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Identification and Characterization of Elf1, a Conserved Transcription Elongation Factor in *Saccharomyces cerevisiae*

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In order to identify previously unknown transcription elongation factors, a genetic screen was carried out to identify mutations that cause lethality when combined with mutations in the genes encoding the elongation factors TFIIS and Spt6. This screen identified a mutation in YKL160W, hereafter named *ELF1* (*el*ongation *f*actor 1). Further analysis identified synthetic lethality between an *elf1*Δ mutation and mutations in genes encoding several known elongation factors, including Spt4, Spt5, Spt6, and members of the Paf1 complex. Genome-wide synthetic lethality studies confirmed that *elf1*Δ specifically interacts with mutations in genes affecting transcription elongation. Chromatin immunoprecipitation experiments show that Elf1 is cotranscriptionally recruited over actively transcribed regions and that this association is partially dependent on Spt4 and Spt6. Analysis of *elf1*Δ mutants suggests a role for this factor in maintaining proper chromatin structure in regions of active transcription. Finally, purification of Elf1 suggests an association with casein kinase II, previously implicated in roles in transcription. Together, these results suggest an important role for Elf1 in the regulation of transcription elongation.

Eukaryotic transcription is a complex process consisting of a series of steps involving initiation, elongation, and termination. Many recent studies have revealed the extent to which these steps are linked to each other, both functionally and physically (54, 71). While transcription initiation has been well studied and many of the fundamental mechanisms have been identified, an understanding of the control of transcription elongation in vivo is less clear. Some understanding of elongation has come from in vitro experiments examining the effects of various factors in modulating the elongation properties of RNA polymerase II (Pol II) (66). In the past few years many studies have also started to address the control of elongation in vivo and have identified many additional factors believed to play a role in this process (3, 69). In this paper, we describe a previously unstudied factor, Elf1 of *Saccharomyces cerevisiae*, that is functionally related to several other elongation factors and complexes, including TFIIS, Spt6, and the Paf1 complex.

TFIIS, the first eukaryotic elongation factor identified, was isolated nearly three decades ago based on its ability to induce long transcripts in an in vitro system (62). Further analysis of TFIIS revealed that it helps elongation in vitro by stimulating an RNA transcript cleavage activity of Pol II (13, 23). This activity allows backtracked (arrested) Pol II to cleave the 3′ end of the nascent transcript, thus realigning the 3′ end of the transcript with the Pol II catalytic site. Additional evidence suggests that TFIIS can help Pol II overcome a promoter-proximal pause (1, 50). Finally, recent evidence has shown that TFIIS is recruited along transcribed open reading frames, further suggesting a general role in elongation (51, 53).

Spt6 is one member of a class of elongation factors, initially identified in screens and selections for transcription mutants in *S. cerevisiae*, that includes Spt4, Spt5, Spt6, and Spt16. Spt4, which is nonessential for growth, and Spt5, which is essential, are components of the DSIF elongation factor which has been found to function both positively and negatively in regulating transcription elongation (21, 57, 72, 73, 76, 80). Spt6, an essential factor, genetically and physically interacts with Spt4 and Spt5 (31, 36, 73). Spt6 likely plays a role with chromatin-mediated transcription elongation, as it can assemble histones onto DNA in vitro (7) and *spt6* mutations suppress loss of the Swi/Snf nucleosome remodeling complex (46, 73). Furthermore, Spt6 is also necessary for maintaining chromatin integrity in regions of active transcription in vivo, as *spt6* mutations allow aberrant transcription initiation within coding regions (26). Spt6 likely plays non-chromatin-related roles in elongation as well, as human Spt6 has been shown to promote elongation on DNA templates in vitro (11). Spt16 interacts with Pob3 to form the CP/SPN complex in yeast (9, 14, 15). The mammalian homologue of this complex, FACT, was identified by its ability to facilitate transcription on a chromatin template in vitro (48). Similar to Spt6, Spt16 possesses histone chaperone activity (6) and mutation of *spt16* also leads to aberrant transcription from within coding regions (26, 41). Current evidence suggests that FACT tracks along transcribed chromatin, destabilizing nucleosomes, and thereby facilitating elongation by Pol II (6, 16, 60).

The Paf1 complex, was identified through its physical interaction with Pol II (64, 65) and is composed of five proteins, including Paf1, Ctr9, Cdc73, Rtf1, and Leo1 (31, 44, 70). Initially thought to play a role in initiation, the Paf1 complex has also been shown to play important roles in elongation (10, 44, 70). The Paf1 complex is localized to transcribed regions and genetically interacts with several elongation factors (10, 31, 51, 70). Recent studies have found that the Paf1 complex is re-

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype
FY120	*MAT***a** *lys2-128δ his4-912δ leu2Δ1 ura3-52*
FY300	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 spt5-194*
FY348	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 spt16-197*
FY1017	*MAT***a** *lys2-128δ his3Δ200 ura3-52 leu2Δ1 trp1Δ63 spt4Δ2::HIS3*
FY1220	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 spt6-140*
FY1235	*MATα lys2-128δ his4-912δ ura3-52 leu2Δ1 trp1Δ63 hir1Δ::LEU2*
FY1635	*MATα lys2-128δ his4-912δ ura3-52 leu2Δ1 spt5-242*
FY1638	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 rpb1-221*
FY1650	*MAT***a** *lys2-128δ his3Δ200 ura3-52 leu2Δ1 rpb2Δ297::HIS3 + pRP2-7 (rpb2-7 CEN URA3)*
FY1651	*MAT***a** *lys2-128δ his3Δ200 ura3-52 leu2Δ1 rpb2Δ297::HIS3 + pRP2-10 (rpb2-10 CEN URA3)*
FY1668	*MAT***a** *lys2-128δ his4-912δ spt5-4*
FY2116	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 trp1Δ63 rtf1Δ101::LEU2*
FY2118	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 RPB3-HA1::LEU2 SPT6-FLAG ctr9Δ::KANMX*
FY2127	*MATα lys2-128δ his4-912δ ura3-52 leu2Δ1 RPB3-HA1::LEU2 SPT6-FLAG cdc73Δ::KANMX*
FY2128	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 RPB3-HA1::LEU2 SPT6-FLAG paf1Δ::KANMX*
FY2139	*MATα lys2-128δ ura3-52 leu2Δ1 spt6-1004*
FY2371	*MAT***a** *lys2-128δ his3Δ200 ura3Δ0 leu2Δ1 dst1Δ::KANMX spt6-14*
FY2372	*MAT***a** *lys2-128δ his3Δ200 ura3Δ0 leu2Δ1 dst1Δ::KANMX spt6-14 elf1::*Tn*3 (+pKC13)*
FY2373	*MAT***a** *lys2-128δ his4-912δ leu2Δ1*
FY2374	*MAT***a** *lys2-128δ his4-912δ leu2Δ1 elf1Δ::NATMX*
FY2375	*MATα lys2-128δ his4-912δ leu2Δ1 dst1Δ::KANMX*
FY2376	*MAT***a** *lys2-128δ his4-912δ leu2Δ1 elf1Δ::NATMX dst1Δ ::KANMX*
FY2377	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 elf1Δ::NATMX*
FY2378	*MAT***a** *lys2-128δ his3Δ200 ura3Δ0 leu2Δ1 trp1Δ63 spt4Δ2::HIS3 elf1Δ::NATMX (+pDP1)*
FY2379	*MATα lys2-128δ his4-912δ ura3Δ0 met15Δ0 trp1Δ63 spt6-1004-FLAG elf1Δ::NATMX (+pDP1)*
FY2380	*MAT***a** *lys2-128δ his4-912δ ura3Δ0 leu2Δ1 cdc73Δ::KANMX elf1Δ::NATMX (+pDP1)*
FY2381	*MATα lys2-128δ his4-912δ ura3Δ0 leu2Δ0 trp1Δ63 paf1Δ::KANMX elf1Δ::NATMX (+pDP1)*
FY2383	*MATα lys2-128δ his4-912δ ura3-52 leu2Δ1 elf1Δ::NATMX spt5-242*
FY2384	*MATα lys2-128δ his4-912δ his3Δ200 ura3-52 leu2Δ1 ELF1-13MYC::KANMX RPB3-HA1::LEU2*
FY2385	*MATα lys2-128δ his4-912δ his3Δ200 ura3-52 leu2Δ1*
FY2386	*MATα lys2-128δ his4-912δ his3Δ200 ura3-52 leu2Δ1 ELF1-13MYC::KANMX RPB3-HA1::LEU2 spt4Δ1::URA3*
FY2387	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 ELF1-13MYC::KANMX RPB3-HA1::LEU2 spt6-1004*
FY2388	*MAT***a** *lys2-128δ his4-912δ his3Δ200 ura3-52 leu2Δ1 ELF1-13MYC::KANMX RPB3-HA1::LEU2 paf1Δ::KANMX*
FY2389	*MAT***a** *lys2-128δ his4-912δ his3Δ200 ura3-52 leu2Δ1 ELF1-13MYC::KANMX RPB3-HA1::LEU2 rtf1Δ101::LEU2*
FY2390	*MATα lys2-128δ his4-912δ ura3-52 leu2Δ1 ELF1-13MYC::KANMX RPB3-HA1::LEU2 spt16-197*
FY2391	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 elf1Δ::NATMX RPB3-HA1::LEU2 CTR9-MYC9::KANMX SPT4-3XFLAG::KANMX*
FY2392	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 elf1Δ::NATMX RPB3-HA1::LEU2 CTR9-MYC9::KANMX SPT4-3XFLAG::KANMX elf1Δ::NATMX*
FY2393	*MAT***a** *lys2-128δ his3Δ200 ura3-52 leu2Δ1 trp1Δ63 prGAL1-FLO8-HIS3::KANMX*
FY2394	*MAT***a** *lys2-128δ his3Δ200 ura3-52 leu2Δ1 prGAL1-FLO8-HIS3::KANMX elf1Δ::NATMX*
FY2395	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 trp1Δ63 rtf1Δ::LEU2 elf1Δ::NATMX*
FY2396	*MAT***a** *lys2-128δ his4-912δ ura3-52 leu2Δ1 trp1Δ63 elf1Δ::NATMX hir1Δ::LEU2*
FY2397	*MAT***a** *lys2-128δ ura3-52 leu2Δ1 spt6-1006*
FY2398	*MAT***a** *lys2-128δ his4-912δ ura3Δ0 leu2Δ1 elf1Δ::NATMX spt16-197*
L1095	*MATα his3Δ(1 or 200) ura3Δ0 leu2Δ0 met15Δ0 elf1Δ::NATMX can1Δ::prMFA1-HIS3*

quired for several histone modifications at active genes, including the methylation of lysines 4 and 36 of histone H3, catalyzed by Set1 and Set2, respectively (30, 32, 47).

Despite the large number of eukaryotic elongation factors recently identified, it seemed likely that not all had been discovered. In order to identify previously unknown elongation factors, we performed a genetic screen to identify mutations that cause lethality when combined with mutations in *DST1*, the gene encoding TFIIS, and *SPT6*. In this screen, we identified a mutation in a previously unstudied gene, YKL160W, that causes lethality in combination with mutations in *DST1* and *SPT6*. The protein encoded by this gene is a member of a conserved family of zinc finger proteins of unknown function. In accordance with a role in transcription elongation, we have designated this gene as *ELF1* (*el*ongation *f*actor 1). Genetic analysis reveals that *elf1*Δ genetically interacts with mutations in genes encoding many other elongation factors, including Spt4, Spt5, Spt6, and the Paf1 complex. Synthetic genetic array (SGA) analysis confirms that *elf1*Δ functionally interacts only with genes suggested to have roles in transcription elongation. Chromatin immunoprecipitation (ChIP) analysis demonstrates that Elf1 preferentially binds to the coding regions of genes compared to promoters. This localization to coding regions was found to be dependent on transcription and partially dependent on Spt4 and Spt6. Analysis of *elf1*Δ mutants suggests a role for this factor in maintaining proper chromatin structure in regions of active transcription. Finally, purification of Elf1 has revealed a physical association with casein kinase II (CKII). Together, these results provide strong evidence of an important role for Elf1 in transcription elongation.

MATERIALS AND METHODS

Yeast strains, plasmids, and media. All *S. cerevisiae* strains (Table 1) are isogenic to a *GAL2*$^+$ derivative of S288C (78). To epitope tag Elf1, DNA sequence encoding 13 copies of the Myc epitope was integrated at the 3′ end of the *ELF1* coding region (37). The *ELF1-13MYC* gene is wild type with respect to

an Spt phenotype, and it does not cause any synthetic defects in growth when crossed to any other mutation tested in this study. The Flag-tagged Spt4 construct was provided by G. Hartzog and has been previously described (36). The Ctr9-Myc construct has been previously described (25). The *dst1*Δ*::KANMX* allele was constructed by a one-step PCR-mediated disruption which replaces the entire open reading frame with the *KANMX4* cassette (8). The *elf1*Δ*::NATMX* allele, referred to as *elf1*Δ throughout this paper, was constructed by a one-step PCR-mediated disruption which replaces the entire open reading frame with the *NATMX4* cassette (20). The *GAL1-FLO8-HIS3* reporter, to be described elsewhere, contains the *HIS3* gene inserted out-of-frame into the 3′ coding region of *FLO8* such that wild-type *HIS3* product is only expressed when transcription initiates from a cryptic promoter within the *FLO8* gene. In addition, the *FLO8* promoter has been replaced by the *GAL1* promoter. All deletion mutants used from the yeast deletion set (InVitrogen) were confirmed by PCR. Plasmid pKC13 (gift of Kristine Johnson and Caroline Kane) contains the *DST1* coding region under *GAL1* control in the YEp435 vector (*URA3*$^+$, 2μm plasmid). Western analysis with TFIIS antisera (a gift of C. Kane) revealed a significant level of TFIIS protein expressed from this plasmid under repressive growth conditions (glucose) and a greatly elevated level under inducing conditions (galactose). Plasmid pDP1 was constructed by addition of BamHI and EcoRI restriction sights to a PCR fragment containing *ELF1* (−487 to +840 bp, where +1 = ATG and +436 = TAA) and ligated into pRS416 (45). Double mutant yeast strains were constructed by standard genetic methods. An *elf1*Δ mutant containing plasmid pDP1 was crossed to strains containing mutations in genes encoding a number of elongation factors. After sporulation and tetrad dissection, double mutant strains were identified and tested for growth on medium containing 5-fluoroorotic acid (5-FOA). In all cases, at least 10 tetrads were dissected and analyzed for growth. In most cases, strains used were in the FY (S288C) strain background and are listed in Table 1. However, strains containing *leo1*Δ, *rpb4*Δ, *hir2*Δ, *hir3*Δ, and *hpc2*Δ were obtained from the yeast deletion set, also in the S288C background (InVitrogen).

Mating, transformation, sporulation, and tetrad analysis were performed by standard procedures previously described (58). Rich (yeast extract-peptone-dextrose [YPD]), minimal (SD), synthetic complete (SC), omission (SC-), 5-FOA, and sporulation media were prepared as previously described (58). For SGA analysis, the sporulation medium was 2% agar, 1% potassium acetate, 0.1% yeast extract, 0.05% glucose supplemented with uracil, histidine, and lysine. Selection for cells containing the *KANMX4* cassette was conducted on YPD containing 200 μg/ml of G418 sulfate (Gibco), and selection for the deletion was on YPD containing 100 μg/ml clonNAT (Werner BioAgents).

Identification of synthetic lethal mutations. To identify novel elongation factors, we performed a genetic screen to identify mutations causing synthetic lethality with mutations in *dst1*Δ and *spt6-14*. First, strain FY2371 (*dst1*Δ *spt6-14*) was transformed with plasmid pKC13, a high-copy-number plasmid containing the wild-type *DST1*$^+$ and the *URA3*$^+$ genes. This strain was then transformed with NotI-generated linear DNA fragments from an mTn3 transposon insertion library (59), and transformants were selected on medium lacking leucine. Approximately 20,000 transformants were replica plated to medium lacking leucine and medium containing 5-FOA. Initially, 28 synthetic lethal candidates were identified due to the lack of growth on 5-FOA-containing medium. These colonies were purified and retested for growth on 5-FOA. Many of the insertions were eliminated as candidates due to the instability of the transposon insertion, weakness of phenotype upon rechecking, or lack of linkage between the phenotype and the transposon insertion. Of these candidates, we found one strong synthetic lethal candidate where the phenotype was linked to the transposon insertion. We identified the genomic location of this insertion by vectorette PCR (34, 56). This insertion was located in the YKL160W/*ELF1* gene and predicts a fusion of the first 62 amino acids with the *lacZ* gene from the transposon insertion.

SGA analysis. SGA analysis was performed as previously described (74) with minor modifications. The *S. cerevisiae* deletion set was transferred from frozen stocks in 96-well containers to large YPD plates and grown as spots for 2 days at 30°C. The strains were then mated to lawns of the query strain L1095 on fresh YPD plates, incubated for 1 day, and then replica plated to YPD plates containing G418 and clonNAT to select for diploids. The diploids were replica plated to sporulation medium and incubated at 22°C for 5 days. *MAT***a** spores were selected by replica plating to SC-his-arg plates containing canavanine (50 mg/liter) for 2 days. Finally, strains were replica plated to haploid selection medium containing G418 and haploid selection medium containing G418 and clonNAT. Growth was scored for 3 days, and synthetic lethal candidates were identified by lack of growth on the medium containing G418 and clonNAT. From this analysis, 10 synthetic lethal candidates were identified. For nine of the candidates, we tested the possible synthetic lethality with *elf1*Δ by tetrad analysis. The tenth candidate (yml009w-BΔ) was not tested, because this deletion removes a small portion of the essential gene, *SPT5*, and some alleles of *SPT5* were already known to be synthetically lethal with *elf1*Δ. Of the nine remaining candidates, only three were determined to be synthetically lethal with *elf1*Δ by tetrad analysis (*cdc73*Δ, *leo1*Δ, and *rpb4*Δ). Five candidates were not synthetically lethal (*rtf1*Δ, *hir1*Δ, *hir2*Δ, *hir3*Δ, and *hpc2*Δ), and one (*bur2*Δ) germinated too poorly to allow any conclusion to be made. As previously pointed out, SGA analysis is subject to the identification of both false-negative and false-positive interactions (38, 74, 75). Using SGA analysis, we identified an interaction with mutations in three of the five known components of the Paf1 complex (*cdc73*Δ, *leo1*Δ, and *rtf1*Δ). Lethality of *elf1*Δ with deletions of genes encoding the other two components of the Paf1 complex, *paf1*Δ and *ctr9*Δ, was not able to be determined by SGA due to the sickness of these strains in the deletion set. In addition, another mutation known to be synthetically lethal with *elf1*Δ, *spt4*Δ, was not identified in the SGA analysis, likely because the *spt4*Δ strain is not healthy in the deletion set and thus could not be scored by SGA. In addition to false negatives, we also identified five false positives (see Table 3, below) for unknown reasons. There are several differences in the SGA method when compared to standard tetrad analysis that could account for these differences, including the use of synthetic complete media for germination or the requirement for sporulation and germination among patches of other wild-type and single mutant yeast cells.

Chromatin immunoprecipitation. All chromatin immunoprecipitations were carried out as previously described with minor modifications (35). Antibodies used in these experiments included the 12CA5 mouse monoclonal antibody against the hemagglutinin (HA) epitope (0.5 μl), A14 anti-Myc rabbit polyclonal (5 μl; Santa Cruz), and M2 anti-FLAG monoclonal antibody (2 μl; Sigma). Immunoprecipitations were carried out in FA lysis buffer containing 150 mM NaCl and no sodium dodecyl sulfate (SDS). Dilutions of input DNA and immunoprecipitated DNA were subjected to quantitative PCR by the incorporation of [α-^{32}P]dATP. The products were separated on a 6% nondenaturing polyacrylamide gel, and quantification was carried out by phosphorimager analysis (Molecular Dynamics). The percent immunoprecipitated was calculated for each sample, and all samples were normalized to a control PCR product amplified in each reaction mixture. The primers for this control PCR product amplify a region of chromosome V that is not contained in any open reading frames (28). Relative positions of primer sites were as follows for *GAL1*: UAS, −536 to −276; TATA, −190 to +54; 5′, +590 to +877; 3′, +1330 to +1657; and 3′UTR, +1805 to +2079; For *PMA1* they were as follows: UAS, −623 to −390; promoter, −370 to −90; 5′, +584 to +807; 3′, +2018 to +2290; 3′UTR, +529 to +742 (relative to stop codon). All values are relative to the translation start site (ATG = +1) except where noted. All experiments were performed at least three times, and standard errors are shown on each graph. By Western analysis, each protein examined by ChIP was present at the same level in the wild-type and mutant strains.

Northern analysis. RNA isolation and Northern hybridization experiments were carried out as described previously (4, 72). Northern hybridization analysis was conducted using probes to *FLO8* and *SCR1*. The *FLO8* probe corresponds to the +1515 to +2326 region of the *FLO8* gene. The *SCR1* probe corresponds to the −221 to +452 region of the *SCR1* gene. For each experiment, Northern analysis was performed in duplicate with similar results between the two experiments. Viability of all strains used for Northern analysis was approximately 100% for all times and temperatures used in the experiments.

Purification of Elf1 and mass spectrometry of associated proteins. Tandem affinity purification (TAP)-tagged Elf1 was purified on immunoglobulin G (IgG) and calmodulin columns from extracts of yeast cells (10 liters) grown in YPD medium to an optical density at 600 nm of 1.0 to 1.5 (19, 55). The cell pellets (7 to 10 g) were frozen in liquid nitrogen and lysed by grinding with dry ice in a Krups coffee grinder (model number 203-70). An equal volume of YEB (250 mM KCl, 100 mM HEPES-KOH, pH 7.9, 1 mM EDTA, 2.5 mM dithiothreitol [DTT]) was added and, following centrifugation in a Beckman Ti70 rotor at 4°C for 2 h at 34,000 rpm, the supernatant was dialyzed against IPP buffer (10 mM Tris-Cl, pH 7.9, 0.1% Triton X-100, 0.5 mM DTT, 0.2 mM EDTA, 20% glycerol, 100 mM NaCl). After dialysis, the extract was again centrifuged in a Ti70 rotor at 4°C for 30 min at 34,000 rpm and the supernatant was mixed for 3 h with 200 μl IgG-Sepharose (Pharmacia) equilibrated with IPP buffer. Following binding, the IgG-Sepharose was washed with 1 ml IPP buffer followed by 400 μl TEV protease cleavage buffer (50 mM Tris-Cl, pH 7.9, 1 mM DTT, 0.1% Triton X-100, 100 mM NaCl). The beads were then incubated overnight at 4°C with 100 U of TEV protease (Life Technologies) in 200 μl TEV cleavage buffer. The eluate was combined with a 200-μl wash with TEV cleavage buffer. To this was added 200 μl calmodulin binding buffer (10 mM Tris-Cl, pH 7.9, 10 mM β-mercaptoethanol, 2 mM $CaCl_2$, 0.1% Triton X-100, 100 mM NaCl) and 200 μl of

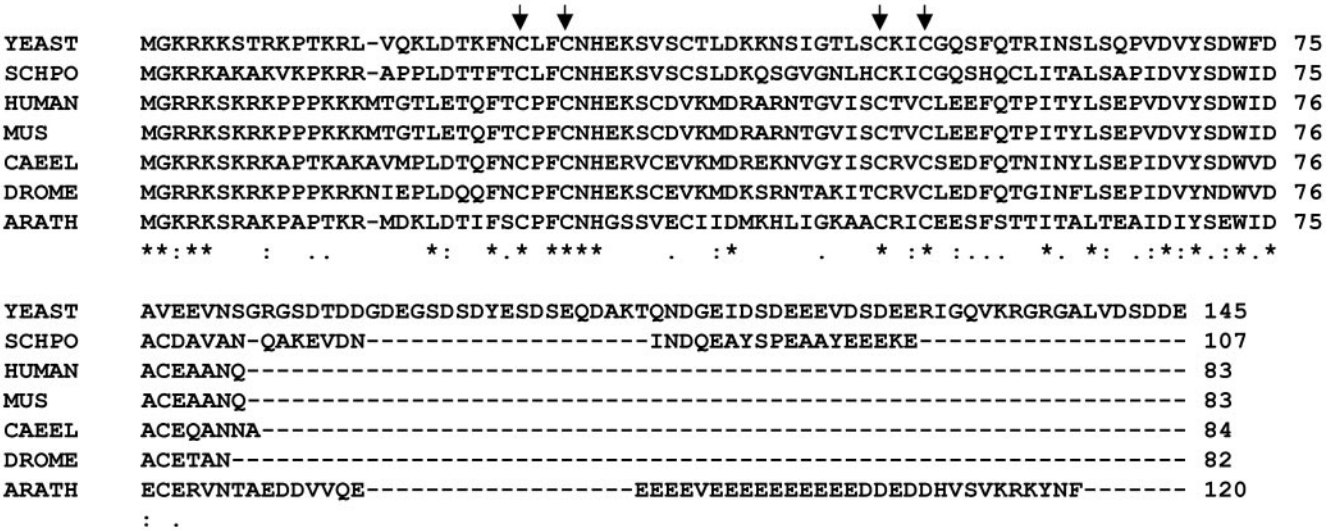

FIG. 1. Elf1 is a conserved member of a zinc finger family of uncharacterized proteins. Homologues of Elf1 were identified by standard protein BLAST analysis. Full-length homologous protein sequences from several eukaryotic organisms were then aligned using ClustalW. Identical residues are noted by an asterisk, conserved substitutions are noted by a colon, and semiconservative substitutions are noted by a period. The four conserved cysteines of the zinc finger domain are noted by arrows above the alignment. Abbreviations: YEAST, *Saccharomyces cerevisiae*; SCHPO, *Schizosaccharomyces pombe*; HUMAN, *Homo sapiens*; MUS, *Mus musculus*; CAEEL, *Caenorhabditis elegans*; DROME, *Drosophila melanogaster*; ARATH, *Arabidopsis thaliana*.

calmodulin beads (Pharmacia) equilibrated with the same buffer. After binding for 1 to 2 h at 4°C, the calmodulin beads were washed with 200 μl calmodulin binding buffer and 200 μl calmodulin wash buffer (10 mM Tris-Cl, pH 7.9, 10 mM β-mercaptoethanol, 0.1 mM $CaCl_2$, 0.1% Triton X-100, 100 mM NaCl). The purified protein complexes were eluted from the calmodulin beads with 5× 100 μl calmodulin elution buffer (10 mM Tris-Cl, pH 7.9, 10 mM β-mercaptoethanol, 3 mM EGTA, pH 8.0, 0.1% Triton X-100, 100 mM NaCl). The purified proteins were either directly reduced, alkylated, digested with trypsin, and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) on a Finnigan LCQ Deca tandem mass spectrometer or else separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on gels containing 10% polyacrylamide and stained with silver. After SDS-PAGE, protein bands were reduced, alkylated, and digested with trypsin, and purified peptide samples were spotted onto a target plate with a matrix of α-cyano-4-hydroxycinnamic acid (Fluka). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry analysis was conducted utilizing a Reflex IV (Bruker Daltonics, Billerica, MA) instrument in positive ion reflectron mode (39, 63). For tandem mass spectrometry, the trichloroacetic acid-precipitated protein was resuspended in 100 mM NH_4HCO_3–1 mM $CaCl_2$ buffer, pH 8.5, and digested overnight at 37°C with 2 μl of immobilized Poros trypsin beads (PerSeptive). The entire digest was fractionated as described previously (17) on a 7.5-cm (inner diameter, 100 μm) reverse-phase C_{18} capillary column attached in-line to a Finnigan LCQ-Deca ion trap mass spectrometer by ramping a linear gradient from 2 to 60% solvent B in 90 min. Solvent A consisted of 5% acetonitrile, 0.5% acetic acid, and 0.02% heptafluorobutanoic acid, and solvent B consisted of acetonitrile-water (80:20) containing 0.5% acetic acid and 0.02% heptafluorobutanoic acid. The flow rate at the tip of the needle was set to 300 nl/min by programming the high-performance liquid chromatography pump and using a split line. The mass spectrometer cycled through four scans as the gradient progressed. The first was a full mass scan followed by successive tandem mass scans of the three most intense ions. A dynamic exclusion list was used to limit collection of tandem mass spectra for peptides that eluted over a long period of time. All tandem mass spectra were searched by using the SEQUEST computer algorithm against the complete yeast protein database. Each high-scoring peptide sequence was manually compared with the corresponding tandem mass spectrum to ensure that the match was correct.

RESULTS

Identification of a mutation that causes lethality in combination with mutations in *DST1* and *SPT6*. To identify previously unstudied genes involved in transcription elongation in *S. cerevisiae*, we performed a genetic screen for mutations that cause inviability when combined with mutations in genes known to encode elongation factors. Specifically, we screened for mutations that cause inviability when combined with both *dst1*Δ and *spt6-14* (described in Materials and Methods). From this screen, we identified one mutation in YKL160W, a previously uncharacterized gene. This gene is hereafter referred to as *ELF1*, and the mutation is referred to as *elf1-1::*Tn*3*. To determine the phenotype of *elf1-1::*Tn*3* when combined with either *dst1*Δ or *spt6-14* individually, we constructed *elf1-1::*Tn*3* *dst1*Δ and *elf1-1::*Tn*3* *spt6-14* double mutants. The *elf1-1::*Tn*3* *dst1*Δ double mutant had very little defect in growth. In contrast, the *elf1-1::*Tn*3* *spt6-14* mutant was extremely sick. Thus, the *elf1-1::*Tn*3* and *spt6-14* mutations contribute the majority of the growth defect initially observed in our screen. Later in this paper, we show that *elf1*Δ *spt6-14* double mutants are inviable and that *elf1*Δ *dst1*Δ double mutants are viable but have mutant phenotypes.

Elf1 is a member of a conserved eukaryotic family of zinc finger proteins. The *ELF1* gene of *S. cerevisiae* is predicted to encode a small protein of 145 amino acids. BLAST analysis (2) reveals that the predicted Elf1 protein is conserved in other sequenced eukaryotic genomes, including other yeasts, human, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis thaliana* (Fig. 1). The conservation of Elf1 occurs in the region corresponding to the N-terminal half of the *S. cerevisiae* protein. These hypothetical proteins are short (often less than 100 amino acids) and contain a C4 zinc-binding region. The four cysteine residues of the potential zinc-binding domain are completely conserved in all homologues, and this conserved domain has been termed DUF701 (*d*omain of *u*nknown *f*unction 701) (40). To date, only the *C. elegans* homologue has

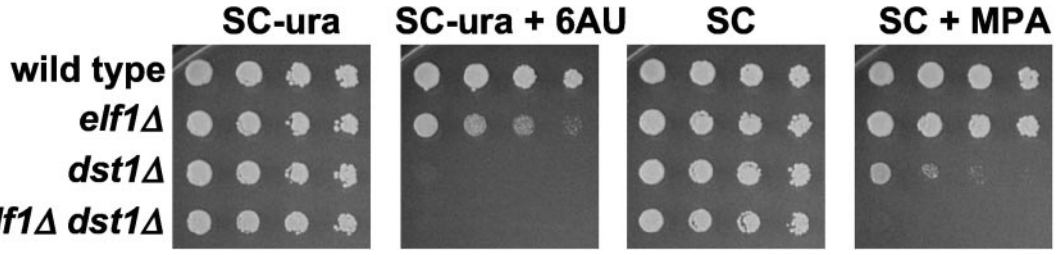

FIG. 2. The *elf1*Δ mutation causes mild sensitivity to 6AU and genetically interacts with *dst1*Δ. Yeast strains were spotted onto plates in a series of 10-fold dilutions. Strains were grown on SC-ura or SC medium for 2 days, medium containing 6AU (50 μg/ml) for 5 days, or medium containing MPA (20 μg/ml) for 3 days.

been functionally characterized; in RNA inhibition experiments, no significant growth defect was observed (24). Two genome-wide localization screens (22, 33) have suggested that Elf1 is localized to the nucleus, consistent with our localization analysis of an Elf1-green fluorescent protein fusion (data not shown). Interestingly, Elf1 was also identified in one of those studies to be associated with meiotic chromosomes (33). The conservation of Elf1 strongly suggests that it plays an important role throughout eukaryotes.

***elf1*Δ causes mild sensitivity to 6-AU and genetically interacts with *dst1*Δ.** To further characterize *ELF1*, we constructed a complete deletion of this gene and tested it for mutant phenotypes. The *elf1*Δ mutation caused no significant defect in growth (Fig. 2). We also tested an *elf1*Δ mutant for sensitivity to 6-azauracil (6AU) and mycophenolic acid (MPA), phenotypes shared by many transcription elongation mutants due to the effect of these compounds on depleting nucleotide pools in vivo (12). Our results show that *elf1*Δ mutants exhibit a mild sensitivity to 6AU, but not to MPA (Fig. 2). We also tested *elf1*Δ *dst1*Δ double mutants and found that they are extremely sensitive to both 6AU and MPA (Fig. 2). In addition, *elf1*Δ *dst1*Δ double mutants fail to grow on galactose medium at 37°C (data not shown). Finally, we also tested whether *elf1*Δ caused an Spt$^-$ (suppressor of Ty insertion) phenotype. This was tested using the insertion mutation *lys2-128*δ (67), and it was shown that *elf1*Δ causes a modest suppression, allowing growth on SC-lys plates after 3 days of incubation at 30°C (data not shown). Together, the 6AU phenotype, the Spt$^-$ phenotype, and genetic interactions with *dst1*Δ suggest a potential role for Elf1 in transcription elongation.

***elf1*Δ genetically interacts with mutations in the genes encoding the Spt4, Spt5, Spt6, and Spt16 elongation factors.** The screen by which we identified *ELF1* strongly suggested that it is critical for growth in an *spt6* mutant background. Therefore, we tested if *elf1*Δ also genetically interacts with mutations that impair several known elongation factors. These tests were performed by two approaches. In the first approach, described in this section and the following one, we constructed specific double mutants, combining *elf1*Δ with mutations in genes encoding other known elongation factors, some of which are essential for viability. The second approach, described later, used SGA analysis (74, 75) to examine the genetic interactions between *elf1*Δ and deletions in all other nonessential yeast genes.

By crosses, we first combined *elf1*Δ with *spt4*, *spt5*, and *spt6* mutations. Genetic and biochemical results have implicated the Spt4, Spt5, and Spt6 proteins functioning through effects on transcription elongation and chromatin structure (82). By crosses, we tested *elf1*Δ combined with a complete deletion of the nonessential *SPT4* gene and with several different missense mutations in the essential *SPT5* and *SPT6* genes. Our results demonstrate that an *elf1*Δ *spt4*Δ double mutant is inviable (Fig. 3A; Table 2). Consistent with these results, an *elf1* mutation was independently identified in a screen for mutations that cause lethality when combined with *spt4*Δ (G. Hartzog and J. Speer, personal communication). We tested *elf1*Δ with three different *spt5* mutations and observed inviability for two of the combinations (Table 2). The third combination is described below. We also tested four different *spt6* alleles: three combinations resulted in synthetic lethality, and the fourth double mutant grew extremely poorly (Fig. 3A; Table 2). From these results, we conclude that, in general, *elf1*Δ causes lethality when combined with *spt4*, *spt5*, and *spt6* mutations.

One interesting exception to the results described above came from the combination of *elf1*Δ with the *spt5-242* mutation. Previous analysis demonstrated that the *spt5-242* mutation is distinct from other *spt5* mutant alleles with respect to its mutant phenotypes, including genetic interactions with other transcription mutants (21). In addition, *spt5-242* confers a cold-sensitive (Cs$^-$) growth phenotype (21). When combined with *spt5-242*, *elf1*Δ suppressed the Cs$^-$ phenotype (Fig. 3B), similar to results previously obtained for mutations in the genes encoding the Rpb1 and Rpb2 subunits of Pol II (21), the Paf1 complex (70), and Chd1, a chromodomain-containing protein that has also been implicated in elongation (68). Additionally, *elf1*Δ also modestly suppressed the Spt$^-$ phenotype of the *spt5-242* allele (Fig. 3B).

We next tested to see whether an *elf1*Δ mutation interacted with an *spt16* mutation. We observed no significant growth defect when *elf1*Δ was combined with the *spt16-197* mutation, although there was a slight synthetic Spt$^-$ phenotype (Table 2 and data not shown). Thus, mutations in *elf1*Δ interact strongly with mutations in *SPT4*, *SPT5*, and *SPT6* but only cause a mild phenotype when combined with a mutation in *SPT16*. However, it is possible that other alleles of *spt16* might cause more severe defects than the *spt16-197* mutation.

***elf1*Δ genetically interacts with mutations in the genes encoding the Paf1 complex.** We next tested for genetic interactions between *elf1*Δ and null mutations in the genes encoding components of the Paf1 complex. We found that an *elf1*Δ mutation causes synthetic lethality when combined with deletion mutations in four of the five Paf1 complex genes, *paf1*Δ,

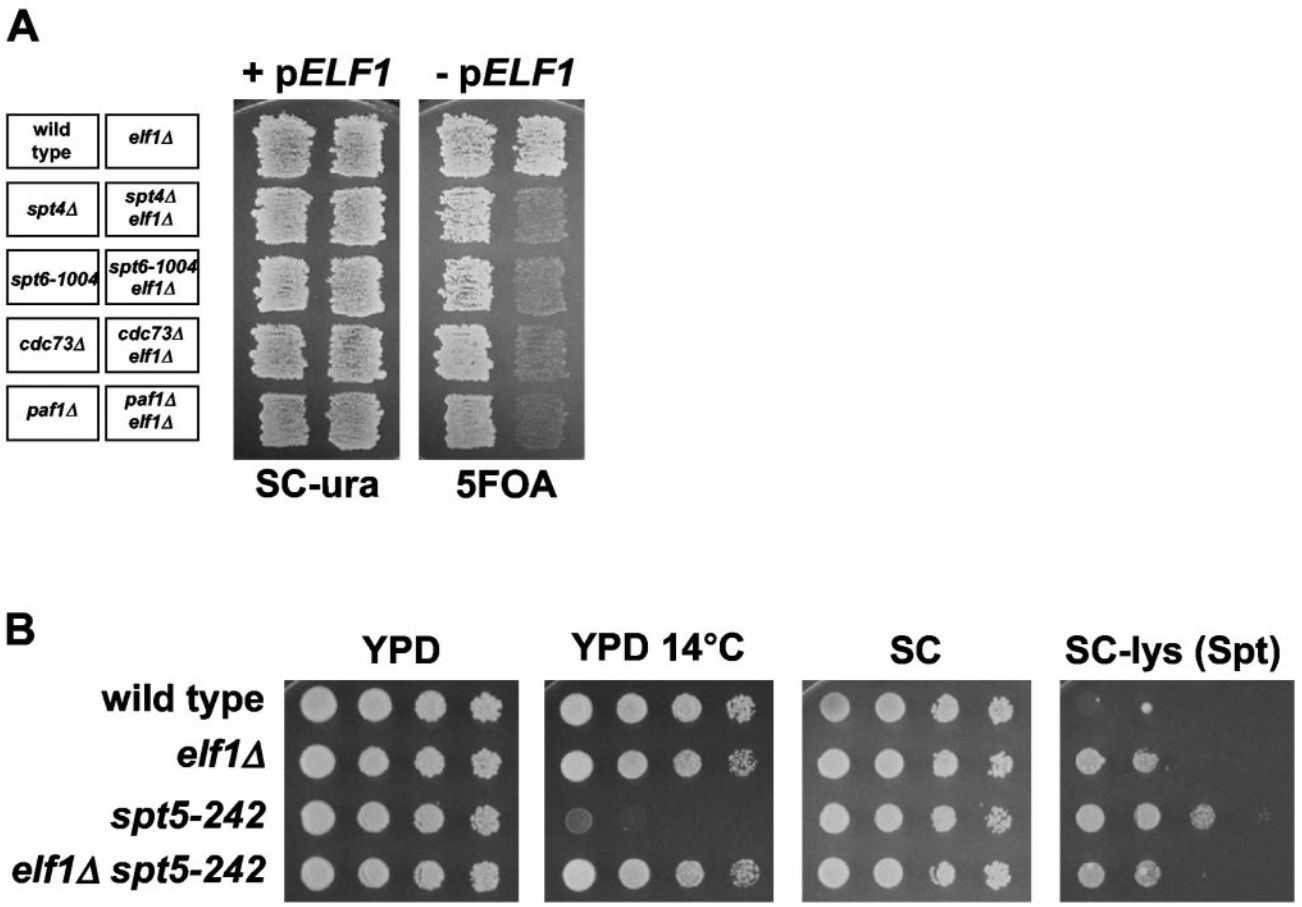

FIG. 3. *elf1*Δ genetically interacts with mutations in the genes encoding the transcription elongation factors Spt4, Spt5, Spt6, and the Paf1 complex. (A) Deletion of *elf1*Δ is lethal in combination with mutations in *SPT4*, *SPT6*, or Paf1 complex genes. *elf1*Δ strains were transformed with plasmid pDP1 containing *ELF1*$^+$ on a *CEN URA3* plasmid and mated to *spt4*Δ, *spt6-1004*, *cdc73*Δ, or *paf1*Δ strains. Diploids were sporulated and dissected, and representative spores from the resulting complete tetrads were patched onto YPD. Patches were replica plated to medium lacking uracil for 1 day and medium containing 5FOA for 2 days. (B) Deletion of *elf1*Δ suppresses *spt5-242* phenotypes. *spt5-242* is a Cs$^-$ Spt$^-$ mutation that can be suppressed by mutations in Pol II or other elongation factors that impair elongation (21). *elf1*Δ strongly suppresses the Cs$^-$ phenotype and modestly suppresses the Spt$^-$ phenotype of *spt5-242*. The Spt$^-$ phenotype was scored by observing growth on medium lacking lysine and using the *lys2-128*δ allele. Yeast strains were spotted in a series of 10-fold serial dilutions onto YPD for 2 days at 30°C, YPD at 14°C for 9 days, SC for 2 days, or SC-lys for 4 days.

*cdc73*Δ, *ctr9*Δ, and *leo1*Δ. In agreement with this result, *elf1*Δ was identified in a large-scale SGA screen to genetically interact with a mutation in the gene encoding the Cdc73 component of the Paf1 complex (75). In contrast, when *elf1*Δ was combined with *rtf1*Δ, the double mutant was viable, although it displayed both Spt$^-$ and Ts$^-$ phenotypes (Table 2). Together, the strong genetic interactions of *elf1*Δ with mutations in the genes encoding the elongation factors Spt4, Spt5, Spt6, and the Paf1 complex further support an important role for Elf1 in transcription elongation.

SGA analysis identifies additional mutations that genetically interact with *elf1*Δ. To examine the genetic interactions of *elf1*Δ with deletions of all of the nonessential genes of *S. cerevisiae*, we performed SGA analysis (74). In this approach, *elf1*Δ was combined with the approximately 4,700 deletions contained within the yeast deletion set. This analysis led to the identification of 10 candidate mutations that were initially scored as causing synthetic lethality with *elf1*Δ (Table 3). Of these 10 candidates, 9 merited further investigation (see Materials and Methods) and were tested for synthetic lethality with *elf1*Δ by standard tetrad analysis. These crosses demonstrated that only three of the nine mutations, *cdc73*Δ, *leo1*Δ, and *rpb4*Δ, actually caused synthetic lethality with *elf1*Δ, while some of the others, while viable, caused other mutant phenotypes (Table 3). As previously pointed out by others (38, 74, 75), analysis of genetic interactions by SGA often leads to false positives (see Materials and Methods). Of the three cases of synthetic lethality, two of them, *cdc73*Δ and *leo1*Δ, had been identified by the direct tests described in an earlier section. Consistent with our results, *elf1*Δ was also identified in a large-scale SGA screen to genetically interact with *cdc73*Δ (75).

The third mutation identified by SGA to cause synthetic lethality with *elf1*Δ was *rpb4*Δ. *RPB4* encodes a nonessential subunit of Pol II (79). Based on this interaction, we also tested whether *elf1*Δ confers synthetic phenotypes when combined with three different alleles in the essential Pol II genes *RPB1* and *RPB2* that confer a 6AU^S phenotype. We found that these three mutations, *rpb1-221*, *rpb2-7*, and *rpb2-10* (21, 52), cause

TABLE 2. *elf1*Δ genetically interacts with mutations encoding several transcription factors

Second mutation	Synthetic phenotype with *elf1*Δ
*spt4*Δ	Inviable
spt5-4	Inviable
spt5-194	Inviable
spt5-242	Suppression of Cs^- and Spt^-
spt6-14	Inviable
spt6-1004	Inviable
spt6-1006	Inviable
spt6-140	Sick
spt16-197	Synthetic Spt^-
*dst1*Δ	Ts^- on Gal, MPA^S
*paf1*Δ	Inviable
*cdc73*Δ	Inviable
*ctr9*Δ	Inviable
*leo1*Δ	Inviable
*rtf1*Δ	Synthetic Spt^- and Ts^-

TABLE 3. SGA analysis of *elf1*Δ

Gene deletion interacting with *elf1*Δ by SGA	Synthetic phenotype with *elf1*Δ by tetrad analysis
*cdc73*Δ	Inviable
*leo1*Δ	Inviable
*rtf1*Δ	Viable, Spt^-
*hir1*Δ	Viable, Spt^-
*hir2*Δ	Viable, Spt^-
*hir3*Δ	Viable, Spt^-
*hpc2*Δ	Viable, Spt^-
*rpb4*Δ	Inviable
*bur2*Δ	ND[a]
*yml009w-B*Δ	ND

[a] ND, not determined.

increased sickness and sensitivity to MPA when combined with an *elf1*Δ mutation (data not shown). Thus, $6AU^S$ mutations in the genes encoding subunits of the Pol II complex can lead to an increased requirement for the Elf1 elongation factor.

Another class of mutations identified by the *elf1*Δ SGA analysis included the *HIR*/*HPC* genes. These four genes, *HIR1*, *HIR2*, *HIR3*/*HPC1*, and *HPC2*, were initially identified due to their roles in the regulation of histone gene transcription (49, 81). More recently, the *HIR* genes have been implicated in transcription elongation due to synthetic lethal interactions with mutations in *spt16* and genes encoding members of the Paf1 complex (16). Although SGA analysis suggested that *elf1*Δ is synthetically lethal with mutations in the *HIR*/*HPC* genes, tetrad analysis demonstrated that the double mutants between *elf1*Δ and deletions in the *HIR* genes were all viable and healthy (Table 3). However, when we tested the *elf1*Δ *hir*/*hpc* double mutants, we found several synthetic phenotypes, including Ts^- and Spt^- phenotypes. Thus, there are genetic interactions present between *elf1*Δ and mutations in the *HIR* genes, although not lethality as implied by SGA analysis. Taken together, our results from SGA analysis have identified only genetic interactions between *elf1*Δ and mutations in genes implicated as playing a role in transcription elongation, further highlighting an important and specific role for Elf1 in transcription elongation.

Elf1 is localized to regions of active transcription. The genetic interactions of *elf1*Δ suggested that Elf1 might play a direct role in transcription elongation. To test if Elf1 is associated with transcribed regions in vivo, we performed ChIP experiments using a functional, 13xMyc-tagged version of Elf1 (see Materials and Methods). In these experiments, we tested the physical association of Elf1 with *PMA1* and *GAL1*, two genes commonly used for such studies (25, 27, 28). *PMA1* is a highly transcribed gene, and *GAL1* is regulated by carbon source, allowing us to test whether Elf1 ChIP is dependent upon ongoing transcription. Our results show that Elf1 is localized at high levels over the coding region of *PMA1* and of *GAL1* when it is induced (Fig. 4A and B). Furthermore, Elf1 is present at reduced levels over the TATA regions and at low levels over the UAS regions of these genes (Fig. 4A and B). Comparison of the distribution of Elf1 to that of Pol II shows that the level of Elf1 association is lower than Pol II over the promoter region compared to the coding regions, suggesting that Elf1 is recruited to genes subsequent to Pol II recruitment (Fig. 4A and B). This pattern is similar to that observed for Spt16 (41) and Spt6 (25, 27). The localization of Elf1 to the *GAL1* coding region is dependent on transcription, as we only observed the association of Elf1 with *GAL1* upon the induction of transcription with galactose (Fig. 4B). Additionally, Elf1 appeared to be present at similar levels to Pol II over the 3′ UTR of both *GAL1* and *PMA1*, similar to what has previously been observed for the elongation factors Spt4, Spt5, Spt6, and Spt16 (25, 27) and in contrast to other elongation factors, including the Paf1 and the TREX complexes, that show low levels of association over the 3′ UTR (25, 27). Taken together, these results suggest that Elf1 is preferentially localized to regions of active transcription.

Elf1 localization is partially dependent on Spt4 and Spt6. To test if the association of Elf1 with transcribed regions is dependent upon other elongation factors, we analyzed the recruitment of Elf1 in several elongation mutants. We found that recruitment of Elf1 to the coding region of *PMA1* was decreased in *spt4*Δ and *spt6-1004* mutants, while there were no strong effects on Elf1 localization in *rtf1*Δ, *paf1*Δ, or *spt16-197* mutants (Fig. 5A). Although Elf1 binding is decreased slightly in the *paf1*Δ mutant, this is likely due to the decreased levels of Pol II binding seen in this mutant (Fig. 5A). These data suggest that Elf1 association with transcriptionally active regions is partially dependent on Spt4 and Spt6 and independent of the Paf1 complex and Spt16.

To examine whether Elf1 is necessary for the association of other elongation factors with transcribed regions, we tested the recruitment of Spt4 and the Ctr9 subunit of the Paf1 complex to *PMA1* in an *elf1*Δ mutant. In an *elf1*Δ mutant, the recruitment of Spt4 and Ctr9 was slightly reduced at *PMA1*, corresponding to a similar small reduction in the level of Pol II associated at *PMA1*. There was no effect on the overall distribution of Pol II in the *elf1*Δ mutant, as has been noted for other elongation factors (42, 43). These results strongly suggest that Spt4 and Ctr9 are recruited to transcribed regions independently of Elf1.

Evidence that Elf1 affects chromatin structure in actively transcribed regions. The genetic interactions of *elf1*Δ with elongation factors and its localization to actively transcribed regions are consistent with a role for Elf1 in transcription elongation. Based on the synthetic lethality observed between

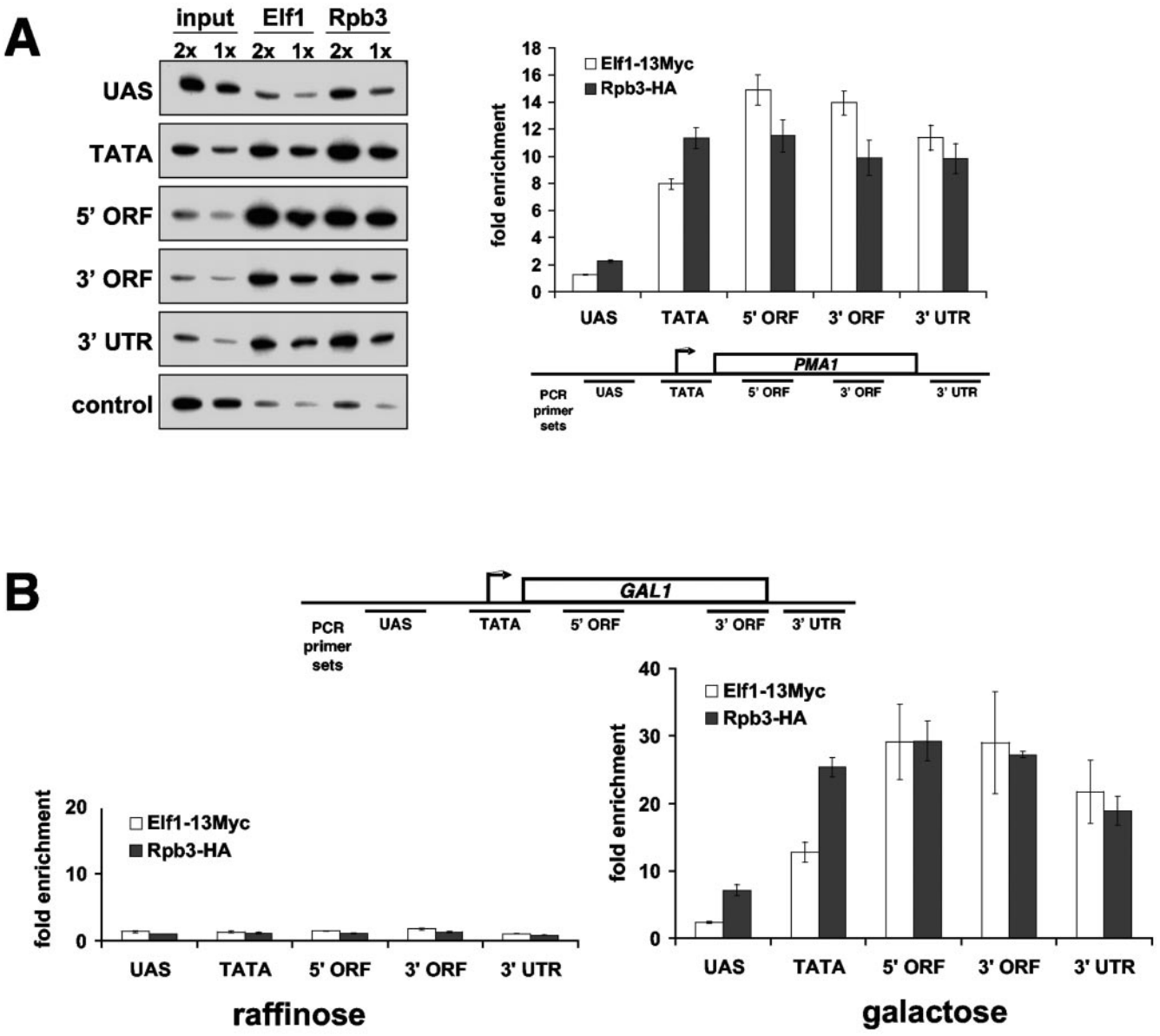

FIG. 4. Elf1 is preferentially localized to transcribed regions of active genes. (A) Elf1 is localized to the transcribed region of *PMA1*. Yeast cells containing the Elf1-13Myc and Rpb3-HA epitope-tagged proteins were grown at 30°C to mid-log in YPD medium. Cells were cross-linked with 1% formaldehyde and lysed, and the chromatin was sheared by sonication to an average size of 400 bp. Elf1-13Myc was immunoprecipitated using the A14 anti-Myc antibody, and Rpb3-HA was immunoprecipitated using the 12CA5 anti-HA antibody. Radiolabeled PCR products were obtained with primers specific to the *PMA1* UAS, TATA, 5′ ORF, 3′ ORF, or 3′ UTRs and were visualized on 6% polyacrylamide gels. As a control for DNA binding specificity, each PCR contained primers to amplify a nontranscribed region of chromosome V that is not contained in any open reading frames (28). ChIP values are represented as the fold enrichment of the specific PCR product compared to this control region. The locations of primer sets used for ChIP analysis are represented as lines below the schematic of the *PMA1* gene. Results were obtained for at least three independent experiments, and the average and standard error are plotted on the graph. (B) Elf1 is localized across the *GAL1* transcribed region upon transcriptional induction. Yeast cells containing Elf1-13Myc and Rpb3-HA epitope-tagged proteins were grown at 30°C to mid-log in medium containing 2% raffinose. Transcription of *GAL1* was induced by the addition of 2% galactose for 2 hours. Chromatin immunoprecipitations were performed as described for panel A in at least three independent experiments, with the resulting averages and standard errors plotted on the graph.

*elf1*Δ and mutations in *SPT4* and *SPT6* and the fact that the localization of Elf1 is partially dependent on Spt4 and Spt6, we hypothesized that Elf1 is performing a role related to these proteins in vivo. Recent studies have suggested a role for Spt6 in controlling chromatin structure over transcribed sequences, as *spt6* mutations were found to cause defects in chromatin structure in actively transcribed regions. These defects allow transcription initiation from cryptic promoters within some coding regions (26).

To determine if Elf1 plays related roles, we employed a phenotypic assay based on the observation that *spt6* mutations cause transcription initiation to start internally within the 3′ coding region of the *FLO8* gene (26). We made use of a reporter construct in which the 3′ end of *FLO8* has been

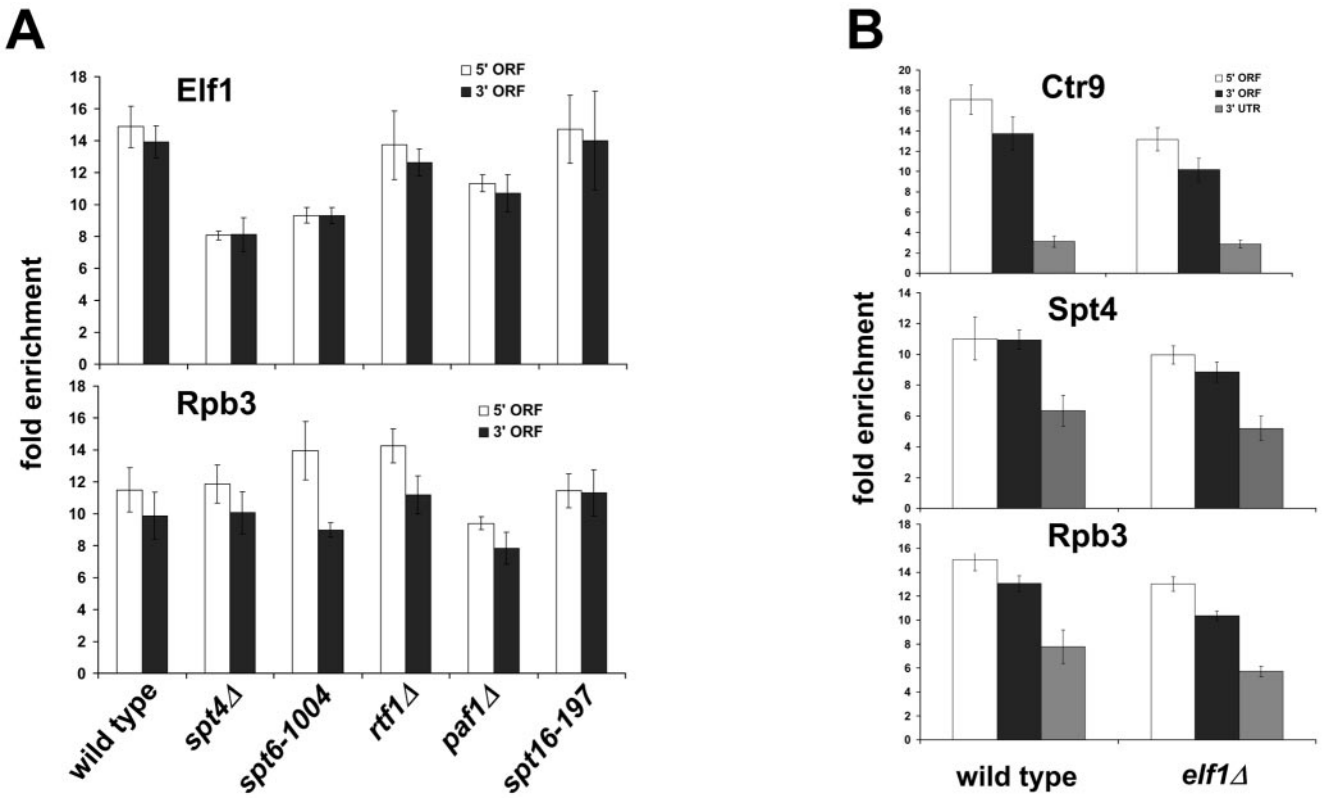

FIG. 5. Elf1 localization to *PMA1* is partially dependent on the Spt4 and Spt6 elongation factors. (A) Elf1 localization at *PMA1* in several elongation mutants. ChIP experiments were performed as described for Fig. 4A for wild-type, *spt4*Δ, *rtf1*Δ, *paf1*Δ, *spt6-1004*, or *spt16-197* strains at 30°C. ChIP results for Elf1-13Myc (top panel) and Rpb3-HA (bottom panel) are shown. The results from at least three independent experiments are shown. (B) Localization of Spt4 and Ctr9 to *PMA1* is not affected in an *elf1*Δ mutant. Wild-type or *elf1*Δ strains containing Spt4-Flag, Ctr9-Myc, and Rpb3-HA were subjected to ChIP analysis as described for Fig. 4A. Ctr9-Myc was immunoprecipitated using the A14 anti-Myc antibody, and Spt4-Flag was immunoprecipitated using the M2 anti-Flag antibody. The results from at least three independent experiments are shown.

replaced with the *HIS3* gene (*GAL1-FLO8-HIS3*) (see Materials and Methods). In this construct, *HIS3* can be expressed only upon transcription initiation from the *FLO8* internal start site. When we analyzed an *elf1*Δ strain for internal initiation, by testing for growth on medium lacking histidine, we found that it was able to grow when *GAL1-FLO8-HIS3* transcription levels are high (growth on galactose) but not when they are low (growth on glucose) (Fig. 6A). This result suggests that cells lacking *elf1*Δ are defective in maintaining proper chromatin structure at the *GAL1-FLO8-HIS3* gene, thus allowing transcription to occur from a normally cryptic promoter within the coding region.

To look directly at this transcriptional effect, we also performed Northern analysis to examine the wild-type *FLO8* gene for cryptic initiation in an *elf1*Δ mutant. In this case, the production of short transcripts was not detectable in the *elf1*Δ mutant (Fig. 6C), presumably due to the lower levels of transcription driven by the *FLO8* promoter compared to the *GAL1* promoter used for *GAL1-FLO8-HIS3*, and to the more modest phenotypes caused by *elf1*Δ compared to *spt6* mutations. We also examined two double mutants for effects at *FLO8*, including *elf1*Δ *rtf1*Δ and *elf1*Δ *hir1*Δ. As described earlier, these double mutants possess synthetic Spt^- and synthetic Ts^- phenotypes when compared to the single mutants (Fig. 6B and data not shown). Analysis of the *elf1*Δ *hir1*Δ double mutant revealed the production of the *FLO8* short transcript when shifted to the nonpermissive temperature of 39°C (Fig. 6C). The size of this short transcript is similar to that observed in the *spt6-1004* mutant (data not shown). Although the *elf1*Δ *rtf1*Δ mutant also fails to grow at elevated temperatures, it does not produce *FLO8* short transcripts when shifted to the nonpermissive temperature. This difference between the *elf1*Δ *hir1*Δ and the *elf1*Δ *rtf1*Δ double mutants suggest that these factors may facilitate elongation through distinct mechanisms. Together, these results strongly suggest that the role of Elf1 in transcription elongation involves effects on chromatin structure.

Elf1 associates with casein kinase II. In order to further characterize *S. cerevisiae* Elf1, we purified it using a C-terminal TAP tag (55) containing a calmodulin-binding peptide and *Staphylococcus aureus* protein A, separated by a TEV protease cleavage site. The tagged protein was then purified on IgG and calmodulin columns and analyzed by SDS-PAGE followed by staining with silver. Protein bands absent from a control preparation and corresponding to the tagged protein and any associated proteins were then identified by MALDI-TOF mass spectrometry (Fig. 7). Elf1-TAP copurified substoichiometrically with subunits of casein kinase II (CKII). We were able to detect the two largest components of CKII (Cka1 and Ckb1) using MALDI-TOF mass spectrometry on trypsin-digested sil-

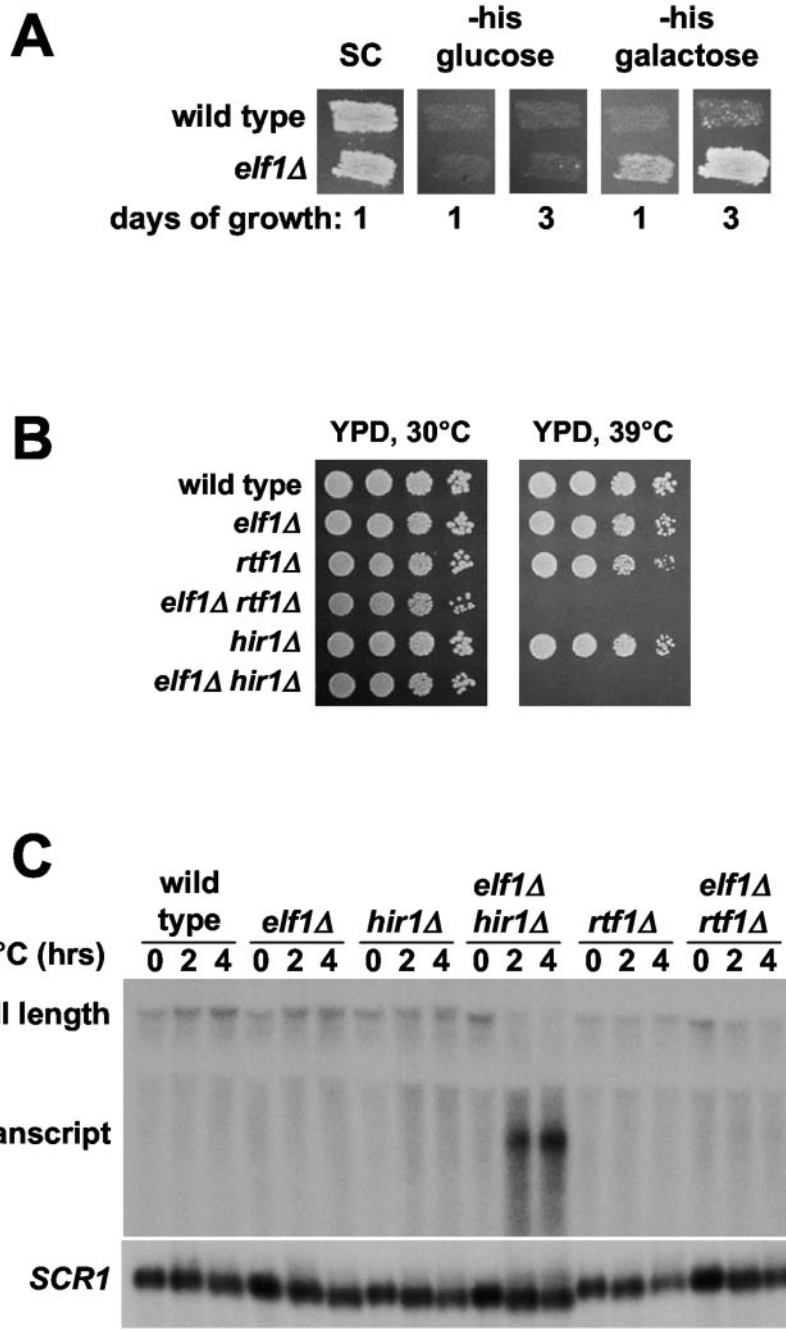

FIG. 6. Elf1 is necessary for maintaining proper chromatin structure in actively transcribed regions. (A) *elf1*Δ mutations allow use of a cryptic TATA element within the *GAL1-FLO8-HIS3* reporter. Wild-type and *elf1*Δ strains containing the *GAL1-FLO8-HIS3* reporter construct were patched onto YPD medium. Strains were then replica plated to synthetic complete medium, medium lacking histidine and containing glucose, and medium lacking histidine and containing galactose as the carbon source. Strains were scored for growth at 30°C for either 1 or 3 days. (B) Mutations in *elf1*Δ are Ts^- in combination with *rtf1*Δ or *hir1*Δ mutations. *elf1*Δ strains were crossed to *rtf1*Δ and *hir1*Δ mutants. Diploids were sporulated and dissected, and representative spores from the resulting complete tetrads were spotted onto YPD medium at 30°C or 39°C for 2 days. (C) *elf1*Δ *hir1*Δ mutants allow transcription to initiate from within the endogenous *FLO8* coding region. Wild-type, *elf1*Δ, *hir1*Δ, *elf11*Δ *hir1*Δ, *rtf1*Δ, and *elf1*Δ *rtf1*Δ strains were grown in YPD to mid-log at 30°C. Strains were shifted to 39°C by addition of an equal volume of YPD medium prewarmed to 48°C. Strains were then incubated at 39°C, and cells were collected at 2 and 4 h after the temperature shift. Northern analysis was performed using probes to the 3′ region of *FLO8* (+1515 to +2326) and also to the *SCR1* loading control.

ver-stained gel bands, but not its two smaller subunits (Cka2 and Ckb2), which coelectrophoresed with the much more abundant Elf1 polypeptide. Cka2 and Ckb2 were, however, detected by trypsin digestion and liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis of the entire purified protein preparation. Consistent with this association, Elf1 contains seven potential CKII sites in its carboxy-terminal end at positions S86, S95, S101, T109, S117, S124, and S142.

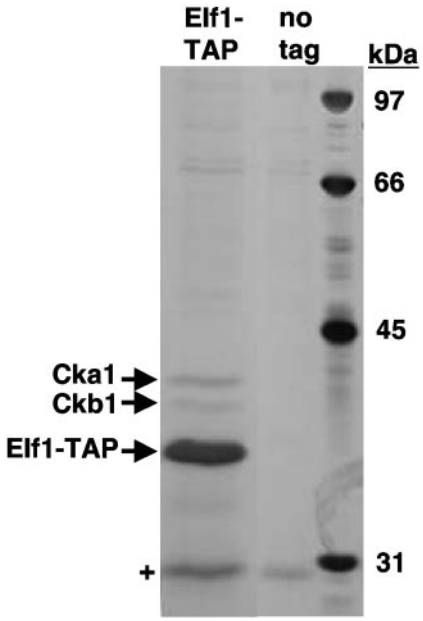

FIG. 7. Purification of Elf1. Purification of Elf1 was carried out with strains containing either an untagged protein or a TAP-tagged version of Elf1. The protein complex was purified and was then analyzed by SDS-PAGE and silver staining. Elf1 and the two largest subunits of CKII (Cka1 and Ckb1) were identified by trypsin digestion and MALDI-TOF. In the mass spectrum, there were 6 (35% coverage), 16 (43% coverage), and 10 (39% coverage) peptides used to identify Elf1, Cka1, and Ckb1, respectively. The two smaller subunits of CKII, which comigrate with Elf1-TAP, were identified (along with Cka1, Ckb1, and Elf1) by using gel-free LC-MS/MS. The + indicates the position of TEV protease. Other detectable bands are present in both lanes, including those at around 70 kDa that are likely the heat shock proteins Ssa1 and Ssb1, present in most purifications (N. Krogan and J. Greenblatt, unpublished results).

DISCUSSION

In this work, we provide several lines of evidence that the previously uncharacterized gene, YKL160W/*ELF1*, plays a role in transcription elongation. First, *elf1*Δ genetically interacts with mutations in many genes encoding known elongation factors. Both by direct crosses and by SGA analysis, the interaction of *elf1*Δ with elongation factor mutants is highly specific, as synthetic interactions were only found between *elf1*Δ and genes believed to be involved in transcription elongation. Second, *elf1*Δ mutants are sensitive to 6AU, a phenotype possessed by mutations in many elongation factors. Third, Elf1 is physically associated with actively transcribed genes and it is preferentially localized to coding regions. This localization to coding regions was determined to be partially dependent on the transcription elongation factors Spt4 and Spt6. Our results also suggest that Elf1 plays a role in maintaining proper chromatin structure in regions of active transcription. Finally, Elf1 copurifies with CKII, a kinase previously implicated in roles in transcription elongation (5, 31, 61). Together, these results provide strong evidence of a significant role for Elf1 in transcription elongation.

The conservation of Elf1 throughout eukaryotes also suggests an important role for this protein in transcription. All Elf1 homologues identified so far are quite small and contain a conserved C4 putative zinc finger domain. The C4 zinc fingers are the most abundant class of zinc-binding domains and are found in a number of proteins associated with the transcriptional machinery, including Spt4, TFIIS, and Rpb9 (29). In the only other case where a role for an Elf1 homologue has been examined, *C. elegans*, no phenotype was detected after treatment with *ELF1*-specific RNA inhibition (24). This result is similar to our finding in *S. cerevisiae*, where an *elf1*Δ mutation alone causes no detectable growth defect and only modest phenotypes under standard laboratory growth conditions. More detailed analysis in other organisms will be required to assess the general role of Elf1 in transcription.

Our analysis of *ELF1* reveals a number of interesting similarities with *SPT4*. First, the proteins encoded by these genes are both small and contain a C4 zinc finger domain. Second, both *elf1*Δ and *spt4*Δ mutations also cause Spt^- and $6AU^S$ phenotypes, although the *elf1*Δ phenotypes are considerably more modest than those caused by *spt4*Δ. Third, although both *elf1*Δ and *spt4*Δ mutations cause no detectable growth defects on their own, both mutations cause inviability when combined with a similar set of mutations in other genes encoding elongation factors. For example, both *elf1*Δ and *spt4*Δ are lethal in combination with mutations in the genes encoding several of the Paf1 complex members (our results and reference 70), as well as in combination with mutations in *SPT5* and *SPT6* (our results and reference 73). Finally, ChIP analysis of Elf1 reveals similarities to Spt4 as well as to Spt5, Spt6, and Spt16, all of which are present at levels comparable to that of Pol II across genes and into the 3′ UTR of genes (25, 27). This localization contrasts with that of the Paf1 and TREX complexes, which show decreased binding past the polyadenylation site (25, 27). The significance of these observations and the effects of these proteins on termination are unclear, but they further highlight the similarities between Spt4 and Elf1.

The physical association of Elf1 and CKII suggests that modification by phosphorylation may play a role in Elf1 function. Consistent with this, Elf1 has been shown to be phosphorylated in vitro in the presence of [^{32}P]ATP and yeast extract (M. Constanzo and B. Andrews, personal communication). Aside from the physical association of Elf1 and CKII, there is no evidence for a stable association of Elf1 with additional proteins. While many elongation factors are present in stable complexes, Elf1 was not identified as a protein partner in global TAP purifications (18, 31). In our attempts to identify Elf1-interacting proteins, we purified Elf1 using the TAP tag under many different salt conditions (100 to 350 mM NaCl, 200 to 500 mM KO-acetate, and 250 mM KCl) and did not detect any other proteins that copurify with Elf1 besides CKII (data not shown). Additionally, Elf1 did not coimmunoprecipitate with Pol II or several other elongation factors, including Spt4, Spt5, Spt6, Rtf1, and Chd1 (data not shown). The relevant physical association of Elf1 with other proteins, whether they are stable or transient, may only occur when it is associated with chromatin, and this might be missed by standard purification approaches.

We have yet to determine how Elf1 is recruited to actively transcribed regions. Results from our chromatin immunoprecipitation experiments suggest that Elf1 can be recruited to significant levels independently of other elongation factors, although its recruitment is partially dependent on Spt4 and

Spt6. One possibility is that Elf1 interacts directly with Pol II and that Spt4 and Spt6 help to stabilize this association. Alternatively, Elf1 may associate with elongating Pol II through some elongation factor not tested here. Another possibility still is that Elf1 is recruited by an altered chromatin structure occurring in the wake of transcription. Further studies are needed to understand the associations of Elf1 along actively transcribed chromatin.

Our experiments suggest that Elf1 plays a role in elongation by maintaining proper chromatin structure in regions of active transcription. There are several pieces of evidence supporting this idea. Mutations in *elf1*Δ cause a weak Spt$^-$ phenotype, a phenotype shared by mutations in the genes encoding several other chromatin elongation factors and within the genes encoding the histones themselves (77). We also found that this phenotype was exacerbated in *elf1*Δ mutants when combined with mutations in genes encoding other elongation factors and regulators of histone transcription. The Spt phenotype has been hypothesized for some *spt* mutants to be due to defects in chromatin structure during elongation, allowing differential use of TATA elements (26). This is supported by the observation that in the *elf1*Δ *hir1*Δ mutant with enhanced Ts$^-$ and Spt$^-$ phenotypes, we also see an increased level of short transcripts initiating from a cryptic promoter within the *FLO8* coding region. We hypothesize that the synthetic lethality of *elf1*Δ observed in combination with mutations in *SPT4*, *SPT5*, and *SPT6* is likely due to the inability of these strains to maintain chromatin integrity in actively transcribing regions of the genome. Further analysis of *elf1*Δ mutants alone and in combination with other elongation mutants will provide clues into the function of this conserved elongation factor and new insights regarding the mechanisms of elongation through chromatin in vivo. Given the conservation of Elf1 and its probable role in chromatin structure, Elf1 may also be involved in other chromatin-related processes, such as DNA repair, replication, and recombination.

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REFERENCES

1. **Adelman, K., M. T. Marr, J. Werner, A. Saunders, Z. Ni, E. D. Andrulis, and J. T. Lis.** 2005. Efficient release from promoter-proximal stall sites requires transcript cleavage factor TFIIS. Mol. Cell **17:**103–112.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Arndt, K. M., and C. M. Kane.** 2003. Running with RNA polymerase: eukaryotic transcript elongation. Trends Genet. **19:**543–550.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. E. Struhl.** 1991. Current protocols in molecular biology. Greene Publishing Associates and Wiley-Interscience, New York, N.Y.
5. **Barz, T., K. Ackermann, G. Dubois, R. Eils, and W. Pyerin.** 2003. Genome-wide expression screens indicate a global role for protein kinase CK2 in chromatin remodeling. J. Cell Sci. **116:**1563–1577.
6. **Belotserkovskaya, R., S. Oh, V. A. Bondarenko, G. Orphanides, V. M. Studitsky, and D. Reinberg.** 2003. FACT facilitates transcription-dependent nucleosome alteration. Science **301:**1090–1093.
7. **Bortvin, A., and F. Winston.** 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science **272:**1473–1476.
8. **Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter, and J. D. Boeke.** 1998. Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:**115–132.
9. **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.
10. **Costa, P. J., and K. M. Arndt.** 2000. Synthetic lethal interactions suggest a role for the Saccharomyces cerevisiae Rtf1 protein in transcription elongation. Genetics **156:**535–547.
11. **Endoh, M., W. Zhu, J. Hasegawa, H. Watanabe, D. K. Kim, M. Aida, N. Inukai, T. Narita, T. Yamada, A. Furuya, H. Sato, Y. Yamaguchi, S. S. Mandal, D. Reinberg, T. Wada, and H. Handa.** 2004. Human Spt6 stimulates transcription elongation by RNA polymerase II in vitro. Mol. Cell. Biol. **24:**3324–3336.
12. **Exinger, F., and F. Lacroute.** 1992. 6-Azauracil inhibition of GTP biosynthesis in Saccharomyces cerevisiae. Curr. Genet. **22:**9–11.
13. **Fish, R. N., and C. M. Kane.** 2002. Promoting elongation with transcript cleavage stimulatory factors. Biochim. Biophys. Acta **1577:**287–307.
14. **Formosa, T.** 2003. Changing the DNA landscape: putting a SPN on chromatin. Curr. Top. Microbiol. Immunol. **274:**171–201.
15. **Formosa, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, and D. J. Stillman.** 2001. Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. **20:**3506–3517.
16. **Formosa, T., S. Ruone, M. D. Adams, A. E. Olsen, P. Eriksson, Y. Yu, A. R. Rhoades, P. D. Kaufman, and D. J. Stillman.** 2002. Defects in SPT16 or POB3 (yFACT) in Saccharomyces cerevisiae cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. Genetics **162:**1557–1571.
17. **Gatlin, C. L., G. R. Kleemann, L. G. Hays, A. J. Link, and J. R. Yates III.** 1998. Protein identification at the low femtomole level from silver-stained gels using a new fritless electrospray interface for liquid chromatography-microspray and nanospray mass spectrometry. Anal. Biochem. **263:**93–101.
18. **Gavin, A. C., M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A. M. Michon, C. M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M. A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, and G. Superti-Furga.** 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature **415:**141–147.
19. **Ghaemmaghami, S., W. K. Huh, K. Bower, R. W. Howson, A. Belle, N. Dephoure, E. K. O'Shea, and J. S. Weissman.** 2003. Global analysis of protein expression in yeast. Nature **425:**737–741.
20. **Goldstein, A. L., and J. H. McCusker.** 1999. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast **15:**1541–1553.
21. **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.
22. **Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson, J. S. Weissman, and E. K. O'Shea.** 2003. Global analysis of protein localization in budding yeast. Nature **425:**686–691.
23. **Izban, M. G., and D. S. Luse.** 1992. The RNA polymerase II ternary complex cleaves the nascent transcript in a 3′-5′ direction in the presence of elongation factor SII. Genes Dev. **6:**1342–1356.
24. **Kamath, R. S., A. G. Fraser, Y. Dong, G. Poulin, R. Durbin, M. Gotta, A. Kanapin, N. Le Bot, S. Moreno, M. Sohrmann, D. P. Welchman, P. Zipperlen, and J. Ahringer.** 2003. Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature **421:**231–237.
25. **Kaplan, C. D., M. J. Holland, and F. Winston.** 2005. Interaction between transcription elongation factors and mRNA 3′-end formation at the Saccharomyces cerevisiae GAL10-GAL7 locus. J. Biol. Chem. **280:**913–922.
26. **Kaplan, C. D., L. Laprade, and F. Winston.** 2003. Transcription elongation factors repress transcription initiation from cryptic sites. Science **301:**1096–1099.
27. **Kim, M., S. H. Ahn, N. J. Krogan, J. F. Greenblatt, and S. Buratowski.** 2004. Transitions in RNA polymerase II elongation complexes at the 3′ ends of genes. EMBO J. **23:**354–364.
28. **Komarnitsky, P., E. J. Cho, and S. Buratowski.** 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. **14:**2452–2460.
29. **Krishna, S. S., I. Majumdar, and N. V. Grishin.** 2003. Structural classification of zinc fingers: survey and summary. Nucleic Acids Res. **31:**532–550.
30. **Krogan, N. J., J. Dover, A. Wood, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, O. W. Ryan, A. Golshani, M. Johnston, J. F. Greenblatt, and A.**

Shilatifard. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol. Cell **11:**721–729.
31. **Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kobor, G. Cagney, A. Emili, A. Shilatifard, S. Buratowski, and J. F. Greenblatt.** 2002. RNA polymerase II elongation factors of *Saccharomyces cerevisiae*: a targeted proteomics approach. Mol. Cell. Biol. **22:**6979–6992.
32. **Krogan, N. J., M. Kim, A. Tong, A. Golshani, G. Cagney, V. Canadien, D. P. Richards, B. K. Beattie, A. Emili, C. Boone, A. Shilatifard, S. Buratowski, and J. Greenblatt.** 2003. Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. Mol. Cell. Biol. **23:**4207–4218.
33. **Kumar, A., S. Agarwal, J. A. Heyman, S. Matson, M. Heidtman, S. Piccirillo, L. Umansky, A. Drawid, R. Jansen, Y. Liu, K. H. Cheung, P. Miller, M. Gerstein, G. S. Roeder, and M. Snyder.** 2002. Subcellular localization of the yeast proteome. Genes Dev. **16:**707–719.
34. **Kumar, A., K. H. Cheung, P. Ross-Macdonald, P. S. Coelho, P. Miller, and M. Snyder.** 2000. TRIPLES: a database of gene function in Saccharomyces cerevisiae. Nucleic Acids Res. **28:**81–84.
35. **Larschan, E., and F. Winston.** 2001. The S. cerevisiae SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev. **15:**1946–1956.
36. **Lindstrom, D. L., S. L. Squazzo, N. Muster, T. A. Burckin, K. C. Wachter, C. A. Emigh, J. A. McCleery, J. R. Yates III, and G. A. Hartzog.** 2003. Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. Mol. Cell. Biol. **23:**1368–1378.
37. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast **14:**953–961.
38. **Malagon, F., A. H. Tong, B. K. Shafer, and J. N. Strathern.** 2004. Genetic interactions of DST1 in Saccharomyces cerevisiae suggest a role of TFIIS in the initiation-elongation transition. Genetics **166:**1215–1227.
39. **Mann, M., P. Hojrup, and P. Roepstorff.** 1993. Use of mass spectrometric molecular weight information to identify proteins in sequence databases. Biol. Mass Spectrom. **22:**338–345.
40. **Marchler-Bauer, A., J. B. Anderson, C. DeWeese-Scott, N. D. Fedorova, L. Y. Geer, S. He, D. I. Hurwitz, J. D. Jackson, A. R. Jacobs, C. J. Lanczycki, C. A. Liebert, C. Liu, T. Madej, G. H. Marchler, R. Mazumder, A. N. Nikolskaya, A. R. Panchenko, B. S. Rao, B. A. Shoemaker, V. Simonyan, J. S. Song, P. A. Thiessen, S. Vasudevan, Y. Wang, R. A. Yamashita, J. J. Yin, and S. H. Bryant.** 2003. CDD: a curated Entrez database of conserved domain alignments. Nucleic Acids Res. **31:**383–387.
41. **Mason, P. B., and K. Struhl.** 2003. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol. Cell. Biol. **23:**8323–8333.
42. **Morillon, A., N. Karabetsou, J. O'Sullivan, N. Kent, N. Proudfoot, and J. Mellor.** 2003. Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. Cell **115:**425–435.
43. **Morillon, A., J. O'Sullivan, A. Azad, N. Proudfoot, and J. Mellor.** 2003. Regulation of elongating RNA polymerase II by forkhead transcription factors in yeast. Science **300:**492–495.
44. **Mueller, C. L., and J. A. Jaehning.** 2002. Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Mol. Cell. Biol. **22:**1971–1980.
45. **Mumberg, D., R. Muller, and M. Funk.** 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene **156:**119–122.
46. **Neigeborn, L., J. L. Celenza, and M. Carlson.** 1987. *SSN20* is an essential gene with mutant alleles that suppress defects in *SUC2* transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**672–678.
47. **Ng, H. H., F. Robert, R. A. Young, and K. Struhl.** 2003. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol. Cell **11:**709–719.
48. **Orphanides, G., G. LeRoy, C. H. Chang, D. S. Luse, and D. Reinberg.** 1998. FACT, a factor that facilitates transcript elongation through nucleosomes. Cell **92:**105–116.
49. **Osley, M. A., and D. Lycan.** 1987. *trans*-acting regulatory mutations that alter transcription of *Saccharomyces cerevisiae* histone genes. Mol. Cell. Biol. **7:**4204–4210.
50. **Pal, M., D. McKean, and D. S. Luse.** 2001. Promoter clearance by RNA polymerase II is an extended, multistep process strongly affected by sequence. Mol. Cell. Biol. **21:**5815–5825.
51. **Pokholok, D. K., N. M. Hannett, and R. A. Young.** 2002. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell **9:**799–809.
52. **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.
53. **Prather, D. M., E. Larschan, and F. Winston.** 2005. Evidence that the elongation factor TFIIS plays a role in transcription initiation at GAL1 in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **25:**2650–2659.
54. **Proudfoot, N. J., A. Furger, and M. J. Dye.** 2002. Integrating mRNA processing with transcription. Cell **108:**501–512.
55. **Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann, and B. Seraphin.** 1999. A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol. **17:**1030–1032.
56. **Riley, J., R. Butler, D. Ogilvie, R. Finniear, D. Jenner, S. Powell, R. Anand, J. C. Smith, and A. F. Markham.** 1990. A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. Nucleic Acids Res. **18:**2887–2890.
57. **Rondon, A. G., M. Garcia-Rubio, S. Gonzalez-Barrera, and A. Aguilera.** 2003. Molecular evidence for a positive role of Spt4 in transcription elongation. EMBO J. **22:**612–620.
58. **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, N.Y.
59. **Ross-Macdonald, P., P. S. Coelho, T. Roemer, S. Agarwal, A. Kumar, R. Jansen, K. H. Cheung, A. Sheehan, D. Symoniatis, L. Umansky, M. Heidtman, F. K. Nelson, H. Iwasaki, K. Hager, M. Gerstein, P. Miller, G. S. Roeder, and M. Snyder.** 1999. Large-scale analysis of the yeast genome by transposon tagging and gene disruption. Nature **402:**413–418.
60. **Saunders, A., J. Werner, E. D. Andrulis, T. Nakayama, S. Hirose, D. Reinberg, and J. T. Lis.** 2003. Tracking FACT and the RNA polymerase II elongation complex through chromatin in vivo. Science **301:**1094–1096.
61. **Sawa, C., E. Nedea, N. Krogan, T. Wada, H. Handa, J. Greenblatt, and S. Buratowski.** 2004. Bromodomain factor 1 (Bdf1) is phosphorylated by protein kinase CK2. Mol. Cell. Biol. **24:**4734–4742.
62. **Sekimizu, K., N. Kobayashi, D. Mizuno, and S. Natori.** 1976. Purification of a factor from Ehrlich ascites tumor cells specifically stimulating RNA polymerase II. Biochemistry **15:**5064–5070.
63. **Shevchenko, A., M. Wilm, O. Vorm, and M. Mann.** 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal. Chem. **68:**850–858.
64. **Shi, X., M. Chang, A. J. Wolf, C. H. Chang, A. A. Frazer-Abel, P. A. Wade, Z. F. Burton, and J. A. Jaehning.** 1997. Cdc73p and Paf1p are found in a novel RNA polymerase II-containing complex distinct from the Srbp-containing holoenzyme. Mol. Cell. Biol. **17:**1160–1169.
65. **Shi, X., A. Finkelstein, A. J. Wolf, P. A. Wade, Z. F. Burton, and J. A. Jaehning.** 1996. Paf1p, an RNA polymerase II-associated factor in *Saccharomyces cerevisiae*, may have both positive and negative roles in transcription. Mol. Cell. Biol. **16:**669–676.
66. **Shilatifard, A., R. C. Conaway, and J. W. Conaway.** 2003. The RNA polymerase II elongation complex. Annu. Rev. Biochem. **72:**693–715.
67. **Simchen, G., F. Winston, C. A. Styles, and G. R. Fink.** 1984. Ty-mediated gene expression of the LYS2 and HIS4 genes of Saccharomyces cerevisiae is controlled by the same SPT genes. Proc. Natl. Acad. Sci. USA **81:**2431–2434.
68. **Simic, R., D. L. Lindstrom, H. G. Tran, K. L. Roinick, P. J. Costa, A. D. Johnson, G. A. Hartzog, and K. M. Arndt.** 2003. Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J. **22:**1846–1856.
69. **Sims, R. J., III, R. Belotserkovskaya, and D. Reinberg.** 2004. Elongation by RNA polymerase II: the short and long of it. Genes Dev. **18:**2437–2468.
70. **Squazzo, S. L., P. J. Costa, D. L. Lindstrom, K. E. Kumer, R. Simic, J. L. Jennings, A. J. Link, K. M. Arndt, and G. A. Hartzog.** 2002. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J. **21:**1764–1774.
71. **Svejstrup, J. Q.** 2004. The RNA polymerase II transcription cycle: cycling through chromatin. Biochim. Biophys. Acta **1677:**64–73.
72. **Swanson, M. S., E. A. Malone, and F. Winston.** 1991. *SPT5*, an essential gene important for normal transcription in *Saccharomyces cerevisiae*, encodes an acidic nuclear protein with a carboxy-terminal repeat. Mol. Cell. Biol. **11:**3009–3019.
73. **Swanson, M. S., and F. Winston.** 1992. SPT4, SPT5 and SPT6 interactions: effects on transcription and viability in Saccharomyces cerevisiae. Genetics **132:**325–336.
74. **Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader, N. Page, M. Robinson, S. Raghibizadeh, C. W. Hogue, H. Bussey, B. Andrews, M. Tyers, and C. Boone.** 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:**2364–2368.
75. **Tong, A. H., G. Lesage, G. D. Bader, H. Ding, H. Xu, X. Xin, J. Young, G. F. Berriz, R. L. Brost, M. Chang, Y. Chen, X. Cheng, G. Chua, H. Friesen, D. S. Goldberg, J. Haynes, C. Humphries, G. He, S. Hussein, L. Ke, N. Krogan, Z. Li, J. N. Levinson, H. Lu, P. Menard, C. Munyana, A. B. Parsons, O. Ryan, R. Tonikian, T. Roberts, A. M. Sdicu, J. Shapiro, B. Sheikh, B. Suter, S. L. Wong, L. V. Zhang, H. Zhu, C. G. Burd, S. Munro, C. Sander, J. Rine, J. Greenblatt, M. Peter, A. Bretscher, G. Bell, F. P. Roth, G. W. Brown, B. Andrews, H. Bussey, and C. Boone.** 2004. Global mapping of the yeast genetic interaction network. Science **303:**808–813.
76. **Wada, T., T. Takagi, Y. Yamaguchi, A. Ferdous, T. Imai, S. Hirose, S. Sugimoto, K. Yano, G. A. Hartzog, F. Winston, S. Buratowski, and H. Handa.** 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.

77. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387–391.
78. **Winston, F., C. Dollard, and S. L. Ricupero-Hovasse.** 1995. Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast **11:**53–55.
79. **Woychik, N. A., and R. A. Young.** 1989. RNA polymerase II subunit RPB4 is essential for high- and low-temperature yeast cell growth. Mol. Cell. Biol. **9:**2854–2859.
80. **Wu, C. H., Y. Yamaguchi, L. R. Benjamin, M. Horvat-Gordon, J. Washinsky, E. Enerly, J. Larsson, A. Lambertsson, H. Handa, and D. Gilmour.** 2003. NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in Drosophila. Genes Dev. **17:**1402–1414.
81. **Xu, H., U. J. Kim, T. Schuster, and M. Grunstein.** 1992. Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**5249–5259.
82. **Yamaguchi, Y., T. Narita, N. Inukai, T. Wada, and H. Handa.** 2001. SPT genes: key players in the regulation of transcription, chromatin structure and other cellular processes. J. Biochem. (Tokyo) **129:**185–191.