.g.*, 200 μg/ml). The 6-AU sensitivity of *ctk1-217* and *pob3-272* strains was not determined. With the exception of the Spt and Bur phenotypes, a description of all phenotypes tested has been provided by Hampsey (1997).

four-spore tetrads exhibited wild-type growth, indicating that we had cloned the correct gene.

RESULTS

The *rtf1*Δ mutation is synthetically lethal with the loss of global transcription regulators: A synthetic lethal screen was performed with an *rtf1*Δ mutation to identify potential interactions with Rtf1 *in vivo*. Mutations that are lethal in combination with an *rtf1*Δ allele might reveal factors that regulate the same process as Rtf1 or factors that physically interact with Rtf1. By using a plasmid-sectoring assay (Kranz and Holm 1990), we screened for mutations that cause synthetic lethality with *rtf1*Δ. Ultimately, 14 synthetic lethal mutations were identified (see materials and methods for details). The mutations are all recessive and comprise nine complementation groups (Table 2; data not shown). This article describes the genes corresponding to four of these groups.

The genes responsible for the synthetic lethality were cloned by complementation, and their identities were verified by subcloning and linkage analysis. Gene and mutant allele names are listed in Table 2. Three of the genes, defined by mutations in *SRB5*, *CTK1*, and *FCP1*, have been directly implicated in the function and modification of RNA pol II. Srb5 is an important component of the SRB/mediator complex that associates with the CTD of RNA pol II, mediates the response to transcriptional activators, and stimulates phosphorylation of the CTD by TFIIH (Hampsey 1998). Ctk1 is the catalytic subunit of the CTDK-I kinase (Lee and Greenleaf 1991). This kinase has been shown to specifically phosphorylate the CTD and promote efficient elongation by RNA pol II *in vitro* (Lee and Greenleaf 1989, 1997; Sterner *et al.* 1995). Fcp1 is a recently described TFIIF-associated, CTD-specific protein phosphatase (Archambault *et al.* 1997; Cho *et al.* 1999; Kobor *et al.* 1999). Fcp1 also possesses a positive elongation function independent of its phosphatase activity (Cho *et al.* 1999). Our screen also uncovered a mutation in the *POB3* gene. Pob3 shares similarity with HMG1-like proteins and forms a complex in yeast with Cdc68/Spt16 (Brewster *et al.* 1998; Wittmeyer *et al.* 1999), a protein that has been implicated in the regulation of transcription by chromatin structure (Malone *et al.* 1991; Rowley *et al.* 1991; Brewster *et al.* 1998). The human homologues of Pob3 and Cdc68/Spt16 form a complex known as FACT (*fa*cilitates *c*hromatin *t*ranscription), which facilitates transcription elongation on chromatin templates *in vitro* (LeRoy *et al.* 1998; Orphanides *et al.* 1999). Together with additional data presented below, the identification of mutations in *SRB5*, *CTK1*, *FCP1*, and *POB3* in our synthetic lethal screen suggests that Rtf1 regulates transcription elongation *in vivo*, perhaps at the initiation to elongation transition.

To confirm the synthetic lethal relationships by an approach distinct from the plasmid loss assay, we performed genetic crosses between an *rtf1*Δ strain and strains that carry the synthetic lethal mutations in an *RTF1*$^+$ genomic background. Following tetrad analysis of the heterozygous diploid strains generated from these crosses, we observed no *rtf1*Δ *ctk1-217* double mutant spores. The *srb5-77* and *fcp1-110* mutations in combination with *rtf1*Δ gave rise to microcolonies that were visible only after 3–4 days of growth at 30° (Figure 1A; data not shown). By this method, the synthetic growth defect involving the *pob3-272* mutation was the least severe. Double mutant spores containing *rtf1*Δ and *pob3-272* gave rise to small, visible colonies after 3–4 days of incubation at 30°. However, as described in a subsequent section, genetic analysis of the *pob3-272* isolate was more complex, since we found that an additional mutation was present that affected the growth of our original strain.

Genetic analysis of mutations obtained in the synthetic lethal screen: To assist in our studies, strains harboring the synthetic lethal mutations were tested for several mutant phenotypes. As summarized in Table 2, the mutations cause a variety of phenotypes, many of which have been associated with defects in transcription. Spt$^-$ and Bur$^-$ phenotypes, inositol auxotrophy (Ino$^-$), and defects in galactose metabolism (Gal$^-$) are often correlated with mutations that affect the general transcription apparatus and/or chromatin factors (Winston 1992; Prelich and Winston 1993; Hampsey

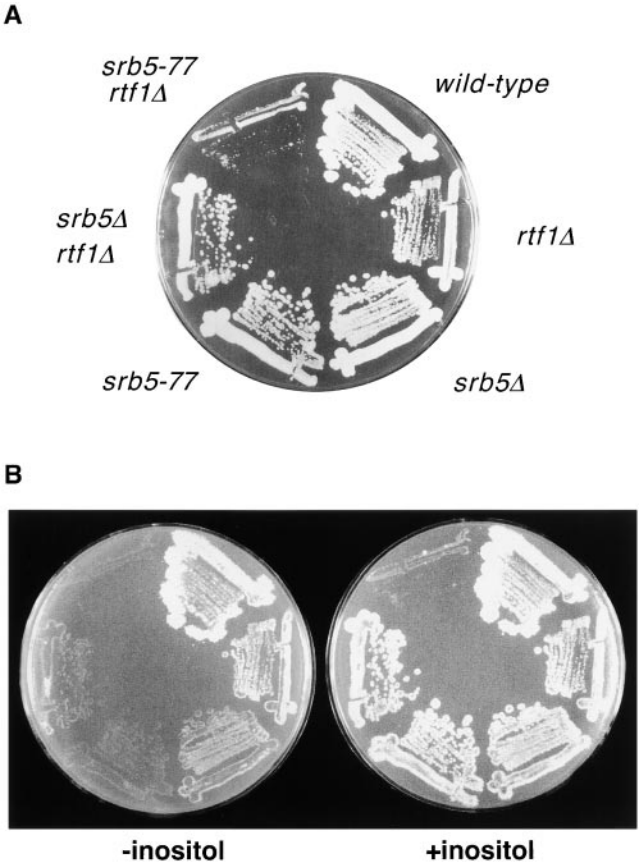

FIGURE 1.—The *srb5-77* allele is distinct from the *srb5*Δ allele. (A) *srb5-77 rtf1*Δ strains, but not *srb5*Δ *rtf1*Δ strains, have a microcolony phenotype. Yeast strains FY23, KY404, L937, KA66, KA68, and KA67 were streaked to YPD media and grown for 4 days at 30° before photography. The sickness of the *srb5*Δ *rtf1*Δ strain is not clearly evident after 3 days of growth. (B) *srb5-77* strains exhibit strong inositol auxotrophy. The yeast strains shown in A were transferred by replica plating to synthetic media lacking or containing inositol and grown for 2 days at 30°. As shown in the photograph, *srb5-77 rtf1*Δ strains do not grow on −inositol media and grow poorly on +inositol media. Strain orientations in B are the same as in A.

1997). The Bur$^-$ [*B*ypass *u*pstream activation sequence (UAS) *r*equirement] phenotype, a characteristic of strains mutant for histones or other transcriptional repressors, reflects the ability to bypass the requirement for a UAS within the *SUC2* promoter (PRELICH and WINSTON 1993). Salt and formamide sensitivity are also caused by mutations that affect transcription, including those that alter transcription elongation and chromatin structure (OTERO *et al.* 1999; TSUKIYAMA *et al.* 1999). Cold sensitivity (Cs$^-$) is frequently associated with defects in protein complex assembly (HAMPSEY 1997). The Ino$^-$ and Gal$^-$ phenotypes caused by the *srb5-77* mutation and the Cs$^-$ phenotype caused by the *ctk1-217* mutation are in agreement with phenotypes conferred by other mutations in these genes (LEE and GREENLEAF 1991; P. J. COSTA and K. M. ARNDT, unpublished observations).

TABLE 3

Genetic interactions between *rtf1*Δ and mutations in genes encoding RNA pol II holoenzyme components

Genotype[a]	Synthetic phenotypes[b]
*gal11*Δ *rtf1*Δ	Slightly sick, Spt$^+$
*rgr1*Δ*2 rtf1*Δ	None[c]
*sin4*Δ *rtf1*Δ	None[c]
*srb2*Δ *rtf1*Δ	Strong Ino$^-$[c]
*srb5*Δ *rtf1*Δ	Sick, strong Ino$^-$, Spt$^+$
*srb10*Δ *rtf1*Δ	None
*kin28-ts3 rtf1*Δ	Strong Ino$^-$, Spt$^{-/+}$
*kin28-ts4 rtf1*Δ	Strong Ino$^-$, Spt$^{-/+}$

[a] The parents for the crosses in the order listed were as follows: FY1257 × KY424, FY1289 × KY409, FY1256 × KY473, FY1285 × KY607, L937 × KY404, GY759 × KY608, KA76 × KY405, and KA72 × KY405.

[b] Spt$^+$ and Spt$^{-/+}$ indicate complete and partial suppression of the Spt$^-$ phenotype of *rtf1*Δ, respectively. Strains were tested for growth at 15°, 30°, and 37° on YPD media, growth on media lacking inositol, and growth on galactose and sucrose media.

[c] Suppression of the Spt$^-$ phenotype conferred by *rtf1*Δ was not tested.

To determine if the synthetic lethality or extreme synthetic sickness between *rtf1*Δ and our *srb5* and *ctk1* mutations was allele specific, we examined the phenotypes of double mutant strains containing an *rtf1*Δ and either an *srb5*Δ or a *ctk1*Δ mutation. We found that the *ctk1*Δ *rtf1*Δ double mutant strains are inviable (data not shown), suggesting that our *ctk1* allele, *ctk1-217*, is probably a null allele. In support of this view, *ctk1-217* and *ctk1*Δ mutations confer the same mutant phenotypes (LEE and GREENLEAF 1991; P. J. COSTA and K. M. ARNDT, unpublished observations). In contrast, the *srb5*Δ *rtf1*Δ double mutant strains are viable, but exhibit several synthetic phenotypes (Figure 1 and Table 3). The double mutant strains grow more slowly than either single mutant and exhibit an exacerbated Ino$^-$ phenotype compared to *srb5*Δ strains. In addition, the *srb5*Δ mutation completely suppresses the Spt$^-$ phenotype conferred by the *rtf1*Δ mutation. *srb5*Δ *rtf1*Δ double mutant strains are significantly healthier than *srb5-77 rtf1*Δ strains, which exhibit a microcolony phenotype (Figure 1A). This result suggests that the *srb5-77* allele, although recessive for its interaction with *rtf1*Δ, is distinct from an *srb5*Δ allele. In accordance with this conclusion, the *srb5-77* mutation, unlike the *srb5*Δ mutation, confers weak sensitivity to the compound 6-azauracil (6-AU; Table 2). As described in more detail below, sensitivity to 6-AU often indicates a defect in transcription elongation (EXINGER and LACROUTE 1992; UPTAIN *et al.* 1997).

Fcp1 and Pob3 are encoded by essential genes in yeast (ARCHAMBAULT *et al.* 1997; WITTMEYER and FORMOSA 1997), suggesting that we have identified partial loss-of-function alleles of these genes. The human and yeast homologues of Fcp1 contain an essential phosphatase

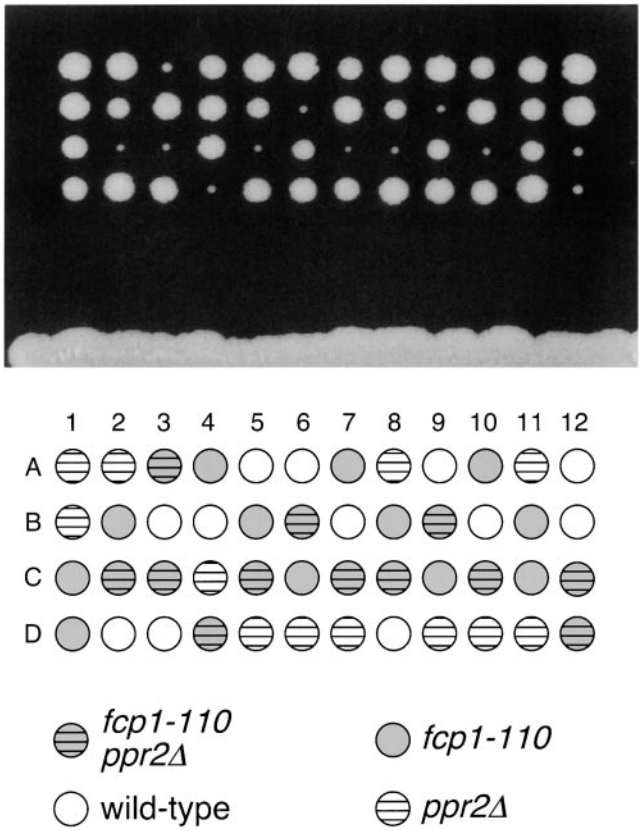

FIGURE 2.—The *fcp1-110* allele genetically interacts with a deletion of *PPR2*. Yeast strains KA79 and KY624 were mated, sporulated, and asci were dissected by tetrad analysis. A photograph of the dissection plate was taken after 4 days of growth at 30°.

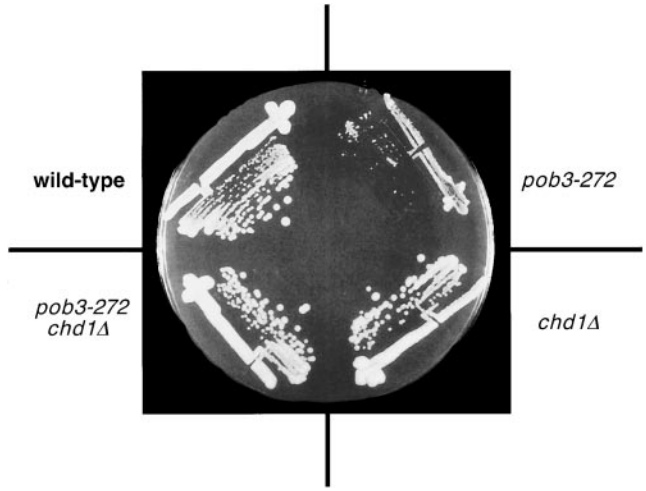

FIGURE 3.—The growth defect conferred by the *pob3-272* mutation is suppressed by a mutation in *CHD1*. Yeast strains KA62, GHY713, KA63, and KY573 were streaked on YPD media and grown for 3 days at 30°.

motif, two binding sites for the RAP74 subunit of the general transcription factor TFIIF, and a *BR*CA1 *c*arboxyl-*t*erminal (BRCT) domain (ARCHAMBAULT *et al.* 1997; CHO *et al.* 1999; KOBOR *et al.* 1999). To identify the domain in Fcp1 that is altered by the *fcp1-110* mutation, we cloned the mutant gene and determined its DNA sequence. The *fcp1-110* mutation changes codon 615 in the open reading frame from a glutamine codon to a stop codon. The phosphatase and BRCT domains are amino-terminal to the Fcp1-110 stop codon. Previous studies showed that the two RAP74 binding sites in Fcp1 map to amino acids 457–666 and 667–732 (ARCHAMBAULT *et al.* 1997). Therefore, the *fcp1-110* mutation is predicted to eliminate one RAP74 interaction domain and truncate the remaining domain. Together, our findings suggest that the Fcp1-TFIIF interaction may be important for the elongation function of Fcp1 *in vivo*. To determine whether the *fcp1-110* mutation compromises transcription elongation *in vivo*, we examined the phenotype of double mutant strains that contain the *fcp1-110* mutation and a deletion of the nonessential gene *PPR2*. *PPR2* encodes the well-characterized elongation factor TFIIS (EXINGER and LACROUTE 1992). Interestingly, *fcp1-110 ppr2*Δ double mutant strains exhibit a strong growth defect and enhanced inositol auxotrophy compared to strains harboring the *fcp1-110* mutation alone (Figure 2; data not shown). In addition, the *fcp1-110* mutation causes strains to be weakly sensitive to 6-AU (Table 2).

Pob3 is similar to HMG1-like proteins found in a wide variety of organisms, including *Arabidopsis thaliana*, *Schizosaccharomyces pombe*, *Drosophila melanogaster*, *Caenorhabditis elegans*, mouse, and humans (WITTMEYER and FORMOSA 1997). However, unlike several other family members, Pob3 does not possess an HMG box, a DNA-binding motif found in the abundant chromatin-associated protein, HMG1 (WITTMEYER and FORMOSA 1997). We cloned and sequenced the *pob3-272* mutation and found that it encodes a substitution of lysine for isoleucine at position 282. The analogous amino acid in the HMG1-like proteins of eleven other species is either an isoleucine or valine. The alteration of a highly conserved small, hydrophobic residue to an extended, charged amino acid is likely to cause a distortion in the Pob3-272 protein, possibly affecting its interaction with another protein.

A mutation in *CHD1* suppresses the growth defect conferred by the *pob3-272* mutation: During our genetic analysis, we found that the *pob3-272* mutation causes extreme sickness in an otherwise wild-type background (Figure 3). The original *pob3-272* mutant strain isolated in our synthetic lethal screen harbored one additional mutation that suppressed this growth defect. Double mutant strains containing the *pob3-272* allele and the suppressor mutation exhibit nearly wild-type growth. We took advantage of these observations to clone the *pob3-272* suppressor (see MATERIALS AND METHODS) and determined, through linkage analysis, that the suppressor mutation was in the gene *CHD1*. Following its identification, we designated the suppressor mutation as *chd1-*

52. We also found that a *chd1*Δ allele behaves similarly in suppressing the growth defect caused by the *pob3-272* mutation (Figure 3; data not shown). *CHD1* encodes a well-conserved protein with a domain structure that suggests a role in chromatin function (Woodage *et al.* 1997). Interestingly, the human homologue of yeast Chd1 has been shown to interact physically with SSRP1, the human homologue of yeast Pob3 (Kelley *et al.* 1999). Since we identified a *CHD1* allele as an outcome of our synthetic lethal screen, we also examined if *rtf1*Δ *chd1* double mutant strains exhibit any genetic interaction. We observed no synthetic phenotypes for these strains (data not shown). However, as mentioned above, *rtf1*Δ *pob3-272 chd1* triple mutant strains give rise to small, visible colonies only after 3–4 days of growth. Since *pob3-272 chd1* double mutant strains exhibit nearly wild-type growth properties, the triple mutant combinations indicate a genetic interaction involving all three genes.

***RTF1* exhibits genetic interactions with a small subset of genes encoding RNA pol II holoenzyme components:** Because we identified an allele of *SRB5* in our synthetic lethal screen, we asked whether *RTF1* displays genetic interactions with mutations that affect other members of the RNA pol II holoenzyme. In addition to *srb5*Δ, we tested null mutations in the nonessential genes *GAL11*, *SIN4*, *SRB2*, and *SRB10*. We also tested a partial loss-of-function allele of the essential gene *RGR1* (Sakai *et al.* 1990) and two temperature-sensitive alleles of *KIN28* (Valay *et al.* 1993). In contrast to our results with *srb5-77*, we did not observe synthetic lethality or severe synthetic sickness between the *rtf1*Δ mutation and mutations in these six other holoenzyme genes (Table 3). However, *gal11*Δ *rtf1*Δ double mutants do exhibit a slight growth defect, and the *gal11*Δ mutation completely suppresses the Spt$^-$ phenotype associated with *rtf1*Δ. In addition, *srb2*Δ *rtf1*Δ double mutant strains exhibit an exacerbated Ino$^-$ phenotype.

Like Ctk1, the holoenzyme-associated Kin28 and Srb10 proteins are cyclin-dependent kinases that phosphorylate the CTD of RNA pol II (Dahmus 1996). While Kin28 plays a positive role in transcription by facilitating the transition from initiation to elongation (Dahmus 1996; Hampsey 1998), Srb10 inhibits initiation by phosphorylating the CTD prior to PIC assembly (Hengartner *et al.* 1998). In striking contrast to the inviability of *rtf1*Δ *ctk1*Δ double mutant strains, *rtf1*Δ *srb10*Δ double mutant strains exhibit no synthetic phenotypes. For both *kin28* alleles, the *rtf1*Δ *kin28* double mutant strains showed synthetic Ino$^-$ phenotypes but no significant defect in growth rate compared to the *rtf1*Δ and *kin28* parents (Table 3). These findings further support the conclusion that the known CTD kinases have distinct roles in transcription and argue that the strong genetic interaction between *rtf1*Δ and *srb5-77* is not a general property of mutations that affect holoenzyme components.

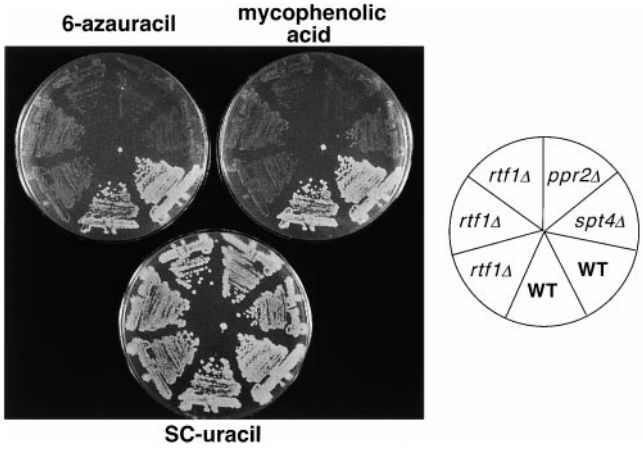

Figure 4.—*rtf1*Δ strains are sensitive to 6-azauracil and mycophenolic acid. Yeast strains GHY285, FY243, FY69, FY91, KY425, KY426, and KY459 were grown on YPD media and transferred by replica plating to SC-uracil media lacking or containing 50 μg/ml 6-azauracil or 20 μg/ml mycophenolic acid. Photographs were taken after 2 days of growth at 30°.

The *rtf1*Δ mutation confers sensitivity to 6-azauracil and mycophenolic acid: The results from our synthetic lethal screen indicate a role for Rtf1 in transcription elongation. To test this hypothesis further, we examined the sensitivity of *rtf1*Δ strains to 6-AU and mycophenolic acid (MPA). 6-AU and MPA decrease nucleotide levels *in vivo* and are thought to increase pausing and arrest by RNA pol II, thereby augmenting the need for factors that stimulate elongation (Exinger and Lacroute 1992; Uptain *et al.* 1997). Therefore, sensitivity to these compounds is often associated with mutations that inactivate transcription elongation factors (Exinger and Lacroute 1992; Uptain *et al.* 1997; Hartzog *et al.* 1998) or lower the elongation rate of RNA pol II (Powell and Reines 1996). Relative to isogenic wild-type strains, *rtf1*Δ strains are strongly sensitive to both 6-AU and MPA (Figure 4). The degree of sensitivity is comparable to that conferred by mutations in *SPT4* and *PPR2*, which encode the elongation factors Spt4 and TFIIS, respectively (Exinger and Lacroute 1992; Hartzog *et al.* 1998).

***RTF1* genetically interacts with known elongation factor genes:** To further test the idea that Rtf1 functions during elongation, we investigated genetic interactions between *RTF1* and several genes encoding transcription elongation factors. We observed several synthetic interactions with mutations in genes encoding Spt4, Spt5, Spt6, TFIIS, and Spt16. First, *rtf1*Δ *spt4*Δ double mutants are very sick, show strong temperature sensitivity (Ts$^-$) for growth, and are weakly Gly$^-$ (inability to use glycerol as the sole carbon source; Figure 5; Table 4). In addition, these double mutant strains are Spt$^+$, indicating a rare case of mutual suppression of Spt$^-$ phenotypes. Likewise, mutations in the essential genes *SPT5* and *SPT6* (Swanson and Winston 1992), in combination

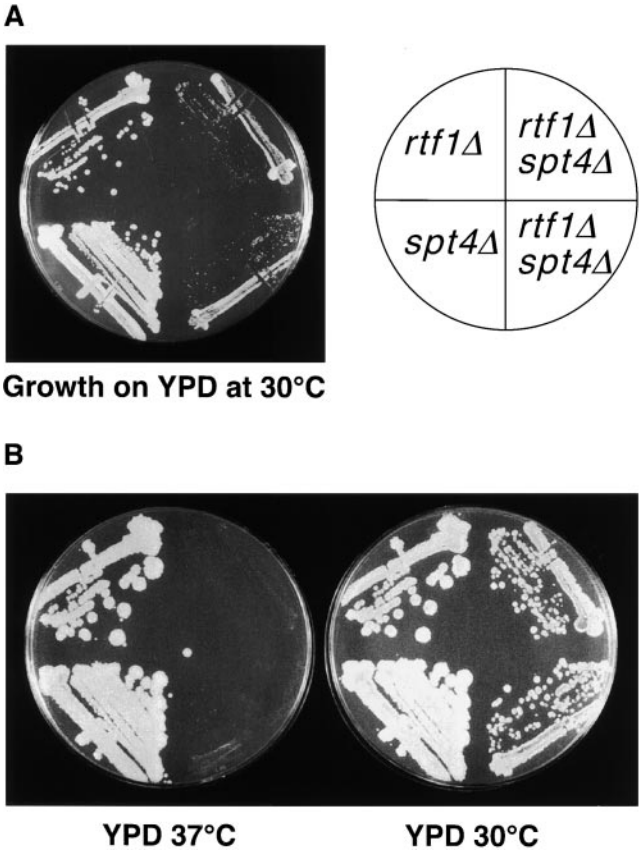

FIGURE 5.—*rtf1*Δ *spt4*Δ double mutant strains are extremely sick and temperature sensitive for growth. (A) Yeast strains KY610, KY611, FY243, and KY405 were streaked on YPD media and grown for 3 days at 30° before photography. (B) The plate shown in A was allowed to grow an additional day at 30° and then replica plated to two YPD plates. These plates were incubated for 3 days at 37° or 30° before photography. The double mutant strains were constructed by crossing the indicated *spt4*Δ and *rtf1*Δ parents and performing tetrad analysis.

with the *rtf1*Δ allele, cause a slight growth defect and a strong Ts$^-$ phenotype (STOLINSKI *et al.* 1997; Table 4).

We also tested for a potential genetic interaction between *rtf1*Δ and a deletion of *PPR2*. For the *rtf1*Δ *ppr2*Δ double mutant, the only synthetic phenotype we observed was the ability of *ppr2*Δ to suppress the Spt$^-$ phenotype associated with *rtf1*Δ. The absence of additional *rtf1*Δ *ppr2*Δ phenotypes may be due to functional redundancy with other elongation factors. Therefore, we examined if elimination of these other factors created a more critical situation for the cell. Indeed, we observed synthetic lethality for the *rtf1*Δ *spt4*Δ *ppr2*Δ triple mutant. Correspondingly, we found that *rtf1*Δ *spt5-194 ppr2*Δ mutants exhibit an exacerbated sickness compared to *rtf1*Δ *spt5-194* strains (Table 4). HARTZOG *et al.* (1998) have previously shown that *spt4*Δ *ppr2*Δ and *spt5-194 ppr2*Δ strains are viable, but are moderately Ts$^-$ at 37°. Important for our results is our observation that *spt4*Δ *ppr2*Δ and *spt5-194 ppr2*Δ strains exhibit little or no growth defect at 30°. Last, we constructed the *rtf1*Δ *spt4*Δ *spt16-197* triple mutant and found it to possess an extreme growth defect, growing much more slowly than the *rtf1*Δ *spt4*Δ double mutant (Table 4). In contrast, *spt4*Δ *spt16-197* and *rtf1*Δ *spt16-197* double mutants showed no synthetic phenotypes in our analysis (Table 4; data not shown). We also tested several of the double mutant combinations for 6-azauracil sensitivity. Strains carrying the *rtf1*Δ allele in combination with either *spt4*Δ, *spt6-14*, or *ppr2*Δ still exhibited sensitivity to 6-AU at the concentration tested (50 μg/ml). Finally, we examined the phenotype of an *rtf1*Δ *rpb2-10* double mutant strain. The *rpb2-10* mutation alters an amino acid in the second largest subunit of RNA pol II and encodes an enzyme with a decreased elongation rate *in vitro* (POWELL and REINES 1996). *rtf1*Δ *rpb2-10* double mutants exhibit a slight growth defect compared to either single mutant (Table 4), suggesting that the elongation rate of the Rpb2-10 enzyme may be further reduced in the absence of Rtf1. Collectively, our findings indicate that the requirement for Rtf1 is significantly increased by mutations that impair transcription elongation in yeast.

DISCUSSION

In this study, we provide evidence that Rtf1 has a role in transcription elongation *in vivo*. Through a genetic screen, we have shown that the function of Rtf1 is critical when the activities of four global regulators of RNA pol II transcription, Srb5, Ctk1, Fcp1, and Pob3, are eliminated or altered by mutation. Each of these proteins has been implicated in CTD phosphorylation and/or transcription elongation. Our genetic studies further indicate a functional redundancy between *RTF1* and genes encoding several elongation factors. In addition, we have found that *rtf1*Δ mutations cause sensitivity to 6-AU and MPA, phenotypes often associated with defects in transcription elongation (UPTAIN *et al.* 1997).

Our results suggest several possible mechanisms for how Rtf1 may govern transcription elongation. In one model, Rtf1 may modulate the phosphorylation state of the CTD, perhaps in a gene-specific manner. In support of this idea, we uncovered mutations in *CTK1* and *SRB5* in our synthetic lethal screen. *CTK1* encodes the cyclin-dependent kinase subunit of CTDK-I (LEE and GREENLEAF 1991), a complex that specifically phosphorylates the CTD (LEE and GREENLEAF 1989; STERNER *et al.* 1995) and promotes efficient elongation by RNA pol II *in vitro* (LEE and GREENLEAF 1997). *SRB5* encodes a component of the Srb/mediator complex that stimulates phosphorylation of the CTD *in vitro* (HAMPSEY 1998). Importantly, Srb5-deficient holoenzyme is significantly impaired in its ability to support CTD phosphorylation (LEE *et al.* 1999). If Rtf1 regulates CTD phosphorylation, a mutation in *RTF1* together with a mutation in a gene encoding either a CTD kinase or a regulator of a CTD kinase could alter the extent or pattern of CTD phos-

TABLE 4

Genetic interactions between *rtf1*Δ and mutations in genes involved in transcription elongation

Genotype[a]	Synthetic phenotypes[b]	Relative growth rates[c]	6-AU sensitive[d]
*rtf1*Δ	NA	+++++	Yes
*rtf1*Δ *spt4*Δ	Very sick, Ts^-, weak Gly^-, Spt^+	++	Yes
*rtf1*Δ *spt5-194*	Slightly sick, Ts^-, weak Gly^-	++++	ND
*rtf1*Δ *spt6-14*	Slightly sick, Ts^-	++++	Yes
*rtf1*Δ *spt16-197*	None	+++++	ND
*rtf1*Δ *ppr2*Δ	Spt^+	+++++	Yes
*rtf1*Δ *spt4*Δ *ppr2*Δ	Dead	−	NA
*rtf1*Δ *spt5-194 ppr2*Δ	Very sick	++	ND
*rtf1*Δ *spt4*Δ *spt16-197*	Very, very sick	+	ND
*rtf1*Δ *rpb2-10*	Slightly sick	++++	ND

NA, not applicable; ND, not determined.

[a] The parents of the double and triple mutant strains in the order listed were as follows: FY243 × KY405, FY300 × FY405, GHY364 × KY405, FY348 × KY405, GHY285 × KY404, FY1671 × KY612, GHY285 × KY613, FY243 × KY614, and GHY492 × KY607.

[b] Ts^-, temperature sensitivity for growth at 37°. Gly^-, inability to use glycerol as the sole carbon source. The synthetic lethality of the *rtf1*Δ *spt4*Δ *ppr2*Δ mutant was determined by failure to recover any triple mutant spores following tetrad analysis.

[c] The relative growth rate reflects the size of individual colonies after 3 days of growth on YPD at 30° compared to an *rtf1*Δ strain. −, no growth.

[d] 6-AU sensitivity was determined as indicated in Figure 4.

phorylation in a way that prevents transcription of one or more essential genes.

Because the Srb/mediator complex plays a key role in transcriptional activation, an alternative explanation for the discovery of an *srb5* mutation in our screen is that Rtf1 and Srb5 function in parallel pathways to facilitate holoenzyme recruitment. However, we do not favor this hypothesis for two reasons. First, we did not observe synthetic lethality or severe synthetic sickness between the *rtf1*Δ mutation and mutations in genes encoding five other Srb/mediator components, some of which have been directly implicated in activator-stimulated RNA pol II recruitment (Barberis *et al.* 1995; Han *et al.* 1999; Lee *et al.* 1999). Second, previous work has shown that different subcomplexes of the Srb/mediator possess distinct functions and that Srb5 is required for a step in transcription that follows activator-mediated recruitment of the polymerase (Li *et al.* 1995; Lee *et al.* 1999).

Independent of any effect on CTD phosphorylation, Rtf1 may regulate transcription elongation in a more general fashion, such as by affecting chromatin structure or by altering the elongation properties of RNA pol II. Accordingly, we identified an *fcp1* mutation and a *pob3* mutation in our screen. In a recent study, Fcp1 has been shown to possess a positive elongation function independent of its CTD phosphatase activity (Cho *et al.* 1999). This raises the possibility that Fcp1 remains associated with RNA pol II during elongation. We have shown that the *fcp1-110* gene harbors a nonsense mutation that is predicted to remove one TFIIF interaction domain and truncate a second domain of this type. The mutation does not alter the phosphatase motif. Previous studies showed that the phosphatase activity of Fcp1 is stimulated by TFIIF *in vitro* (Chambers *et al.* 1995; Archambault *et al.* 1997). Therefore, our results do not distinguish between an effect of the *fcp1* mutation on CTD modification and a potentially more direct effect on the elongation properties of RNA pol II. Nevertheless, the isolation of an *fcp1* allele in our synthetic lethal screen, together with the synthetic interaction between *fcp1-110* and *ppr2*Δ, provides genetic support for a role of Fcp1 in transcription elongation and suggests that the interaction between Fcp1 and TFIIF is important for this function *in vivo*.

The human counterpart of the Pob3-Cdc68/Spt16 complex, FACT, has been shown to facilitate elongation specifically on nucleosomal templates *in vitro* (LeRoy *et al.* 1998; Orphanides *et al.* 1999). Since FACT interacts with histone H2A/H2B dimers, Orphanides *et al.* (1999) have proposed that FACT may function by promoting nucleosome disassembly upon transcription by RNA pol II. Importantly, the *pob3-272* mutation isolated in our screen confers Spt^- and Bur^- phenotypes, both of which correlate well with a role for Pob3 in chromatin function. Whereas both phenotypes have been previously attributed to mutations in *CDC68/SPT16* (Malone *et al.* 1991; Prelich and Winston 1993), our results extend these phenotypes to a mutation in *POB3*. In addition, they provide support for the involvement of the Pob3-Cdc68/Spt16 complex in transcription elongation *in vivo*. Interestingly, this complex has been shown to interact with DNA polymerase α (Wittmeyer and Formosa 1997; Wittmeyer *et al.* 1999), suggesting that

both DNA and RNA polymerases may employ this complex to move through chromatin.

The *pob3-272* mutation alters a highly conserved amino acid. In addition to the Spt$^-$ and Bur$^-$ phenotypes, this alteration results in a severe growth defect. We found that a mutation in the *CHD1* gene suppresses the growth defect, but not the Spt$^-$ and Bur$^-$ phenotypes (data not shown). Chd1 has a well-conserved tripartite structure, which includes chromo (*chr*omatin *o*rganization *m*odifier) domains, a Snf2-related helicase/ATPase domain, and a DNA-binding domain (Woodage *et al.* 1997). Chromo domains have been found in Polycomb and heterochromatin-binding protein 1, proteins that have important roles in chromatin compaction and transcriptional silencing (Paro 1993). Data from yeast suggest that Chd1 may be involved in the inhibition of transcription (Woodage *et al.* 1997). Our finding that a deletion of *CHD1* can suppress the growth defect conferred by a mutation in *POB3* also suggests that Chd1 has a negative role in transcription, possibly at the level of elongation. Kelley *et al.* (1999) have shown that the human homologues of Pob3 and Chd1 physically interact *in vivo* and *in vitro*. It will be of interest to determine if yeast Pob3 and Chd1 also physically associate, since such an interaction may have significance for both DNA replication and transcription.

In addition to the genes identified through the synthetic lethal screen, we uncovered a range of interactions between *RTF1* and genes that encode Spt4, Spt5, Spt6, Spt16, and TFIIS. In most cases, the combination of the *rtf1*Δ mutation with mutations in these genes results in a more severe phenotype. Particularly noteworthy is the inviability of *rtf1*Δ *spt4*Δ *ppr2*Δ triple mutant strains, a suggestion that the complete loss of three elongation factors cannot be tolerated by the cell. We have found that *RTF1* genetically interacts with genes encoding both components of the Spt4-Spt5 complex, both subunits of the Pob3-Cdc68/Spt16 complex, and TFIIS. We also detected an interaction between *RTF1* and *SPT6*. Spt6 functionally interacts with elongation factors (Hartzog *et al.* 1998), physically interacts with histones, and assembles nucleosomes *in vitro* (Bortvin and Winston 1996). The synthetic and conditional phenotypes of the multiply mutated strains most likely reflect a functional redundancy among the RNA pol II elongation factors in yeast.

We initially reported that *rtf1* mutations suppress the Spt$^-$ phenotype of the TBP-altered specificity mutant TBP-L205F by altering transcription initiation (Stolinski *et al.* 1997). Our current work indicates that *RTF1* has a role in elongation and genetically interacts with *SPT4*, *SPT5*, *SPT6*, *SPT16*, and *POB3*, all genes implicated in the control of transcription by chromatin structure. Together, these findings suggest that Rtf1 may suppress the Spt$^-$ phenotype of TBP-L205F by altering chromatin structure and controlling the accessibility of competing TATA boxes. Alternatively, Rtf1 may influence the productive elongation of transcripts that initiate from distinct start sites within a promoter. In support of these ideas, it should be noted that *SPT4*, *SPT5*, *SPT6*, and *SPT16* were all initially identified by their ability to cause an Spt$^-$ phenotype (Malone *et al.* 1991; Winston 1992). This phenotype has been described as an effect on transcription initiation (Winston 1992). However, recent data have implicated all four genes in elongation (Hartzog *et al.* 1998; Orphanides *et al.* 1999). Further work is needed to determine whether Rtf1 directly regulates both the initiation and elongation stages of the transcription cycle.

In summary, by a combination of genetic approaches, we have obtained evidence that Rtf1 regulates transcription elongation in yeast. Further genetic studies coupled with a biochemical characterization of Rtf1 and its interacting partners should provide additional insights into its mode of action. Since we have recently recognized proteins with similar sequence in humans and *C. elegans*, our studies on the *S. cerevisiae* Rtf1 protein will also be applicable to an understanding of transcriptional regulation in other eukaryotes.

We are very grateful to the following individuals for the gifts of strains and plasmids: Grant Hartzog, Greg Prelich, Pamela Silver, and Fred Winston. We thank Michael Kobor for the suggestion of testing *fcp1-110* strains for hydroxyurea sensitivity and Diana Cardona for analyzing the 6-AU sensitivity of *srb5-77* strains. We are grateful to Grant Hartzog, Greg Prelich, and members of the Arndt laboratory, especially Margaret Shirra, for many helpful discussions and critical reading of the manuscript. This work was supported by National Institutes of Health grant GM52593 to K.M.A.

LITERATURE CITED

Archambault, J., R. S. Chambers, M. S. Kobor, Y. Ho, M. Cartier *et al.*, 1997 An essential component of a C-terminal domain phosphatase that interacts with transcription factor IIF in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **94:** 14300–14305.

Arndt, K. M., C. R. Wobbe, S. Ricupero-Hovasse, K. Struhl and F. Winston, 1994 Equivalent mutations in the two repeats of yeast TATA-binding protein confer distinct TATA-recognition specificities. Mol. Cell. Biol. **14:** 3719–3728.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 1998 *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience, New York.

Barberis, A., J. Pearlberg, N. Simkovich, S. Farrell, P. Reinagel *et al.*, 1995 Contact with a component of the polymerase II holoenzyme suffices for gene activation. Cell **81:** 359–368.

Bortvin, A., and F. Winston, 1996 Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science **272:** 1473–1475.

Brewster, N. K., G. C. Johnston and R. A. Singer, 1998 Characterization of the CP complex, an abundant dimer of Cdc68 and Pob3 proteins that regulates yeast transcriptional activation and chromatin repression. J. Biol. Chem. **273:** 21972–21979.

Brown, S. A., A. N. Imbalzano and R. E. Kingston, 1996 Activator-dependent regulation of transcriptional pausing on nucleosomal templates. Genes Dev. **10:** 1479–1490.

Cadena, D. L., and M. E. Dahmus, 1987 Messenger RNA synthesis in mammalian cells is catalyzed by the phosphorylated form of RNA polymerase II. J. Biol. Chem. **262:** 12468–12474.

Chambers, R. S., B. Q. Wang, Z. F. Burton and M. E. Dahmus, 1995 The activity of COOH-terminal domain phosphatase is regulated by a docking site on RNA polymerase II and by the general transcription factors IIF and IIB. J. Biol. Chem. **23:** 14962–14969.

Chesnut, J. D., J. H. Stephens and M. E. Dahmus, 1992 The interaction of RNA polymerase II with the adenovirus-2 major late promoter is precluded by phosphorylation of the C-terminal domain of subunit IIa. J. Biol. Chem. **267:** 10500–10506.

Cho, H., T. K. Kim, H. Mancebo, W. S. Lane, O. Flores *et al.*, 1999 A protein phosphatase functions to recycle RNA polymerase II. Genes Dev. **13:** 1540–1552.

Cujec, T. P., H. Okamoto, K. Fujinaga, J. Meyer, H. Chamberlin *et al.*, 1997 The HIV transactivator TAT binds to the CDK-activating kinase and activates the phosphorylation of the carboxy-terminal domain of RNA polymerase II. Genes Dev. **11:** 2645–2657.

Dahmus, M. E., 1996 Reversible phosphorylation of the C-terminal domain of RNA polymerase II. J. Biol. Chem. **271:** 19009–19012.

Exinger, F., and F. Lacroute, 1992 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr. Genet. **22:** 9–11.

García-Martínez, L. F., G. Mavankal, J. M. Neveu, W. S. Lane, D. Ivanov *et al.*, 1997 Purification of a Tat-associated kinase reveals a TFIIH complex that modulates HIV-1 transcription. EMBO J. **16:** 2836–2850.

Hampsey, M., 1997 A review of phenotypes in *Saccharomyces cerevisiae*. Yeast **13:** 1099–1133.

Hampsey, M., 1998 Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol. Mol. Biol. Rev. **62:** 465–503.

Han, S. J., Y. C. Lee, B. S. Gim, G. H. Ryu, S. J. Park *et al.*, 1999 Activator-specific requirement of yeast mediator proteins for RNA polymerase II transcriptional activation. Mol. Cell. Biol. **19:** 979–988.

Hartzog, G. A., T. Wada, H. Handa and F. Winston, 1998 Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*. Genes Dev. **12:** 357–369.

Hengartner, C. J., V. E. Myer, S.-M. Liao, C. J. Wilson, S. S. Koh *et al.*, 1998 Temporal regulation of RNA polymerase II by Srb10 and Kin28 cyclin-dependent kinases. Mol. Cell **2:** 43–53.

Karlin, S., 1993 Unusual charge configurations in transcription factors of the basic RNA polymerase II initiation complex. Proc. Natl. Acad. Sci. USA **90:** 5593–5597.

Kelley, D. E., D. G. Stokes and R. P. Perry, 1999 CHD1 interacts with SSRP1 and depends on both its chromodomain and its ATPase/helicase-like domain for proper association with chromatin. Chromosoma **108:** 10–25.

Kingston, R. E., C. A. Bunker and A. N. Imbalzano, 1996 Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev. **10:** 905–920.

Kobor, M. S., J. Archambault, W. Lester, F. C. Holstege, O. Gileadi *et al.*, 1999 An unusual eukaryotic protein phosphatase required for transcription by RNA polymerase II and CTD dephosphorylation in *S. cerevisiae*. Mol. Cell **4:** 55–62.

Koepp, D. M., D. H. Wong, A. H. Corbett and P. A. Silver, 1996 Dynamic localization of the nuclear import receptor and its interactions with transport factors. J. Cell Biol. **133:** 1163–1176.

Kranz, J. E., and C. Holm, 1990 Cloning by function: an alternative approach for identifying yeast homologs of genes from other organisms. Proc. Natl. Acad. Sci. USA **87:** 6629–6633.

Lee, J. M., and A. L. Greenleaf, 1989 A protein kinase that phosphorylates the C-terminal repeat domain of the largest subunit of RNA polymerase II. Proc. Natl. Acad. Sci. USA **86:** 3624–3628.

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.

Lee, J. M., and A. L. Greenleaf, 1997 Modulation of RNA polymerase II elongation efficiency by C-terminal heptapeptide repeat domain kinase I. J. Biol. Chem. **272:** 10990–10993.

Lee, Y. C., J. M. Park, S. Min, S. J. Han and Y.-J. Kim, 1999 An activator binding module of yeast RNA polymerase II holoenzyme. Mol. Cell. Biol. **19:** 2967–2976.

LeRoy, G., G. Orphanides, W. S. Lane and D. Reinberg, 1998 Requirement of RSF and FACT for transcription of chromatin templates in vitro. Science **282:** 1900–1904.

Li, Y., S. Bjorklund, Y. W. Jiang, Y. J. Kim, W. S. Lane *et al.*, 1995 Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. Proc. Natl. Acad. Sci. USA **92:** 10864–10868.

Lu, H., O. Flores, R. Weinmann and D. Reinberg, 1991 The nonphosphorylated form of RNA polymerase II preferentially associates with the preinitiation complex. Proc. Natl. Acad. Sci. USA **88:** 10004–10008.

Malone, E. A., C. D. Clark, A. Chiang and F. Winston, 1991 Mutations in *SPT16/CDC68* suppress *cis*- and *trans*-acting mutations that affect promoter function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 5710–5717.

Marshall, N. F., and D. H. Price, 1995 Purification of P-TEFb, a transcription factor required for the transition into productive elongation. J. Biol. Chem. **270:** 12335–12338.

O'Brien, T., S. Hardin, A. Greenleaf and J. T. Lis, 1994 Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation. Nature **370:** 75–77.

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.

Orr-Weaver, T., J. W. Szostak and R. Rothstein, 1983 Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101:** 228–245.

Otero, G., J. Fellows, Y. Li, T. de Bizemont, A. M. G. Dirac *et al.*, 1999 Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. Mol. Cell **3:** 109–118.

Parada, C. A., and R. G. Roeder, 1996 Enhanced processivity of RNA polymerase II triggered by Tat-induced phosphorylation of its carboxy-terminal domain. Nature **384:** 375–378.

Paranjape, S. M., R. T. Kamakaka and J. T. Kadonaga, 1994 Role of chromatin structure in the regulation of transcription by RNA polymerase II. Annu. Rev. Biochem. **63:** 265–297.

Paro, R., 1993 Mechanisms of heritable gene repression during development of *Drosophila*. Curr. Opin. Cell Biol. **5:** 999–1005.

Payne, J. M., P. J. Laybourn and M. E. Dahmus, 1989 The transition of RNA polymerase II from initiation to elongation is associated with phosphorylation of the carboxyl-terminal domain of subunit IIa. J. Biol. Chem. **264:** 19621–19629.

Powell, W., and D. Reines, 1996 Mutations in the second largest subunit of RNA polymerase II cause 6-azauracil sensitivity in yeast and increased transcriptional arrest in vitro. J. Biol. Chem. **271:** 6866–6873.

Prelich, G., and F. Winston, 1993 Mutations that suppress the deletion of an upstream activating sequence in yeast: involvement of a protein kinase and histone H3 in repressing transcription *in vivo*. Genetics **135:** 665–676.

Roberts, S. M., and F. Winston, 1997 Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. Genetics **147:** 451–465.

Rose, M. D., P. Novick, J. H. Thomas, D. Botstein and G. R. Fink, 1987 A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:** 237–243.

Rose, M. D., F. Winston and P. Hieter, 1990 *Methods in Yeast Genetics: a Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Rowley, A., R. A. Singer and G. C. Johnston, 1991 *CDC68*, a yeast gene that affects regulation of cell proliferation and transcription, encodes a protein with a highly acidic carboxyl terminus. Mol. Cell. Biol. **11:** 5718–5726.

Sakai, A., Y. Shimizu, S. Kondou, T. Chibazakura and F. Hishinuma, 1990 Structure and molecular analysis of *RGR1*, a gene required for glucose repression of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:** 4130–4138.

Sherman, F., G. R. Fink and J. B. Hicks, 1981 *Methods in Yeast Genetics: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharoymces cerevisiae*. Genetics **122:** 19–27.

Sterner, D. E., J. M. Lee, S. E. Hardin and A. L. Greenleaf, 1995 The yeast carboxyl-terminal repeat domain kinase CTDK-I is a divergent cyclin-cyclin-dependent kinase complex. Mol. Cell. Biol. **15:** 5716–5724.

Stolinski, L. A., D. M. Eisenmann and K. M. Arndt, 1997 Identification of *RTF1*, a novel gene important for TATA site selection by TBP in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:** 4490–4500.

Swanson, M. S., and F. Winston, 1992 *SPT4*, *SPT5*, and *SPT6* inter-

actions: effects on transcription and viability in *Saccharomyces cerevisiae*. Genetics **132:** 325–336.

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 protein in yeast. Cell **73:** 1361–1375.

Tsukiyama, T., J. Palmer, C. C. Landel, J. Shiloach and C. Wu, 1999 Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. Genes Dev. **13:** 686–697.

Uptain, S. M., C. M. Kane and M. J. Chamberlin, 1997 Basic mechanisms of transcript elongation and its regulation. Annu. Rev. Biochem. **66:** 117–172.

Valay, J. G., M. Simon and G. Faye, 1993 The Kin28 protein kinase is associated with a cyclin in *Saccharomyces cerevisiae*. J. Mol. Biol. **234:** 307–310.

Wada, T., T. Takagi, Y. Yamaguchi, A. Ferdous, T. Imai *et al.*, 1998 DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. Genes Dev. **12:** 343–356.

Winston, F., 1992 Analysis of *SPT* genes: a genetic approach toward analysis of TFIID, histones and other transcription factors of yeast, pp. 1271–1293 in *Transcriptional Regulation*, edited by S. L. McKnight and K. R. Yamamoto. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Winston, F., C. Dollard and S. Ricupero-Hovasse, 1995 Construction of a set of convenient *S. cerevisiae* strains that are isogenic to S288C. Yeast **11:** 53–55.

Wittmeyer, J., and T. Formosa, 1997 The *Saccharomyces cerevisiae* DNA polymerase α catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. Mol. Cell. Biol. **17:** 4178–4190.

Wittmeyer, J., L. Joss and T. Formosa, 1999 Spt16 and Pob3 of *Saccharomyces cerevisiae* form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase α. Biochemistry **38:** 8961–8971.

Wittschieben, B. O., G. Otero, T. de Bizemont, J. Fellows, H. Erdjument-Bromage *et al.*, 1999 A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. Mol. Cell **4:** 123–128.

Woodage, T., M. A. Basrai, A. D. Baxevanis, P. Hieter and F. S. Collins, 1997 Characterization of the CHD family of proteins. Proc. Natl. Acad. Sci. USA **94:** 11472–11477.

Yankulov, K., J. Blau, T. Purton, S. Roberts and D. L. Bentley, 1994 Transcriptional elongation by RNA polymerase II is stimulated by transactivators. Cell **77:** 749–759.

Yankulov, K., K. Yamashita, R. Roy, J.-M. Egly and D. L. Bentley, 1995 The transcriptional elongation inhibitor 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole inhibits transcription factor IIH-associated protein kinase. J. Biol. Chem. **270:** 23922–23925.

Yankulov, K. Y., M. Pandes, S. McCracken, D. Bouchard and D. L. Bentley, 1996 TFIIH functions in regulating transcriptional elongation by RNA polymerase II in *Xenopus* oocytes. Mol. Cell. Biol. **16:** 3291–3299.

Communicating editor: M. Johnston