Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*

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Previous characterization of the *Saccharomyces cerevisiae* Spt4, Spt5, and Spt6 proteins suggested that these proteins act as transcription factors that modify chromatin structure. In this work, we report new genetic and biochemical studies of Spt4, Spt5, and Spt6 that reveal a role for these factors in transcription elongation. We have isolated conditional mutations in *SPT5* that can be suppressed in an allele-specific manner by mutations in the two largest subunits of RNA polymerase II (Pol II). Strikingly, one of these RNA Pol II mutants is defective for transcription elongation and the others cause phenotypes consistent with an elongation defect. In addition, we show that *spt4*, *spt5*, and *spt6* mutants themselves have phenotypes suggesting defects in transcription elongation in vivo. Consistent with these findings, we show that Spt5 is physically associated with RNA Pol II in vivo, and have identified a region of sequence similarity between Spt5 and NusG, an *Escherichia coli* transcription elongation factor that binds directly to RNA polymerase. Finally, we show that Spt4 and Spt5 are tightly associated in a complex that does not contain Spt6. These results, taken together with the biochemical identification of a human Spt4–Spt5 complex as a transcription elongation factor (Wada et al. 1998), provide strong evidence that these factors are important for transcription elongation in vivo.

[*Key Words:* Transcription elongation; Spt4; Spt5; Spt6; NusG; chromatin]

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Transcription elongation is an important aspect of gene regulation in both prokaryotes and eukaryotes. In *Escherichia coli* and bacteriophage λ, the *cis*-acting elements and *trans*-acting factors that regulate transcription elongation have been characterized extensively (Uptain et al. 1997). Whereas elongation is less well understood in eukaryotes, many of the factors that control this process have been identified biochemically and are now the focus of intense interest. For elongation by RNA polymerase II (Pol II), these factors include the general transcription factors, TFIIS, TFIIH, TFIIF, and the elongins (Uptain et al. 1997), transcriptional activators such as heat shock factor (Lis and Wu 1993), and factors that affect chromatin structure, including histones, the nucleosome remodeling complex Snf–Swi (Brown et al. 1996), and the chromatin protein HMG14 (Ding et al. 1994).

Many eukaryotic elongation factors function by either preventing or overcoming transcriptional pausing by RNA Pol II. In eukaryotes, this has been most carefully documented at *Drosophila hsp* genes in which a regulated pause occurs a short distance from the initiation site (Lis and Wu 1993), and in HIV in which the action of the virus-encoded TAT protein is required to overcome an early block to elongation (Jones 1997). Many other eukaryotic genes are known to have blocks to elongation (Uptain et al. 1997), and blocking may represent a general feature of transcription by RNA Pol II (Bentley 1995; Krumm et al. 1995). Given that pause sites can occur throughout a gene, and that nucleosomes provide a repeating barrier to elongation, it seems likely that eukaryotes use specific mechanisms and factors to permit efficient elongation across the length of a gene (Chang and Luse 1997; Uptain et al. 1997). Such factors might be required to alter nucleosomes to permit passage of RNA Pol II, to restore normal nucleosome positioning or structure following passage of RNA Pol II, or both.

Spt4, Spt5, and Spt6 of *Saccharomyces cerevisiae* are conserved proteins, believed to be involved in transcription and chromatin structure. These proteins were initially identified by mutations that suppress *cis*- and *trans*-acting mutations that affect promoter function (Winston and Carlson 1992). Substantial genetic analysis has suggested that all three proteins are involved in a common function in vivo (Swanson and Winston 1992). Recent work on Spt6 has shown that it interacts with

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histones, that it can assemble nucleosomes in vitro, and that *spt6* mutations alter chromatin structure in vivo (Bortvin and Winston 1996). In addition to their role in transcription, genetic studies suggest that these proteins are required for normal recombination (Malagon and Aguilera 1996) and chromosome segregation (Basrai et al. 1996), raising the possibility that they are involved in the establishment or maintenance of chromatin states necessary for diverse chromosomal functions.

Previous studies of *SPT4*, *SPT5*, and *SPT6* involved the analysis of mutant alleles isolated by particular genetic selections (Winston and Carlson 1992). Because *SPT5* and *SPT6* are essential for growth, these studies may have yielded mutations conferring only a subset of the possible mutant phenotypes that could be acquired. In this work, we have isolated new mutations in *SPT5*, requiring only that they cause cold-sensitive growth. Our analysis of these mutants and of extragenic suppressors has led us to evidence that Spt4, Spt5, and Spt6 are required for transcription elongation in vivo. Consistent with these genetic data, we have obtained evidence that Spt5 associates with RNA Pol II. Finally, we show that Spt4 and Spt5 are in a complex that does not contain Spt6. These results, in conjunction with those in the accompanying manuscript (Wada et al. 1998) that show that a human Spt4–Spt5 complex affects transcription elongation in vitro, provide strong evidence that these proteins play an important role in transcription elongation throughout eukaryotes.

Results

Identification of cold-sensitive spt5 *mutants*

Previous genetic analysis of *SPT5* relied on *spt5* mutations that were selected either as suppressors of insertion mutations or as suppressors of *snf–swi* mutations (Winston and Carlson 1992). Because *SPT5* is essential for growth, such mutations might represent a particular class of *spt5* alleles and, hence, only cause a subset of possible *spt5* mutant phenotypes. Therefore, we screened for a new class of *spt5* mutations—those that cause cold-sensitive (Cs^-) growth. Two new *spt5* mutations that cause a Cs^- phenotype, *spt5-242* and *spt5-276*, were isolated. Both mutations also cause the phenotype observed for the original *spt5* mutations, suppression of Ty δ insertion mutations (Spt^- phenotype; Table 1). As shown in subsequent sections, however, these new *spt5* alleles have revealed a previously unknown role for Spt5. In both *spt5* Cs^- mutants, the level of Spt5 protein is unaffected, even when the strains are grown at nonpermissive temperature for 48 hr (data not shown).

To look directly at the effects of the *spt5* Cs^- mutations on transcription, we examined the steady-state levels of three different mRNAs by Northern hybridization analysis. The results show that the *spt5* mutations cause a significantly reduced level of *TPI1* and *HIS4* mRNAs when cells are shifted to the nonpermissive temperature (13°C) for 48 hr (Fig. 1). Under the conditions of this experiment, there was no loss of viability for the *spt5* mutants (data not shown). These results show that the *spt5* Cs^- mutations cause decreased levels of some, but not all, RNA Pol II-dependent transcripts.

Extragenic suppressors in RPB1 *suggest that the* spt5 *Cs⁻ mutations impair transcription elongation*

To identify proteins that might interact with Spt5, we selected for suppressors of the two *spt5* Cs^- mutants.

Table 1. *Phenotypes of* spt5 *Cs⁻ and* rpb1/rpo21 *mutants*

	Relevant genotype	Growth[a] 15°C	30°C	37°C	SC − His[b]	Other phenotypes
1	wild type	+	+	+	−	
2	*spt5-242*	−	+/−	+	+/−	
3	*spt5-276*	−	+/−	+	+/−	
4	*rpb1-221*	+	+	+	−/+	$6AU^S$
5	*rpb1-244*	+	+	+	−/+	$6AU^S$
6	*rpo21-18*	N.D.	+	+	−	$6AU^S$
7	*rpb1-5*	+[c]	+	−	N.D.	
8	*rpb1-244 spt5*Cs^-[d]	+/−	+/−	+/−	+/−	$6AU^S$
9	*rpb1-221 spt5* Cs^-[d]	+/−	+/−	+/−	+/−	$6AU^S$
10	*rpo21-18 spt5-242*	−	−/+	+/−	N.D.	$6AU^S$
11	*rpb1-5 spt5-276*	−	+/−	−	N.D.	

[a]All phenotypes were scored by replica plating patches of cells to YPD plates and incubating at the indicated temperature. For growth at 15°C, + indicates confluent growth after 3 days, +/− indicates slightly less growth than +, and − indicates little or no growth after 5 days. For growth at 30°C and 37°C, + indicates confluent growth after 1 day, +/− indicates that the strain grew nearly as well as wild type, −/+ indicates slow but definite growth after 3 days, and − indicates little or no growth after 3 days. (N.D.) Not done.

[b]SC-His tests the Spt phenotype. Spt^+ strains are His^-, and Spt^- strains are His^+ (Winston et al. 1984). Growth on SC-His was scored at 30°C. +/− indicates confluent growth after 2 days, −/+ indicates slow but definite growth after 3 days, and − indicates little or no growth after 3 days.

[c]These data were reported in Scafe et al. (1990a).

[d]The spt5 Cs^- mutations suppressed the *rpb1*^{sup} mutations indistinguishably. Therefore, lines 7 and 8 each refer to double mutant combinations of the *rpb1* allele with both *spt5-242* and *spt5-276*.

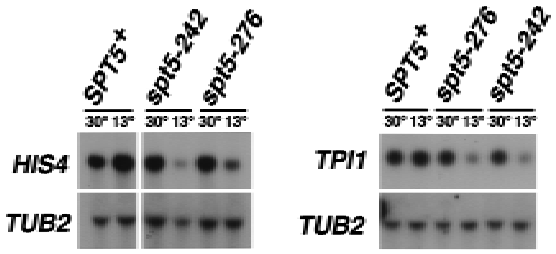

Figure 1. Northen blot analysis of *SPT5*$^+$ and *spt5* Cs$^-$ strains. *SPT5*$^+$, *spt5-242,* and *spt5-276* strains were grown at 30°C or 13°C for 48 hr prior to isolation of RNA for Northern analysis. The blots were probed with *HIS4* or *TPI1* as indicated, and also with *TUB2* as a normalization control. Note that the order of the two mutants is different in the two experiments shown. The blot on the *left* contains RNA from strains FY2, FY1672, and FY1673. The blot on the *right* contains RNA from strains FY120, FY1634, and FY1635.

Interestingly, two of the suppressor mutations are in *RPB1/RPO21,* which encodes the largest subunit of RNA Pol II (see Materials and Methods; hereafter, we will refer to this gene and its mutant forms as *RPB1,* except for mutant alleles published previously as *RPO21*). These suppressor mutations have been designated *rpb1-221* and *rpb1-244* and will be referred to as *rpb1*^{sup} mutations. In addition to suppressing the Cs$^-$ phenotype of the *spt5* mutants, the *rpb1*^{sup} mutations cause two other mutant phenotypes: sensitivity to 6-azauracil (6AU^s), and a weak Spt$^-$ phenotype in an *SPT5*$^+$ genetic background (Table 1).

Substantial evidence suggests that a 6AU^s phenotype indicates a defect in transcription elongation (Uptain et al. 1997). 6AU is a drug that reduces UTP and GTP levels in vivo (Exinger and Lacroute 1992), and decreased nucleotide levels have been shown to increase pausing and arrest by RNA Pol II in vitro (Uptain et al. 1997). In addition, particular mutations in RNA Pol II subunits and in the transcription elongation factor TFIIS confer a 6AU^s phenotype in yeast (Archambault et al. 1992; Exinger and Lacroute 1992; Powell and Reines 1996). Thus, the 6AU^s phenotype caused by the *rpb1*^{sup} mutations suggests that they impair transcription elongation.

The sequence of the *rpb1*^{sup} mutations was determined. Each mutant gene contains a single base pair change; *rpb1-221* causes the amino acid change H1367D, and *rpb1-244* causes the amino acid change E1351K. Both of these amino acid changes occur between regions G and H of Rpb1, two of the eight blocks of sequence homology conserved among the largest subunits of eukaryotic and prokaryotic DNA-directed RNA polymerases (Archambault and Friesen 1993). Interestingly, previous studies have identified other mutations in the same region of *RPB1,* and some of these mutations also cause a 6AU^s and/or an Spt$^-$ phenotype (Archambault and Friesen 1993; Berroteran et al. 1994). Two of these 6AU^s *rpb1* mutations, *rpo21-18* and *rpo21-24,* cause RNA Pol II to have a decreased affinity for the transcription elongation factor TFIIS (Wu et al. 1996). Thus, based on both their phenotypes and position, the *rpb1*^{sup} mutations may cause a defect in transcription elongation.

The *rpb1*^{sup} mutations might represent a particular class of *rpb1* mutation. If so, we would expect the suppression of *spt5* Cs$^-$ mutations by *rpb1* mutations to be allele specific. To test this possibility, we analyzed double mutants between the *spt5* Cs$^-$ mutations and two other *rpb1* mutations. The results (Table 1) show that suppression of *spt5* mutations by *rpb1* mutations is indeed allele specific. First, we tested the *rpb1* mutation *rpo21-18,* which, as mentioned, causes a decreased affinity between RNA Pol II and TFIIS, and a 6AU^s phenotype. The *rpo21-18* mutation behaved in the opposite fashion from the *rpb1*^{sup} mutations: The *rpo21-18 spt5-242* double mutant was extremely sick even at 30°C (Table 1). At 37°C, the temperature at which the *spt5-242* mutant grows almost as well as wild type, the double mutant was viable. We also tested *rpb1-5,* which causes an amino acid change, R335C, distant from the GH region of Rpb1 (Scafe et al. 1990a). This mutation also does not suppress the *spt5* Cs$^-$ mutations (Table 1). This allele specificity indicates that Spt5 interacts directly with RNA Pol II and/or that only a particular type of defect in RNA Pol II will suppress the *spt5* Cs$^-$ phenotype. Both of these possibilities are supported by results described in later sections.

As a second way to test whether the *rpb1*^{sup} mutations cause a distinct type of transcription defect, we analyzed them in double mutants with a deletion of the gene encoding the elongation factor TFIIS. During elongation, after each nucleotide is added to the nascent mRNA, RNA Pol II can either add another nucleotide, pause in an elongation-competent state, or arrest in an elongation-incompetent state (Fig. 2; Uptain et al. 1997). TFIIS acts on arrested RNA Pol II, stimulating an intrinsic nuclease activity of RNA Pol II to digest a few nucleotides from the 3′ end of the nascent mRNA (Uptain et al. 1997). This action resets RNA Pol II into an elongation-competent state, allowing it to make another effort to elongate through the site where it had arrested previously. In *Saccharomyces cerevisiae,* TFIIS is encoded by the *PPR2* gene. In an otherwise wild-type background, deletion of *PPR2* causes no detectable defect except to cause a 6AU^s phenotype (Table 2; Exinger and Lacroute 1992; Uptain et al. 1997). The lack of a strong *ppr2Δ* phenotype is presumably because either TFIIS is partially redundant with another factor, or arrest is not a

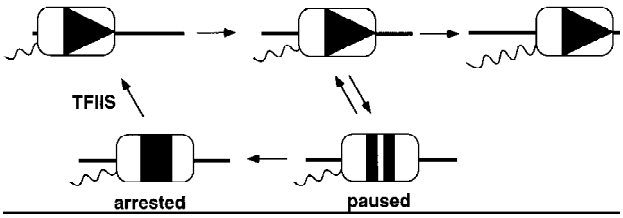

Figure 2. Model of the RNA Pol II transcription elongation cycle (for review, see Uptain et al. 1997).

Table 2. *Phenotypes of* ppr2Δ rpb1 *double mutants*

Relevant genotype	Growth (°C)[a] 15	30	37
1 none	+	+	+
2 *ppr2Δ*	+	+	+
3 *rpb1-244 ppr2Δ*	N.D.	−	N.D.
4 *rpb1-221 ppr2Δ*	−	−/+	−
5 *spt5-242 ppr2Δ*	−	−	+/−
6 *spt5-276 ppr2Δ*	−	−	+/−

[a]Growth is scored as indicated in Table 1. Strains in lines 2-6 are $6AU^S$. (N.D.) Not done.

very frequent event during transcription elongation in an otherwise wild-type strain. Previous work has shown that *rpo21-18 ppr2Δ* mutants are viable, consistent with the fact that *rpo21-18* impairs the RNA Pol II–TFIIS interaction (Archambault et al. 1992; Wu et al. 1996). In contrast, $rpb1^{sup}$ *ppr2Δ* double mutants are either extremely sick or inviable (Table 2). The striking difference in behavior between the $rpb1^{sup}$ mutations and *rpo21-18* strongly supports the idea that these mutations cause two different types of defects in elongation, in turn suggesting that the $rpb1^{sup}$ mutations do not impair RNA Pol II–TFIIS interactions, whereas the *rpo21-18* mutation does.

Possible roles for Spt5 in promoting transcription elongation

The suppression of the *spt5* Cs^- mutations by *rpb1* mutations that are likely to cause a transcription elongation defect suggests that Spt5 may play a role in transcription elongation. Therefore, the *spt5* Cs^- mutations may cause a transcription elongation defect. Such a defect could affect elongation in any of several different ways. We hypothesized that the role of Spt5 might be to promote transcription past potential barriers to elongation. By this model, when RNA Pol II encounters a barrier to elongation, it is much more likely to pause or arrest in an *spt5* Cs^- mutant, where the essential Spt5 activity is reduced, than it would be in an $SPT5^+$ strain. This leads to two predictions that can be tested genetically. First, if RNA Pol II arrests at a greater frequency in an *spt5* Cs^- mutant, the cell's dependence on TFIIS should be greater. Second, if Spt5 activity is reduced, then reducing the rate of elongation by RNA Pol II might provide adequate time for a partially disabled Spt5 to help RNA Pol II transcribe past the barrier, thereby suppressing the *spt5* mutant defect. The next two sections present the results of these genetic tests.

Genetic interactions of Spt5 with the elongation factor TFIIS

To test whether the requirement for TFIIS is increased in an *spt5* Cs^- mutant, we performed double-mutant analysis. As mentioned above, deletion of *PPR2*, which encodes TFIIS, causes no detectable defect in cell growth except to cause a $6AU^s$ phenotype. When combined with either of the *spt5* Cs^- mutations, however, *ppr2Δ* causes a severe growth defect. Both *spt5-242 ppr2Δ* and *spt5-276 ppr2Δ* double mutants are inviable at both 15°C and 30°C (Fig. 3; Table 2). At 37°C, the double mutants are viable. These results resemble those obtained previously for the *spt5-242 rpo21-18* double mutant (Table 1). Thus, TFIIS becomes an essential factor when Spt5 function is limiting, strongly suggesting that the *spt5* Cs^- mutations cause RNA Pol II to arrest more frequently.

The spt5 *Cs⁻ mutations are suppressed by a decreased rate of transcription elongation*

The second genetic test was to determine whether a decreased rate of transcription elongation would compensate for the impaired Spt5 function in the *spt5* Cs^- mutants. This test was performed in two ways. First, we took advantage of a mutation in *RPB2*, the gene encoding the second largest subunit of RNA Pol II. This mutation, *rpb2-10*, causes RNA Pol II to have a decreased elongation rate in vitro and causes a $6AU^s$ phenotype in vivo (Powell and Reines 1996). Consistent with our model, in an *spt5-242 rpb2-10* double mutant, *rpb2-10* suppressed the Cs^- phenotype of the *spt5* mutation (Fig. 4A). As a control, we also tested another *rpb2* mutation, *rpb2-7*, which, although it also causes a $6AU^s$ phenotype, does not affect the RNA Pol II elongation rate in vitro (Powell and Reines 1996). This mutation did not suppress *spt5-242* (Fig. 4A). Second, we treated cells with a low level of 6AU to decrease the concentration of nucleotides in vivo, thereby presumably decreasing the rate of transcription elongation. This test was possible because the *spt5* Cs^- mutations, unlike the $rpb1^{sup}$ mutations, do not cause a $6AU^s$ phenotype (see Discussion). Our results show that 6AU suppresses the Cs^- phenotype: The *spt5* Cs^- mutants grew as well as $SPT5^+$ cells at 15°C on the 6AU-containing media (Fig. 4B). Thus, slower elongation by RNA Pol II apparently suppresses the Cs^- defect of the *spt5* Cs^- mutants.

Genetic evidence that spt4, spt5, *and* spt6 *mutations all affect transcription elongation*

Previous work has strongly suggested that two other pro-

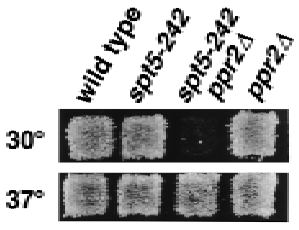

Figure 3. *spt5-242 ppr2Δ* double mutants. The indicated strains derived from a genetic cross of FY1645 × FY1670. Cells were grown at 37°C on a YPD plate and then replica plated to YPD plates that were incubated at 30°C or 37°C for 2 days.

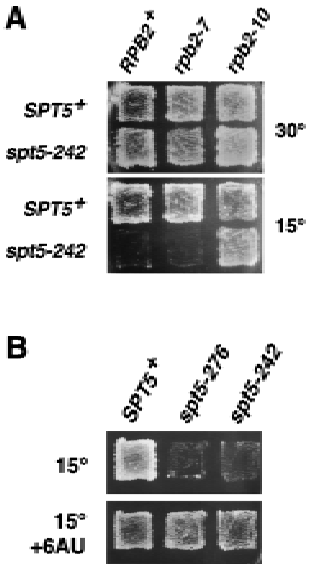

Figure 4. The cold-sensitive growth defect of the *spt5* Cs⁻ mutants is suppressed by decreasing the rate of transcription elongation. (*A*) Suppression of *spt5-242* by *rpb2-10*, a mutation known to decrease the rate of elongation by RNA Pol II (Powell and Reines 1996). Growth of the indicated strains (FY1649, FY1650, FY1651, FY1648, FY1652, and FY1653) on SC − Ura plates at 30°C and 15°C was assayed. The photograph of the 30°C plate was taken after 2 days; the photograph of the 15°C plate was taken after 8 days. The *rpb2-10* mutation also suppressed the other *spt5* Cs⁻ mutation, *spt5-276* (data not shown). (*B*) Suppression of *spt5-276* and *spt5-242* by 6AU. Growth of the indicated strains (FY267, FY1647, and FY1645) on SC − Ura plates at 15°C was assayed in the presence or absence of 6AU. The plates were photographed after 8 days of incubation.

teins, Spt4 and Spt6, act with Spt5 to control transcription in vivo (Swanson and Winston 1992). Therefore, we tested whether *spt4* or *spt6* mutants display defects consistent with an effect on transcription elongation. We also included previously identified *spt5* mutations that had been selected by their Spt⁻ phenotype. First, we constructed and analyzed *spt4Δ ppr2Δ*, *spt5-194 ppr2Δ*, and *spt6-14 ppr2Δ* double mutants. We were able to use an *spt4Δ* mutation because, unlike *SPT5* and *SPT6*, *SPT4* is not essential for growth (Malone et al. 1993). All three double mutants show a strong temperature sensitive growth phenotype not observed for any of the single mutants (Fig. 5). These results show that the requirement for TFIIS is greatly increased in *spt4*, *spt5*, and *spt6* mutants. *spt4*, *spt5*, and *spt6* mutations do not generally cause synthetic lethality in combination with mutations in transcription factors (Winston et al. 1984), supporting the idea that the double-mutant phenotypes with *ppr2Δ* indicate an elongation defect. Second, we tested *spt4Δ* and several *spt5* and *spt6* mutants for a 6AU^s phenotype. The *spt4Δ* mutation causes a strong 6AU^s phenotype, as severe as that observed in a *ppr2Δ* mutant (Table 3). For the *spt5* mutants, we found that, in contrast to the *spt5* Cs⁻ mutants, both *spt5-194* and *spt5-4* cause 6AU^s phenotypes (Table 3). Of the three *spt6* mutants tested, only one, *spt6-50*, was 6AU^s. These mutant phenotypes strongly suggest that like Spt5, Spt4 and Spt6 play a role in transcription elongation.

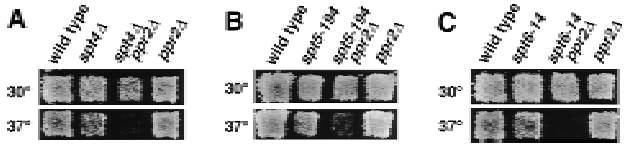

Figure 5. A *ppr2Δ* mutation causes conditional lethality in combination with *spt4*, *spt5*, and *spt6* mutations. Strains of the indicated genotypes were grown as patches on YPD plates at 30°C and were then replica plated to duplicate YPD plates. These plates were incubated at 30°C or 37°C for 2 days and then photographed. The strains were derived from single tetrads obtained by crossing an *spt* strain with a *ppr2Δ* strain as indicated. (*A*) *spt4Δ ppr2Δ* (FY1644 × FY1646); (*B*) *spt5-194 ppr2Δ* (FY1585 × FY366); (*C*) *spt6-14 ppr2Δ* (FY1644 × FY1655).

Identification of an Spt4–Spt5 complex

Previously identified mutant phenotypes, including double-mutant lethality and unlinked noncomplementation, strongly suggested that Spt4, Spt5, and Spt6 might physically interact in a complex. Indeed, coimmunoprecipitation experiments detected a weak physical interaction between Spt5 and Spt6 (Swanson and Winston 1992). We have now examined the physical interactions between all three proteins, using three different methods.

First, we expressed GST–Spt4 and GST–Spt6 fusion proteins in vivo to assay their physical association with the other Spt proteins. For each fusion, we assayed its interactions with Spt5 and with Spt6. We observed that

Table 3. *Growth of* spt4, spt5, *and* spt6 *mutants on media containing* 6AU

	Relevant genotype	Growth[a]		Other phenotypes
		SC − Ura	SC − Ura + 6AU	
1	wild type	+	+	Spt⁺
2	*ppr2Δ*	+	−	Spt⁺
3	*spt4Δ*	+	−	slow growth at 37°C
4	*spt5-242*	+	+	Cs⁻
5	*spt5-276*	+	+	Cs⁻
6	*spt5-4*	+	−/+	
7	*spt5-194*	+	−/+	
8	*spt6-14*	+	+	slow growth at 37°C
9	*spt6-140*	+	+	slow growth at 37°C
10	*spt6-50*	+	−/+	

[a]All phenotypes were scored by replica plating patches of cells to the indicated media at 30°C. (+) Confluent growth after 1–2 days; (−/+) slow but definite growth after 3 days; (−) little or no growth after 3 days. All *spt4*, *spt5*, and *spt6* mutations cause an Spt⁻ phenotype.

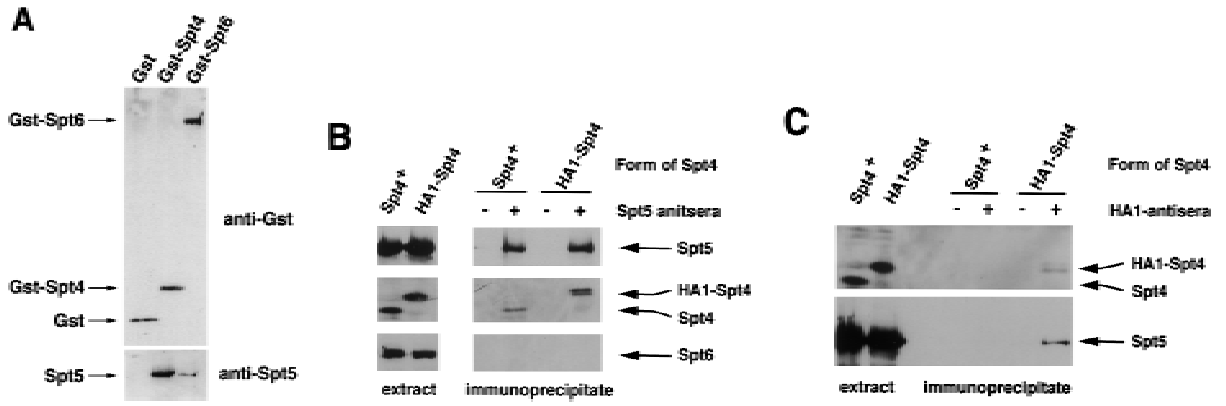

Figure 6. Identification of an Spt4–Spt5 complex. (*A*) Strains expressing the indicated GST fusion protein were used to prepare whole cell extracts. The GST fusion and associated proteins were isolated by affinity chromatgraphy, separated by SDS-PAGE, and immunoblotted, as described in Materials and Methods. Shown is a blot probed with anti-GST (*top*) and anti-Spt5 (*bottom*) antibodies. (*B*) Spt4 coimmunoprecipitates with Spt5. Extracts were prepared from wild-type (FY1654) and Spt4–HA1-tagged (FY1643) strains and used for immunoprecipitations of Spt5. Western blots of the immunoprecipitates were probed with anti-Spt5 (*top*), anti-HA1 (*middle*), or anti-Spt6 (*bottom*) antibodies. (*C*) Spt5 coimmunoprecipitates with Spt4. Anti-HA1 immunoprecipitations were carried out by use of extracts of wild-type (FY120) or HA1–Spt4-tagged (FY1643) strains. Western blots of the immunoprecipitates were probed with anti-HA1 (*top*) and anti-Spt5 (*bottom*) antibodies.

Spt5 binds strongly to GST–Spt4 and only weakly to GST–Spt6 (Fig. 6A). There was no detectable interaction of Spt5 with GST alone. A control experiment showed that the Spt5/GST–Spt4 interaction was stable even after washing the bound complexes with up to 1 M ammonium acetate (data not shown). In contrast to Spt5, Spt6 did not detectably bind to either GST–Spt4 or GST–Spt6. These results indicate a strong association between Spt4 and Spt5, a weak association between Spt5 and Spt6, and no detectable association between Spt4 and Spt6.

Second, we performed coimmunoprecipitation experiments to test for interactions among Spt4, Spt5, and Spt6. For these experiments, we used an HA1-epitope-tagged version of Spt4. Coimmunoprecipitation of Spt4 and Spt5 was tested in both directions, and the results show that the two proteins coimmunoprecipitate (Fig. 6B,C). In contrast, Spt6 did not detectably coimmunoprecipitate with either HA1–Spt4 (data not shown) or Spt5 (Fig. 6B).

Finally, we used a Flag–epitope-tagged version of Spt5 to partially purify Spt5 and associated proteins. When extracts made from Spt5–Flag-containing cells were applied to beads conjugated to anti-Flag antibody, both Spt5–Flag and Spt4 bound, whereas Spt6 did not (Fig. 7). Spt4 remained bound even after extensive washing in buffer containing 1 M KCl (data not shown). As a control, extracts of wild-type cells, in which Spt5 was not epitope-tagged, were applied to anti-Flag beads. In this case, neither Spt4, Spt5, nor Spt6 bound to the beads (Fig. 7). On the basis of these three separate assays, we conclude that Spt4 and Spt5 are strongly associated in a complex and that Spt6 is at most weakly associated with this Spt4–Spt5 complex.

Spt5 binds to RNA Pol II

Two results prompted us to test whether Spt5 physically associates with RNA Pol II. First, the extensive *spt5–rpb1* genetic interactions suggested an Spt5–RNA Pol II interaction. Second, analysis of silver-stained SDS gels of the proteins that associate with Spt5-Flag showed the copurification, albeit substoichiometrically, of a protein of ~220 kD, the size of Rpb1. Therefore, we performed Western analysis with anti-Rpb1 antibodies and found that Rpb1 copurifies specifically with the Spt4–Spt5 complex (Fig. 7). Furthermore, the association of Rpb1 with the anti-Flag beads was stable to extensive washes of buffer containing 0.5 M KCl (data not shown). Thus, in addition to its genetic interactions with Rpb1 and Rpb2,

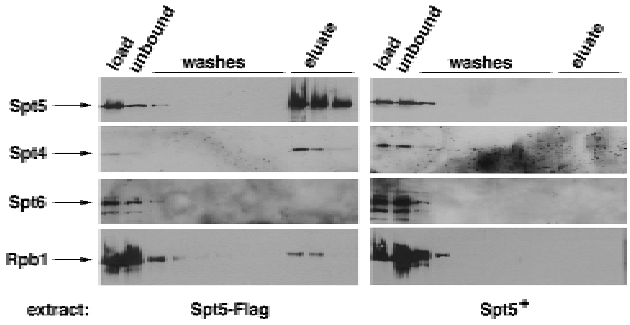

Figure 7. Purification of Spt5–Flag and associated proteins. An extract of a wild-type strain in which Spt5 was tagged with the Flag epitope was mixed with anti-Flag beads, washed, and then bound proteins were competitively eluted with a Flag peptide as described in Materials and Methods. Approximately 1.7% of the material loaded on the anti-Flag beads, unbound material, each of the six washes, and 20% of each of the three eluted fractions were separated on an SDS–polyacrylamide gel and blotted. (*Left*) This blot was probed with antibodies that recognize Spt5, Spt4, Spt6, and the CTD of Rpb1. (*Right*) Western blots of a control experiment carried out identically and in parallel in which the extract used contained Spt5 that was not Flag tagged.

Spt5 appears to associate with RNA Pol II in vivo. Interestingly, computer searches have revealed a weak homology between a conserved portion of the Spt5 family of proteins and the bacterial elongation factor NusG (Fig. 8; Wada et al. 1998), which is also known to interact with *E. coli* RNA polymerase (Condon et al. 1995; Mogridge et al. 1995).

Discussion

In vivo and in vitro studies suggest an important role for Spt4, Spt5, and Spt6 in transcription elongation

Our studies have provided evidence that the transcription factors Spt4, Spt5, and Spt6 are required for transcription elongation in vivo. This conclusion is supported by four sets of results presented in this paper. First, we have shown both a genetic and a physical interaction between Spt5 and RNA Pol II. Second, we have shown that *spt4*, *spt5*, and *spt6* mutations all cause phenotypes that strongly suggest elongation defects in vivo. These phenotypes include allele-specific suppression of *spt5* mutations by *rpb1* and *rpb2* mutations, including one *rpb2* mutation that has been characterized as causing an elongation defect in vitro. In addition, *spt4*, *spt5*, and *spt6* mutations all show conditional lethality in combination with *ppr2Δ*, a mutation that abolishes the function of the elongation factor TFIIS, and many *spt4*, *spt5*, and *spt6* mutations cause a 6AU^S phenotype. Third, Spt5 shows some sequence similarity to the *E. coli* transcription elongation factor NusG. Finally, we have shown that Spt4 and Spt5 form a complex; this result fits extremely well with the results in Wada et al. (1998), in which a human Spt4–Spt5 complex has been shown to affect transcription elongation in vitro and to interact with RNA Pol II.

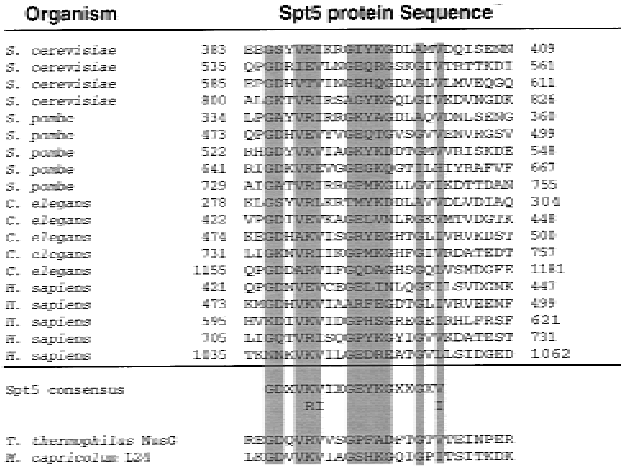

Figure 8. A repeated element in Spt5 is homologous to a region of NusG. Alignments of a conserved, repeated element in Spt5 proteins along with the consensus sequence developed by the program MEME. This consensus is similar to a motif of unknown function, KOW (Kyrpides et al. 1996), found in NusG, ribosomal protein L24, and their homologs. For comparison, the homologous sequences of the highest scoring NusG and L24 proteins are included. The accession number for *S. cerevisiae* Spt5 is M62882; for human, U56402; for *Caenorhabditis elegans*, Z68316; for *Schizosaccharomyces pombe*, Z99753; for *Tetrahymena thermophila* NusG, P35872; and for *Mycoplasma capricolum* L24, P10141.

Spt4 and Spt5 form a complex that does not contain Spt6

Although our previous genetic results were most consistent with a model in which Spt4, Spt5, and Spt6 form a complex, the biochemical data presented here show clearly that Spt4 and Spt5 form a tight complex that, at most, associates only weakly with Spt6. We observed the tight association of Spt4 and Spt5 in three different assays (Figs. 6 and 7). In contrast, both our past (Swanson and Winston 1992) and current works show that Spt5 and Spt6 interact weakly at best, and in no case was any association detected between Spt4 and Spt6. Consistent with these findings, in preliminary experiments in which we have biochemically fractionated yeast extracts, Spt4 and Spt5 cofractionate, whereas Spt6 separates away from the Spt4–Spt5 complex (data not shown). Consistent with our findings, Wada et al. (1998) purified a human Spt4–Spt5 complex that does not contain human Spt6. Thus, any model for a common function for these three proteins must account for them working in physically distinct complexes. Given the genetic evidence that Spt4, Spt5, and Spt6 work together, an Spt4–Spt5 complex may interact with Spt6 in a dynamic, but biochemically unstable fashion in vivo.

Spt4, Spt5, and Spt6 are conserved regulators of transcription elongation

Our results suggest that Spt5 serves a positive role for transcription elongation in vivo. Although we have not yet been able to obtain direct biochemical evidence that *spt5* mutants are defective for elongation, Wada et al. (1998) provided a clear biochemical demonstration of a direct role for Spt4–Spt5 in transcription elongation in vitro. The sequence conservation between the yeast and human Spt4 and Spt5 proteins (Chiang et al. 1996a,b; Hartzog et al. 1996; Stachora et al. 1997) and the fact that human Spt4 functions well in yeast (Hartzog et al. 1996) suggest that these proteins are functionally conserved. Thus, it is likely that at a fundamental level, Spt4 and Spt5 carry out similar roles in yeast and humans.

Our past genetic studies of Spt4, Spt5, and Spt6 have strongly suggested that these genes also play a negative role in transcription initiation (Swanson and Winston 1992; Winston and Carlson 1992). These results fit well with the results of Wada et al. (1998), showing both negative and positive effects of human Spt4–Spt5 in vitro. Our previous conclusions about a role for Spt4, Spt5, and Spt6 in initiation were based on the ability of many *spt4*, *spt5*, and *spt6* mutations to suppress loss of the Snf–Swi complex (Neigeborn et al. 1987; Swanson and Winston 1992), the deletion of a UAS element (Prelich and Win-

ston 1993), mutations in positive activators (Denis and Malvar 1990), and the negative effects of insertion mutations in the promoter regions of genes (Swanson and Winston 1992). Although we believed these effects to occur at the level of transcription intiation, we must now consider that some of them could occur at the level of elongation. Consistent with this reinterpretation of our data, recent studies suggest a role for Snf–Swi in elongation (Brown et al. 1996) and that promoter-bound transcription factors can modify the processivity of RNA Pol II (Bentley 1995; Krumm et al. 1995).

Spt5 and RNA Pol II interact physically and functionally to promote elongation

The allele-specific interactions we have observed between *spt5* and *rpb1* mutations have suggested two types of interactions between Spt5 and RNA Pol II. Allele-specific interactions between mutations in different genes are often believed to indicate a physical interaction of their protein products. The demonstration of an Rpb1–Spt5 physical interaction (Fig. 7) supports this interpretation of the genetic data. The *spt5* Cs$^-$ mutations, however, are also suppressed in an allele-specific manner by *rpb2* mutations, as well as by treatment with 6AU. These results suggest that the *spt5* Cs$^-$ mutations are suppressed by a particular type of defect in RNA Pol II that is conferred by the *rpb1*^{sup} and *rpb2-10* mutants, as well as by treatment with 6AU. Given that the *rpb2-10* mutation causes RNA Pol II to have a decreased elongation rate in vitro and that 6AU treatment is believed to decrease the rate of transcription elongation in vivo (Powell and Reines 1996; Uptain et al. 1997), it is likely that the common defect that causes suppression of the *spt5* Cs$^-$ mutations is a decrease in the processivity of RNA Pol II. Thus, the allele-specific nature of the *rpb1-spt5* interactions likely reflects both a functional and physical interaction between Spt5 and RNA Pol II. Additional genetic and biochemical analysis will be required to determine which RNA Pol II subunits are actually in direct contact with Spt4–Spt5.

Spt4, Spt5, and Spt6 may help RNA Pol II transcribe past nucleosome barriers

The synthetic conditional phenotypes we observed for *spt ppr2Δ* double mutants suggests that, in the absence of Spt4, Spt5, and Spt6 function, RNA Pol II encounters conditions that cause it to arrest. Interestingly, a DNA-bound protein can block elongation in vitro and impose a requirement for TFIIS function (Reines and Mote 1993). Previous studies have shown that nucleosomes form a barrier to elongating RNA Pol II in vitro, suggesting that they also form a barrier in vivo (Chang and Luse 1997; Uptain et al. 1997). In addition, recent work has shown that the nucleosome remodeling activity Snf–Swi (Brown et al. 1996), and another activity, FACT (Orphanides et al. 1998), are important for elongation on chromatin templates. Spt4, Spt5, and Spt6 are distinct from these activities, as Spt5 and Spt6 are not part of either Snf–Swi (B. Cairns, pers. comm.) or FACT (D. Reinberg, pers. comm.). We propose that one role for Spt4, Spt5, and Spt6 in transcription elongation in vivo is to help RNA Pol II elongate past nucleosomes. The results in this work and those in Wada et al. (1998) have shown an interaction of Spt4–Spt5 with RNA Pol II. In addition, these investigators have shown that Spt4–Spt5 affects transcription elongation in vitro on a nonchromatin template. These results suggest that Spt4–Spt5 interacts directly with RNA Pol II to control its processivity. Past biochemical analysis has shown a direct interaction of Spt6 with histones (Bortvin and Winston 1996). In addition, past genetic analyses have shown that *spt4*, *spt5*, and *spt6* mutations share many phenotypes with mutations in histone genes (Hirschhorn et al. 1992; Winston and Carlson 1992; Bortvin and Winston 1996). These results suggest that Spt4, Spt5, and Spt6 may mediate interactions of RNA Pol II with chromatin. In helping RNA Pol II elongate past nucleosomes, Spt4, Spt5, and Spt6 could conceivably be required for either of two hypothetical steps: (1) nucleosome remodeling or disassembly to allow the passage of RNA Pol II, or (2) the reassembly of nucleosomes into a normal chromatin structure after the passage of RNA Pol II. Our results do not yet distinguish between these possibilities. Previous studies of Spt6 have shown that it has nucleosome assembly activity in vitro (Bortvin and Winston 1996); whether or not an Spt6 nucleosome assembly activity is relevant to the role Spt6 plays in elongation in vivo remains to be determined. Given the known biochemical interactions so far established, one possibility is that Spt4–Spt5 is primarily associated with the transcriptional apparatus, whereas Spt6 is primarily associated with nucleosomes.

The suppression of the *spt5* Cs$^-$ mutations by a decreased rate of transcription elongation also supports a model in which the *spt5* mutations impair the ability of RNA Pol II to elongate past nucleosomes. In this model, the rate of Spt4–Spt5 function must be appropriately coupled to the rate of RNA Pol II elongation to avoid pausing. In an *spt5* Cs$^-$ mutant grown under nonpermissive conditions, Spt4–Spt5 function is reduced such that RNA Pol II has an increased probability of pausing or arresting when it encounters a nucleosome. The pause is caused by the inability of Spt4–Spt5 to act at a normal rate to remove the nucleosome barrier. A decreased rate of elongation by RNA Pol II, then, would allow adequate time for the weakened Spt4–Spt5 activity to remove the barrier, thereby avoiding a stalled RNA Pol II. Previous studies have suggested that Cs$^-$ mutations can impair protein–protein interactions and/or protein activity (see discussion in Noble and Guthrie 1996). Thus, the specific defect caused by the *spt5* Cs$^-$ mutations could be a weakened interaction with RNA Pol II, histones, or some other transcription elongation factor. Alternatively, the *spt5* Cs$^-$ mutations may interefere with an intrinsic activity of Spt5. Because the *spt5* Cs$^-$ mutations do not cause a 6AU^s phenotype and are actually suppressed by 6AU, the elongation defect that they cause is very likely to be distinct from that caused by *rpb1*,

rpb2, or other *spt* mutations that cause a 6AU^s phenotype and are believed to impair elongation.

In conclusion, our work has provided strong genetic evidence for a role in transcription elongation for yeast Spt4, Spt5, and Spt6. Biochemical experiments on the human Spt4 and Spt5 proteins also strongly suggest a role in elongation (Wada et al. 1998). Future experiments will be aimed to discover other proteins that physically associate with both the Spt4–Spt5 complex and with Spt6, to address how these factors might play both positive and negative roles in transcription elongation in vivo, and to develop in vitro assays for these factors on chromatin templates. It will be interesting to determine whether Spt4, Spt5, and Spt6 are required for the function of transcriptional activators that work by overcoming a transcriptional pause, such as *Drosophila hsp70* (Lis and Wu 1993) or HIV Tat (Jones 1997).

Materials and methods

Media and genetic methods

The *S. cerevisiae* strains used in this study (Table 4) are isogenic to S288C and are *GAL2*$^+$ (Winston et al. 1995). Strain constructions and other genetic manipulations were carried out by standard methods. Yeast media, including rich media (YPD), minimal media (SD), and synthetic complete media lacking uracil (SC − Ura) were made as described previously (Rose et al. 1990). Strains containing the *rpb1Δ187::HIS3* and *rpb2Δ297::HIS3* deletions were made as described previously (Nonet et al. 1987; Scafe et al. 1990b). 6AU sensitivity was scored on SC − Ura plates with 50 μg/ml of 6AU. The *ppr2Δ::hisG* deletion was integrated by use plasmid pJD3. Strains carrying the plasmid-borne *rpb1* and *rpb2* mutations were created by plasmid shuffle methods (Guthrie and Fink 1991) by use of host strains with either *rpb1Δ187::HIS3* or *rpb2Δ297::HIS3*. Preliminary experiments utilized a derivative of *SPT5* that had been tagged with the Myc epitope. Although this epitope was not used, several of the strains listed do carry Spt5–Myc. All epitope-tagged alleles of *SPT4*, *SPT5*, and *SPT6* used in this work were phenotypically indistinguishable from the wild-type genes. Double-mutant strains that are inviable at 30°C were isolated by germination of dissected tetrads at 37°C.

Plasmids

pRP187 and pRP112 are described in Nonet et al. (1987). pRP212, pRP297, pRP2-7(U), and pRP2-10(U) are described in Scafe et al. (1990b). pRPB1-5 is described in Scafe et al. (1990a). pJA509 and pJA483 are described in Archambault et al. (1992). pFW45 (Winston et al. 1984), pYST138 (Som et al. 1988), and pHB59 (a gift of Henry Baker, University of Florida, Gainesville) were used to make the *HIS4*, *TUB2*, and *TPI1* probes for Northern blots. pGH25 is a derivative of pMALcRI (New England Biolabs) fused in-frame to the 3′ *Eco*RI site of *SPT5*. This results in expression of a fusion of MBP to amino acids 807–1063 of Spt5. pGH123 contains full-length *SPT4* fused in frame to MBP in pMALcRI. The HA1 epitope-tagged allele of *SPT4* was created by subcloning the *Hin*dIII fragment of pBM56 (Malone et al. 1993), containing HA1–*SPT4*, into pRS306 (Sikorski and Hieter 1989) to create pGH90. The *SPT5*–Flag allele was constructed by site-directed mutagenisis of pGH17, a derivative of pBluescript-IIKS+ containing the *Kpn*I–*Xho*I fragment of *SPT5*, by use of oligonucleotide OGH52, 5′-CTCCTTTTGGTGATTACTTGTCATCGTCGTCCTTGTAGTCATGACCTCCCCATGTAC-3′. The mutagenized *Kpn*I–*Xho*I *SPT5*–Flag fragment was cloned into pRS306, creating plasmid pGH184. pMS4 is described in Swanson et al. (1991), and pBM24 contains the *SPT5* genomic DNA fragment from pMS4 in pBM453, a *TRP1 CEN* shuttle vector. pGH84 contains full-length *SPT4* fused in-frame to GST in pEMBL–GST (Kranz et al. 1994). pGH85 contains full-length *SPT6* fused in-frame to GST in pEMBL–GST. GST was expressed from plasmid pYBS305 (pEMBL–GST derivative generously provided by E. Elion, Harvard Medical School, Boston, MA). pGH189 is a derivative of pRP112 that lacks the *Aat*II–*Bst*EII fragment upstream of *RPB1*. pGH188 is a derivative of pRP112 that lacks an internal *Eco*RI–*Xba*I fragment of *RPB1*.

Isolation of spt5 *Cs*$^-$ *mutants*

The *spt5* Cs$^-$ mutations were isolated by mutagenizing plasmid pBM24 with hydroxylamine as described (Rose et al. 1990). The mutagenized plasmid was used to transform strain FY849 and the Trp$^+$ transformants were replica plated to 5FOA plates to select for loss of the wild-type *SPT5* plasmid pMS4. 5FOA-resistant colonies were then replica plated to YPD plates that were incubated at 15°C and 30°C to identify colonies defective for growth at 15°C. Each candidate was retested by isolating the mutant plasmid and using it to retransform strain FY849 to determine if the Cs$^-$ phenotype was plasmid linked. Two *spt5* mutations conferring a Cs$^-$ growth defect were identified and recombined into the *S. cerevisiae* genome, replacing *SPT5*$^+$. When integrated, these *spt5* mutations, *spt5-242* and *spt5-276*, still cause a Cs$^-$ phenotype and also confer a weak Spt$^-$ phenotype. Linkage analysis and plasmid complementation were used to verify that these mutant phenotypes were caused by the *spt5* mutations.

Isolation of suppressors of the spt5 *Cs*$^-$ *mutations and identification of these suppressors as* rpb1 *mutations*

To isolate extragenic suppressors of the *spt5* Cs$^-$ mutations, the *spt5-242* strain FY1635 and the *spt5-276* strain FY1634 were mutagenized with 300 ergs/mm^2 of UV light, allowed to grow into colonies, and then replica plated to YPD and incubated at 15°C. Colonies that grew at 15°C were purified and retested for a Cs$^+$ phenotype. The detailed analysis of most of these suppressors will be described elsewhere. Two of the suppressor mutations were shown to be mutations in *RPB1* by several steps. These mutations suppressed the Cs$^-$ phenotype of the *spt5* mutatons and also caused a mild Spt$^-$ phenotype in an *SPT5*$^+$ genetic background. To clone the gene corresponding to this complementation group, strain FY1637 was transformed with a plasmid library of yeast genomic DNA (Rose et al. 1987) Transformants were screened for an Spt$^+$ phenotype (His$^-$ and Lys$^-$). Candidate plasmids were isolated and used to retransform strains FY1637 and FY1636 to determine whether all of the mutant phenotypes could be complemented by the plasmid. The one plasmid that passed these tests was shown to contain *RPB1* by DNA sequence analysis. Experiments with plasmid pGH189, which contains only *RPB1*, confirmed that *RPB1* was sufficient to complement all the mutant phenotypes of the *spt5* Cs$^-$ suppressors. In addition, pGH188, which lacks the internal *Eco*RI–*Xba*I fragment of *RPB1*, could not complement the suppressors. Finally, both suppressors were shown to be allelic to *RPB1* by linkage analysis.

Sequence analysis of rpb1^{sup} *mutations*

The two *rpb1*^{sup} mutations were cloned from strains FY1637 and FY1638 into plasmid pRP112, by use of the method of gap

Table 4. S. cerevisiae *strains*

Strain	Genotype
FY2	*MAT*α *ura3-52*
FY120	*MAT***a** *his4-912*δ *lys2-128*δ *trp1*Δ*63 ura3-52 leu2*Δ*1*
FY267	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1*
FY366	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt5-194*
FY849	*MAT***a** *his4-912*δ *lys2-128*δ *trp1*Δ*63 ura3-52 leu2*Δ*1 spt5*Δ*202::LEU2* [pMS4]
FY1585	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ppr2*Δ*::HISG*
FY1634	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 spt5-276*
FY1635	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 spt5-242*
FY1636	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 spt5-242 rpb1-244*
FY1637	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 rpb1-244*
FY1638	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*l ura3-52 rpb1-221*
FY1639	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 trp1*Δ*63 HA1–SPT4 HA1–SPT6 SPT5–MYC*
FY1640	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 trp1*Δ*63 spt4*Δ*2::HIS3 HA1–SPT6 SPT5–MYC*
FY1641	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 spt6*Δ*::LEU2 SPT5–MYC ade8* [pGH85 = *pGAL–GST–SPT6* 2μ *URA3 leu2d*]
FY1642	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 SPT5–FLAG*
FY1643	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 HA1–SPT4 MYC–SPT5 pep4*Δ*::LEU2*
FY1644	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ppr2*Δ*::HISG*
FY1645	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt5-242*
FY1646	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt4*Δ*2::HIS3*
FY1647	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt5-276*
FY1648	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-521 rpb2*Δ*297::HIS3 spt5-242 his3*Δ*200* or *HIS3* [pRP212 = *RPB2 URA3 CEN*]
FY1649	*MAT***a** *his3*Δ*200 lys2-128*δ *leu2*Δ*1 ura3-52 rpb2*Δ*297::HIS3* [pRP212 = *RPB2 URA3 CEN*]
FY1650	*MAT***a** *his3*Δ*200 lys2-128*δ *leu2*Δ*1 ura3-52 rpb2*Δ*297::HIS3* [prp2-7(U) = *rpb2-7 CEN URA3*]
FY1651	*MAT***a** *his3*Δ*200 lys2-128*δ *leu2*Δ*1 ura3-52 rpb2*Δ*297::HIS3* [pRP2-10(U) = *rpb2-10 URA3 CEN*]
FY1652	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 rpb2*Δ*297::HIS3 spt5-242 his3*Δ*200* or *HIS3* [prp2-7(U) = *rpb2-7 URA3 CEN*]
FY1653	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 rpb2*Δ*297::HIS3 spt5-242 his3*Δ*200* or *HIS3* [prp2-10(U) = *rpb2-10 URA3 CEN*]
FY1654	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 trp1*Δ*63 HA1–SPT6 SPT5–MYC*
FY1655	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt6-14*
FY1659	*MAT***a** *lys2-128*δ *leu2*Δ*1 ura3-52 trp1*Δ*63 spt5-242 rpb1*Δ*187::HIS3 his3*Δ*200* or *HIS3* [pJA509 = *rpo21-18 CEN TRP1*]
FY1660	*MAT*α *lys2-128*δ *leu2*Δ*1 ura3-52 trp1*Δ*63 rpb1*Δ*187::HIS3 his3*Δ*200* or *HIS3* [pJA509 = *rpo21-18 CEN TRP1*]
FY1663	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 spt5-276 his3*Δ*200* or *HIS3 rpb1*Δ*187::HIS3* [prpb1-5 = *rpb1-5 LEU2 CEN*]
FY1665	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt5-276 ppr2*Δ*::HISG*
FY1666	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt5-242 ppr2*Δ*::HISG*
FY1667	*MAT*α *his4-912*δ *leu2*Δ*1 lys2-173R2* or *lys2-128*δ *spt6-50*
FY1668	*MAT***a** *his4-912*δ *lys2-128*δ *spt5-4*
FY1669	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 spt6-140*
FY1670	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ppr2*Δ*::HISG*
FY1671	*MAT*α *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 ppr2*Δ*::HISG*
FY1672	*MAT*α *lys2-128*δ *leu2*Δ*1 ura3-52 spt5-242*
FY1673	*MAT***a** *lys2-128*δ *leu2*Δ*1 ura3-52 spt5-276 his3*Δ*200*
FY1686	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 his3*Δ*200 rpb1*Δ*187::HIS3* [prpb1-5 = *rpb1–5 LEU2 CEN*]
FY1687	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 rpb1-221 spt5-242*
FY1688	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 rpb1-244 spt5-276*
FY1689	*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52 rpb1-221 spt5-276*

repair (Guthrie and Fink 1991). Based on the gap-repair results, the 2.7-kb *Xba*I–*Sna*BI fragment of *RPB1* was sequenced for each mutant at the Biopolymer Facility in the Department of Genetics, Harvard Medical School. In each case, a single base-substitution mutation was identified. To verify that this mutation caused the suppressor phenotype, the *Xba*I–*Sna*BI fragment of each mutant was subcloned into an otherwise wild-type *RPB1* gene in pRP112. For both *rpb1-244* and *rpb1-221*, these reconstructed plasmids, pGH202 and pGH203, were phenotypically indistinguishable from the original mutant isolates.

Northern analysis

For Northern analysis, cells were grown in SD media supplemented with necessary amino acids to a density of 0.5×10^7 to 2.0×10^7 cells/ml. RNA was isolated from cells by a hot phenol

method (Ausubel et al. 1988), quantitated with a spectrophotometer, and 10 µg of each sample was separated on an agarose–formaldehyde gel, blotted to Genescreen, and probed as described in Swanson et al. (1991). ^{32}P-Labeled probes were made by nick translation with a kit purchased from Boehringer Mannheim Biochemicals.

Analysis of protein–protein interactions

Strains were grown to a density of 1×10^7 to 4×10^7 cells/ml, and extracts were prepared by the bead-beating method (Eisenmann et al. 1992). Cells were lysed in buffer containing 25 mM $NaPO_4$, 0.1 M KOAc, 2 mM MgAc, and 10% glycerol. Unless otherwise noted, all manipulations of protein were carried out at 4°C and in the presence of the following protease inhibitors: aprotinin (1.72 µg/ml); pepstatin A (1 µg/ml); chymotatin (0.1 µg/ml); E64 (7.2 µg/ml); phosporamidon (1.1 µg/ml); and PMSF (1 mM).

For the GST-binding experiments, strain FY1639 was transformed with pYBS305 (GST) and strain FY1640 was transformed with pGH84 (GST–Spt4). All GST fusion proteins were functional in vivo. The FY1639 and FY1640 transformants, as well as strain FY1641 (expressing GST–Spt6 from pGH85), were grown in SC − Ura with 2% raffinose as the carbon source to 1×10^7 to 4×10^7 cells/ml. Galactose was then added to 2%, and cells were grown for 3 hr at 30°C and then processed for extracts as described above. GST pull-down experiments were performed by mixing 500 µg of protein extracts with 200 µl of glutathione–Sepharose (Pharmacia) in a volume of 500 µl in the presence of 0.5% Triton X-100. After gentle mixing for 1 hr at 4°C, the beads were pelleted, the supernatant was removed, and the beads were washed in five volumes of lysis buffer adjusted to 0.5% Triton and 0.4 M ammonium acetate. After washing, 40 µl of beads was removed and mixed with SDS-loading buffer for polyacrylamide gel electrophoresis.

Spt5–Flag was purified by mixing ~6 mg of an extract from strain FY1642 with ~50 µl of M2 anti-Flag beads (Kodak) and mixing gently for 6 hr. The beads were pelleted, the unbound material was removed, and the beads were washed six times in wash buffer (25 mM HEPES at pH 7.4, 1 mM EDTA, 0.02% NP-40, 3 mM $MgCl_2$, 10% glycerol, 1 mM DTT, 0.2 M KCl) with 5 min of gentle mixing between washes. The bound proteins were eluted by adding 50 µl of wash buffer containing 2 mg/ml of the Flag peptide, Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (Research Genetics), and incubating for 30 min before pelleting the beads gently and removing the eluate. This elution step was repeated two to three times. A control experiment was carried out in parallel in which anti-Flag beads were mixed with an extract of FY267 cells, in which Spt5 lacks the Flag epitope, to determine the level of nonspecific binding of proteins to the beads.

Immunoprecipitations were performed by mixing 250 µg of protein extract (prespun for 10 min at 4°C in a microcentrifuge to remove protein aggregates) in lysis buffer plus 0.5 M ammonium acetate and 0.1% Tween 20 with ~50 µl of anti-mouse (for anti-HA1 immunoprecipitations) or anti-rabbit (for anti-Spt5 immunoprecipitations) IgG (Sigma Immunochemicals) in a final volume of 250 µl. After 30 min of incubation with gentle mixing, the beads were removed by low-speed centrifugation and the supernatant was transferred to a clean tube and mixed with 3 µl of affinity purified anti-Spt5 or 5 µl of anti-HA1 antibody as appropriate. After 1 hr of gentle mixing in the presence of the primary antibody, the reactions were centrifuged for 10 min in a microcentrifuge and transferred to a clean tube with 50 µl of the appropriate anti-IgG beads for an additional 1 hr of incubation. Finally, the beads were washed five times by centrifugation, removal of the supernatant and addition of 0.5 ml of lysis buffer plus 0.5 M ammonium acetate and 0.1% Tween-20 followed by 5 min of gentle rocking. After the final wash, the beads were resuspended in SDS–sample loading buffer for gel electrophoresis.

Antibodies and immunoblots

The anti-Spt4 and -Spt5 antibodies were directed against maltose-binding protein (MBP) fusion proteins produced from plasmids pGH123 and pGH25, respectively, with the MBP protein fusion and purification system from New England Biolabs (Beverly, MA), according to their instructions. Rabbits were immunized with the MBP–Spt4 and MBP–Spt5 fusion proteins by Cocalico Biologicals (Reamstown, PA). The Spt5 antibody was affinity purified by coupling MBP alone and MBP–Spt5 to Affigel (Bio-Rad), preclearing the crude antisera against the MBP-affigel, and then purifying the Spt5 specific antibodies on the MBP–Spt5-affigel as described previously (Harlow and Lane 1988). The anti-HA1 antibody was produced by BabCo (Berkeley, CA). The anti-Rpb1 antibody is a mouse monoclonal antibody, 8WG16, directed against the carboxy-terminal domain (CTD) of RNA Pol II (Thompson et al. 1989). All immunoblots were performed by separating the proteins of interest on 7.5%–16% or 7.5%–20% gradient SDS–polyacrylamide gels and transferring them to Immobilon-NC membranes (Millipore). Western blots were probed under standard conditions (Harlow and Lane 1988). HRP-coupled secondary antibodies were obtained from Bio-Rad. Blots were developed with the Amersham ECL kit according to their instructions.

Computer searches

Homology searches, by use of BLAST (Altschul et al. 1990), identified a repeated, conserved sequence element among Spt5 proteins. With the program MEME (Bailey and Elkan 1994), a consensus for this sequence element was developed. The consensus sequence was then used to search a nonredundant protein database by use of MAST (Bailey and Elkan 1994). For each potential match to the motif, MAST calculates the probability that a random sequence would match the motif as well as the potential match. MAST then calculates the number of expected occurrences of a match to the motif at that probability level as if it were searching a random database of the same size as the nonredundant database; this is termed the E value. Of the 52 proteins with matches to the motif with an E value of less than 1, 41 occurred in Spt5 proteins, ribosomal protein L24 proteins or in NusG proteins.

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Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*

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