Suppression Analysis Reveals a Functional Difference Between the Serines in Positions Two and Five in the Consensus Sequence of the C-Terminal Domain of Yeast RNA Polymerase II

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Manuscript received December 21, 1995
Accepted for publication February 19, 1996

ABSTRACT

The largest subunit of RNA polymerase II contains a repetitive C-terminal domain (CTD) consisting of tandem repeats of the consensus sequence $Tyr_1Ser_2Pro_3Thr_4Ser_5Pro_6Ser_7$. Substitution of nonphosphorylatable amino acids at positions two or five of the *Saccharomyces cerevisiae* CTD is lethal. We developed a selection ssytem for isolating suppressors of this lethal phenotype and cloned a gene, *SCA1* (suppressor of CTD alanine), which complements recessive suppressors of lethal multiple-substitution mutations. A partial deletion of *SCA1* (*sca1Δ::hisG*) suppresses alanine or glutamate substitutions at position two of the consensus CTD sequence, and a lethal CTD truncation mutation, but *SCA1* deletion does not suppress alanine or glutamate substitutions at position five. *SCA1* is identical to *SRB9*, a suppressor of a cold-sensitive CTD truncation mutation. Strains carrying dominant *SRB* mutations have the same suppression properties as a *sca1Δ::hisG* strain. These results reveal a functional difference between positions two and five of the consensus CTD heptapeptide repeat. The ability of *SCA1* and *SRB* mutant alleles to suppress CTD truncation mutations suggest that substitutions at position two, but not at position five, cause a defect in RNA polymerase II function similar to that introduced by CTD truncation.

THE largest subunit of RNA polymerase II (pol II) contains an unusual repetitive carboxy-terminal extension that consists of tandem copies of the consensus heptapeptide $Tyr_1Ser_2Pro_3Thr_4Ser_5Pro_6Ser_7$ (ALLISON *et al.* 1985; CORDEN *et al.* 1985). The CTD is present in the largest subunit of RNA pol II from a variety of organisms but is not found in the corresponding large subunits of RNA pol I or pol III or in prokaryotic RNA polymerase (CORDEN 1990; CORDEN and INGLES 1992). The function of the CTD is not known but mutational studies have established that it plays an essential function *in vivo* (NONET *et al.* 1987; ALLISON *et al.* 1988; BARTOLOMEI *et al.* 1988).

The yeast RNA polymerase II largest subunit is encoded by the *RPB1* gene and contains 26 (ALLISON *et al.* 1985) or 27 (NONET *et al.* 1987) copies of the consensus heptapeptide. Deletion of the entire yeast (NONET *et al.* 1987; ALLISON *et al.* 1988) or mouse (BARTOLOMEI *et al.* 1988) CTD is lethal, although in both cases, partial deletions have been shown to confer a conditional growth phenotype. Partial deletions of the CTD preferentially reduce transcription of some genes (SCAFE *et al.* 1990; MEISELS *et al.* 1995). YOUNG and colleagues have isolated a set of suppressors of a conditional lethal defect in a strain containing 11 heptapeptide repeats (NONET and YOUNG 1989; HENGARTNER *et al.* 1995; LIAO *et al.* 1995). The *SRB* genes encode proteins that form a complex with RNA polymerase II (THOMPSON *et al.* 1993; KOLESKE and YOUNG 1994) that responds to the presence of transcriptional activator proteins *in vitro* (KIM *et al.* 1994; KOLESKE and YOUNG 1994) and mutations in SRB genes cause defective gene activation *in vivo* (LIAO *et al.* 1995).

In vivo, the CTD is multiply phosphorylated, giving rise to two forms of the largest subunit that differ in their electrophoretic mobility in SDS gels (CADENA and DAHMUS 1987); the phosphorylated IIO subunit migrates more slowly than the unphosphorylated IIA form. Phosphorylation of the CTD has been temporally linked to transcription initiation (LAYBOURN and DAHMUS 1990; DAHMUS 1994) although the requirement for this modification is not absolute (SERIZAWA *et al.* 1993). Several kinases have been shown to phosphorylate the CTD *in vitro*. We have previously isolated two murine CTD kinases containing $p34^{cdc2}$ catalytic subunits (CISEK and CORDEN 1989, 1991) and have shown that these kinases phosphorylate serine residues in both positions two and five in the consensus heptapeptide repeat (ZHANG and CORDEN 1991). The *CTK1* gene from yeast encodes a CTD kinase catalytic subunit that is related to $p34^{cdc2}$ (LEE and GREENLEAF 1991). Deletion of this gene is not lethal but does result in slow growth (LEE and GREENLEAF 1991). Although the level of CTD phosphorylation is lower than normal in a *ctk1* null strain, some phosphorylation activity still remains, indicating

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the existence of at least one additional CTD kinase. A CTD kinase activity has also been identified in preparations of the yeast general transcription factor TFIIH (FEAVER *et al.* 1991) and this kinase has been identified as the *KIN28* gene product (FEAVER *et al.* 1994). Mammalian TFIIH contains a homologous CTD kinase activity catalyzed by the MO15 kinase (ROY *et al.* 1994). Finally, *SRB10* and *SRB11* encode a cdk/cyclin pair that have been implicated in CTD phosphorylation *in vitro* (LIAO *et al.* 1995). Which, if any, of these kinases phosphorylate the CTD *in vivo* is unknown.

We have constructed and studied a collection of CTD substitution mutations in *Saccharomyces cerevisiae* for the purpose of investigating the requirement for CTD phosphorylation (WEST and CORDEN 1995). In these mutant polymerase genes, the natural CTD coding sequence has been replaced with tandem oligonucleotides that encode multiple copies of wild-type or mutated CTD heptapeptides. Strains with eight or more consensus heptapeptides are viable although growth is slow with <10 repeats (WEST and CORDEN 1995). We have also constructed a set of multiple phosphorylation site mutants, each containing >15 identical mutated repeats. The results of these studies demonstrate that changing positions two or five to either alanine or glutamate is lethal. We now report the isolation of several yeast strains that are able to suppress a subset of these lethal CTD phosphorylation site substitution mutations. We used complementation of one of these suppressor mutations to clone the wild-type allele of a gene we call *SCA1* (suppressor of CTD alanine). A *SCA1* deletion mutation suppresses the same subset of CTD mutations as the original suppressor strains. Genetic analysis of *sca1* mutant strains indicates that a second gene, *SCA2*, is necessary for suppression.

MATERIALS AND METHODS

Yeast strains and media: All yeast strains used in this study are listed in Table 1. Media was prepared according to ROSE *et al.* (1990), except as indicated. Cycloheximide was added to YPD media at 10 μg/ml and to SC medium at 5 μg/ml. 5-Fluoro-orotic acid (5FOA) was added at a concentration of 1 mg/ml to SC or SC-leu medium for selection against the presence of the *URA3* gene (SIKORSKI and BOEKE 1991).

CTD mutants: The following lethal CTD substitution mutations (mutant residue underlined) have been used in the present study: *rpb1-a2(18)* contains an RNA polymerase largest subunit gene, which has a CTD consisting of 18 YAPTSPS repeats; *rpb1-e2(15)* has 15 YEPTSPS CTD repeats; *rpb1-a5(15)* has 15 YSPTAPS repeats and *rpb1-e5(18)* has 18 YSPTEPS repeats. We have also used CTD truncation mutations constructed in our laboratory: *rpb1-WT7* has seven consensus (YSPTSPS) CTD repeats; *rpb1-WT8* has eight consensus repeats, and *rpb1-WT0* lacks the entire CTD. The *rpb1-WT7* and *rpb1-WT0* alleles are lethal in our background and the *rpb1-WT8* allele confers slow growth, cold-sensitive and Ino$^-$ phenotypes. Thus, our *rpb1-WT8* mutation is phenotypically equivalent to the *rpb1*Δ*103* allele in R. YOUNG's collection of *RPB1* mutants (NONET and YOUNG 1989). Details of the construction and characterization of these strains are described elsewhere (WEST and CORDEN 1995).

Construction of *RPB1*-containing plasmids: To allow selection against the wild-type *RPB1* gene, we constructed a plasmid containing two selectable markers (Figure 1). Plasmid YERPIIcyh was made by cloning the *Hin*dIII fragment of wild-type *RPB1* into YEp352 (HILL *et al.* 1986). A 1.4-kb *Bam*HI fragment from pKC3 (SIKORSKI and BOEKE 1991; kind gift of J. BOEKE) containing a functional *CYH2* gene was inserted downstream of the *RPB1* gene. The plasmid containing the *RPB1* gene can be counterselected by either 5FOA or cycloheximide. Plasmids pY1A^2(18), pY1A^5(15), pY1E^2(15) and pY1E^5(18) (see Figure 1 and CTD mutants section) carry lethal *RPB1* alleles with mutated CTDs (WEST and CORDEN 1995) cloned into a pRS315 vector (SIKORSKI and HIETER 1989). In cells harboring both plasmids, protein-coding and 3′-untranslated regions of the *RPB1* genes are putative sites of double recombination that can introduce *RPB1* sequences into the *rpb1* gene on the *LEU2* plasmid. To eliminate this possibility, in later experiments the 3′-untranslated region of *RPB1* in pYERPIIcyh was replaced by the 3′-untranslated region of *PGK1* in plasmids pYERPIIpgkcyh, pYERPIIpgk and pRPIIpgk416. pYERPIIpgk is the same as pYERPIIpgkcyh but without *CYH2*. pRPIIpgkcyh416 is the same as pYERPIIpgkcyh but is a CEN, *URA3* plasmid based on the pRS416 vector (SIKORSKI and HIETER 1989). pYERPIIpgkcyhsca is the same as pYERPIIpgkcyh but contains the *SCA1* gene between the *RPB1* and *URA3* genes.

Isolation of suppressors of lethal CTD mutants: Suppressors were isolated by selection against a wild-type *RPB1*-containing plasmid in strains carrying lethal *rpb1* mutants on a second plasmid and a disruption in the chromosomal *RPB1* gene (Figure 1). Selection against the wild-type plasmid is lethal unless a cell contains a mutation that enables RNA polymerase II to function with the mutant CTD. Several sources of unwanted revertants were minimized. The *CYH2* gene was included in the wild-type plasmid and cycloheximide was added to the 5FOA selection medium to decrease the background of mutations in the *URA3* gene during counterselection. To enrich the population of extragenically suppressed 5Foa^{r}Cyh^r revertants and minimize the contribution of unwanted recombinant revertants, we performed EMS mutagenesis before selection.

Four different strains carrying pYERPIIcyh and pY1 derivatives containing *rpb1-a2(18)*, *rpb1-e2(15)*, *rpb1-a5(15)* and *rpb1-e5(18)* mutant alleles (WEST and CORDEN 1995) were derived from strain AY7 (Table 1), a cycloheximide-resistant version of strain Z26 obtained by spontaneous selection on YPD plates containing cycloheximide. Cells were grown overnight in five ml of SC-Ura-Leu media. Approximately 5×10^8 cells were washed twice in sterile water and resuspended in 1 ml of 0.1 M K-phosphate buffer, pH 8.0. EMS (30 μl, Sigma) was added and cells were incubated for 1 hr at room temperature. Cells were washed twice and resuspended in 10% (w/v) sodium thiosulfate. A survival rate of 60% was obtained with a frequency of Can^r colonies (after mutagenesis) of 10^{-4}. After EMS treatment, 5×10^3 cells of each strain were plated on YPD plates (10^3 cells per plate) and allowed to grow for 3 days at 30°. Colonies were then replica-plated on SC plates with 1 mg/ml 5FOA and 5 μg/ml cycloheximide (SIKORSKI and BOEKE 1991). After 14 days of selection, 5Foa^{r}Cyh^r revertants were picked. To verify the absence of a wild-type CTD and the presence of a mutant CTD, the revertants were screened by PCR using oligonucleotides specific for either the mutant or wild-type CTD (WEST and CORDEN 1995).

We isolated 60 A2(18) suppressors, 16 E2(15) suppressors and four A5(15) suppressors. All of these suppressors were mated with strain N418 (NONET *et al.* 1987) and diploids were

TABLE 1

Yeast strains used in this study

Name	Genotype
YPH4[a]	*MATa ura3-52 lys 2-801^{amber} ade2-101^{ochre} his3-Δ200*
YPH47[a]	*MATα ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ1*
Z26[b]	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 sca2-1* (pRP112 [*URA3 RPB1*])
N418[b]	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 SCA2-2* (pRP112 [*URA3 RPB1*])
AY7	*MATα ura-3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 cyh2 sca2-1* (pRPIIpgkcyh [*URA3 RPB1 CYH2*])
S80[c]	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 cyh2 sca2-1* (pY1A2(18) [LEU2 *rpb1-a2 (18)*])
S29	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 cyh2 sca1-1 sca2-1* (pY1E2(15) [*LEU2 rpb1-e2(15)*])
S80I[d]	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-a2(18) cyh2 sca2-1*
S29I[d]	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-e2(15) cyh2 sca1-1 sca2-1*
AY24	*MATa ura3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 trp1-Δ63 SCA2-2* (pY1At [*LEU2 RPB1*])
AY25	*MATα ura3-52 his3-Δ200 leu2-3, 112 rpb1-Δ187::HIS3 trp1-Δ63 cyh^{R}2 sca2-1* (pY1At [*LEU2 RPB1*])
AY52	*MATα ura3-52 his3-Δ200 leu2-3,112 rpb1-Δ187::HIS3 trp1-Δ63 cyh2 sca2-1 sca1Δ::hisG* (pY1At [*LEU2 RPB1*])
AY53A	*MATa ura3-52 his3-Δ200 leu2-3, 112 trp1-Δ63 rpb1-a2(18) SCA2-2* (pY1At [*LEU2 RPB1*])
AY53E	*MATa ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 rpb1-e2(15) SCA2-2* (pY1At [*LEU2 RPB1*])
AY54A	*MATα ura3-52 his3-Δ200 leu2-3, 112 trp1-Δ63 rpb1-a2(18) sca1Δ::hisG cyh2 sca2-1* (pRPIIcyh416 [*URA3 RPB1 CYH2*])
AY56A	*MATα ura3-52 his3-Δ200 leu2-3, 112 trp1-Δ63 rpb1-a2(18) cyh2 sca2-1* (pY1At [*LEU2 RPB1*])
AY56E	*MATα ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 rpb1-e2(15) cyh2 sca2-1* (pY1At [*LEU2 RPB1*])
AY57-1	*MATa ura3-52 his3-Δ200 leu2-3,112 trp 1-Δ63 rpb1-a2(18) sca1Δ::hisG sca2-1* (pRPIIcyh416 [*URA3 RPB1 CYH2*])
AY57-5	*MATa ura3-52 his3-Δ200 leu2-3,112 trp 1-Δ63 rpb1-a2(18) sca1Δ::hisG sca2-1* (pRPIIcyh416 [*URA3 RPB1 CYH2*])
AY60A	*MATa ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 rpb1-a2(18) sca1Δ::hisG SCA2-2* (pY1At [*LEU2 RPB1*])
AY60E	*MATa ura3-52 his3-Δ200 leu2-3,112 trp1-Δ63 rpb1-e2(15) sca1Δ::hisG SCA2-2* (pY1At [*LEU2 RPB1*])

[a] SIKORSKI and HIETER (1989).

[b] NONET and YOUNG (1989). The *sca2-1* allele is present in the Z26 background while *SCA2-2* is present in N418. *SCA2-2* is dominant to *sca2-1* but the identity of the wild-type allele is not known.

[c] S80 may or may not contain a mutant allele of *SCA1*.

[d] Strains S80I and S29I are derived from S80 and S29 by integration of mutant *rpb1* alleles.

selected on SC-ura-leu plates. Diploids were replica-plated on YPD plates and, after 2 days of growth, replica-plated on SC plates with 5FOA. All of the suppressors formed inviable diploids on 5FOA media indicating that the suppressor mutations are recessive.

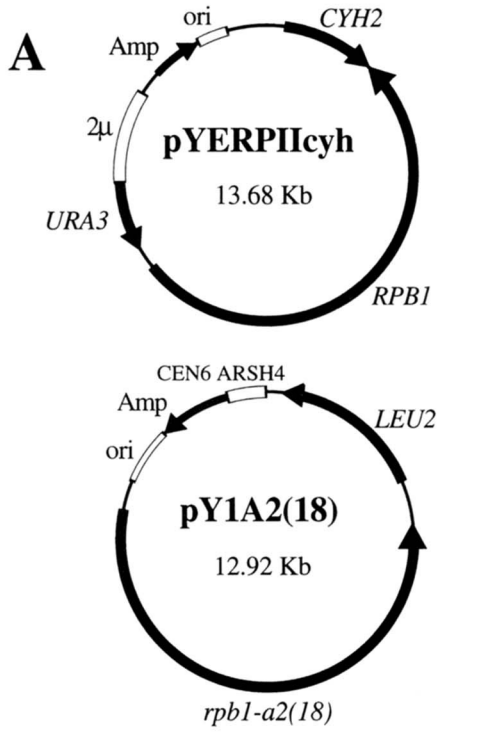
Further genetic analysis of CTD suppression has focused on two of these original suppressor strains, S80 and S29, suppressors of *rpb1-a2(18)* and *rpb1-e5(15)*, respectively. Both of these strains grow slowly (see Figure 6), are clumpy, transform poorly, have an irregular cell morphology, and survive poorly at 4° on SC media. The presence of multiple mutations in the original suppressor strains has limited their utility and the present paper will be restricted to describing their use in identifying *SCA1*, a gene that complements their recessive suppression of the lethal mutant CTD phenotype.

Integration of CTD mutations into the yeast genome: To facilitate cloning genes that complement recessive suppressors of CTD mutations, we first integrated the mutant *rpb1* genes into the genome. A *Hin*dIII fragment containing the *RPB1* gene in pYERPIIcyh was replaced by *Hin*dIII fragments containing either the *rpb1-a2(18)* or *rpb1-e2(15)* genes. A *HIS3* gene was introduced into this plasmid by replacing the *Sna*BI-*Aat*II fragment with a *Fsp*I-*Aat*II fragment derived from pRS403 (SIKORSKI and HIETER 1989). This substitution removed most of the 2-μ sequences yielding an integrative plasmid (Figure 2A). The final constructs pA2(18)RPIIYEpHIS3 and pE2(15)RPIIYEpHIS3 were linearized by *Nhe*I, which targets their integration into the previously inserted *HIS3* gene in the *rpb1Δ187 HIS3* allele (Figure 2B). The result of this integration is an *rpb1* gene flanked by two *HIS3* genes located at the *RPB1* locus (Figure 2C). Two consecutive selections on media with cycloheximide and then with 5FOA resulted in a Ura$^-$Leu$^-$His$^-$Cyh^r strain carrying an integrated *rpb1-a2(18)* or *rpb1-e2(15)* gene (Figure 2D).

Yeast genomic DNA library: We obtained a library of yeast genomic DNA cloned in a derivative of YCp50 containing a *LEU2* marker. Inserts are derived from partially digested *Sau*3A fragments of YPH1 (SIKORSKI and HIETER 1989) genomic DNA. The library contains $\sim 10^4$ individual transformants with an average insert size $\sim$10 kb and was made by F. SPENCER and P. HIETER (unpublished data).

Cloning *SCA1*: The mutant *rpb1-a2(18)* allele was first integrated into the *rpb1Δ::187* locus of S80 as described above to yield strain S80I (Table 1). Spontaneous loss of the *LEU2* plasmid carrying the original *rpb1-a2(18)* allele was achieved by growing cells in nonselective media and wild-type *RPB1* was reintroduced on the counter-selectable plasmid pYERPIIpgk. A wild-type yeast genomic library was then introduced and 4500 transformants (representing about three to four copies of the yeast genome) were obtained by selection on SC-Ura-Leu plates. After 3 days of growth at 30°, colonies were replica-plated onto SC-Leu+5FOA plates. About 100 colonies appearing inviable on 5FOA plates were traced back to the SC-Ura-Leu plates and repatched onto SC-Leu plates. After 3 days growth, patches were again replica-plated onto SC-leu+5FOA plates. After several rounds of repatching and replica-plating on SC-leu+5FOA plates, 10 strains were chosen for recovery of library plasmids. All 10 strains were mated with YPH4 and diploids were selected on SC-Ura-His. Diploids then were streaked on SC-Leu plates with 5FOA to eliminate pYERPIIpgk and the remaining library plasmid was recovered into *E. coli* (STRATHERN and HIGGINS 1991).

DNA manipulations and sequencing: Yeast transformation was performed by the lithium acetate procedure (SCHIESTL and GIETZ 1989) but the addition of high molecular weight carrier was omitted except for transformation of yeast genomic library DNA. All DNA manipulations, including restric-



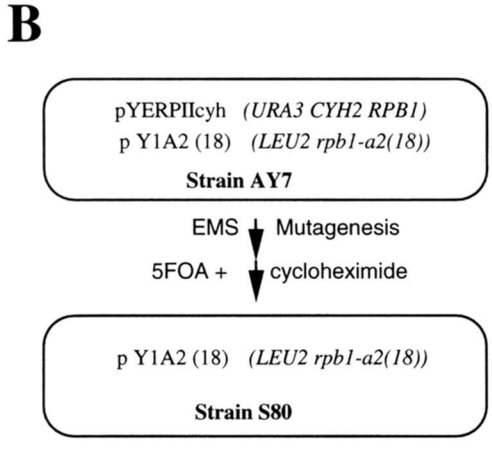


FIGURE 1.—Selection scheme for isolating *sca* mutants. (A) Plasmid maps for plasmids used in selection of sca mutants. Details of the construction of these plasmids are given in MATERIALS AND METHODS. Shaded block arrows refer to the positions of different selectable genes whereas white blocks refer to replication and regulatory regions. (B) Stepwise selection of *sca* mutant S80.

tion digestions, ligations, PCR, $CaCl_2$ *E. coli* transformations, gel electrophoresis, oligonucleotide labeling and Southern analysis, were performed essentially as described by SAMBROOK *et al.* (1989). Plasmid DNA preparations were done using commercial kits (Qiagen, Promega) or by the CsCl method described in SAMBROOK *et al.* (1989). Yeast plasmid and genomic DNA were isolated by standard procedures (AUSUBEL *et al.* 1991). DNA sequence was obtained by dideoxynucleotide terminator chemistry in an Applied Biosystems 373-A automated DNA sequencer. DNA comparison studies and homology searches of the DNA sequence database were done through the Biomedical Computer Center, Frederick, MD, using the program BLAST (ALTSCHUL *et al.* 1990).

***SCA1* disruptions:** Construction of the *sca1Δ::URA3* allele

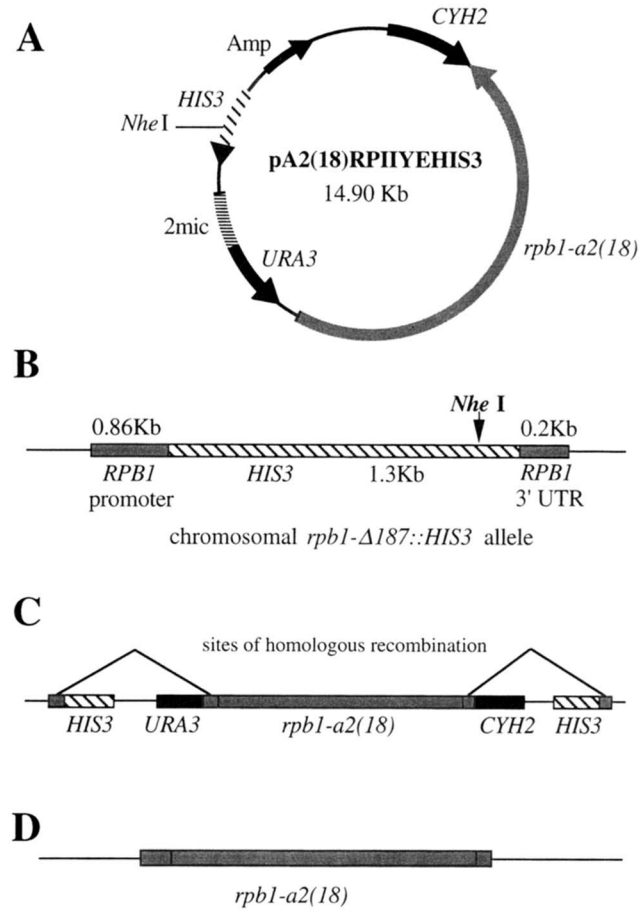


FIGURE 2.—Strategy for integration of *rpb1-a2(18)* and *rpb1-e2(15)* into the RPB1 locus. (A) The integration plasmid was linearized at a *Nhe*I site within the *HIS3* gene. (B) The chromosomal target is the *HIS3* gene used to disrupt the *RPB1* gene in strain Z26 (NONET *et al.* 1987). (C) The mutant CTD plasmid integrated between duplicated *HIS3* genes. (D) After selection against *URA3* and *CYH2*, the mutant *rpb1* allele is stably integrated into the correct chromosomal location.

consisted of cloning of an *Fsp*I fragment containing the *URA3* gene of pRS406 (SIKORSKI and HIETER 1989) into the *SCA1*-containing library plasmid. The *SCA1* gene was cut with *Bsp*EI-*Nco*I (Figure 4C) to replace >75% of the *SCA1* coding sequence (from amino acid 289 to 1379). Construction of the *sca1Δ::hisG* allele consisted of cloning a *Bgl*II-*Bam*HI fragment of pNKY51 (ALANI *et al.* 1986) containing a 3.8-kb *hisG-URA3-hisG* fragment into the *SCA1*-containing library plasmid. The *SCA1* gene was again cut by *Bsp*EI-*Nco*I to replace most of the *SCA1* gene and yeast strain AY25 (*MATα*) was transformed with a *Kpn*I fragment containing the deletion construct. Correct integration was checked by Southern blot analysis of Ura^+ transformants. Positive clones were grown overnight in YPD and *URA3* deletions were selected on 5FOA plates. Deletion strains were verified by Southern blot analysis.

Construction of *sca1Δ::hisG* strains: Genetic analysis of *sca1Δ::hisG* suppression was carried out to verify that the suppressor phenotype segregates with the disrupted allele and to clarify the role of a second gene in suppression of CTD mutations. The strains used in these crosses are described in Table 1 and the results of the crosses are described in Table 3.

In AY53A and AY53E, *rpb1-a2(18)* and *rpb1-e2(15)* alleles were integrated into genome of AY24 (*MAT***a**) essentially as

described in Figure 2. AY56A and AY56E were made by integration of the corresponding mutant *rpb1* alleles into the chromosome of strain AY25 as described in Figure 2. Strain AY25 was derived from strain Z26 (Table 1) while strain AY24 was derived from strain N418 (Table 1). We initially assumed that Z26 and N418 were isogenic (NONET and YOUNG 1989), but further analysis revealed genetic differences important for suppression. AY54A was made by integration of the *rpb1-a2(18)* allele into the chromosome of AY52. All integrations into the genome were verified by Southern blot analysis.

AY53/AY54 diploids were selected on -Leu-Ura medium. Subsequent growth in -Ura medium allowed identification of a Ura^+Leu^- diploid missing the *LEU2 RPB1*-containing plasmid. This strain was sporulated and dissected tetrads were tested for suppression by growth on SC+5FOA medium. Failure of the suppressor phenotype to segregate 2:2 in the AY53/AY54 cross suggested a requirement for an additional gene in the Z26-derived background. To determine whether *sca1* suppression segregates 2:2 in the Z26-derived background, we set up a mating between several $5FOA^r$ *MAT***a** *sca1Δ::hisG* strains derived from the progeny of the AY53/AY54 cross (AY57-1 and AY57-5, Table 1), and *MATα SCA1* strains AY56A and AY56E, which are also derived from AY25. Diploids were selected on SC-Ura-Leu medium and the *LEU2 RPB1* plasmid was lost as described above. The resulting strain was sporulated, tetrads were dissected, and progeny were tested for 5FOA resistance.

A final cross was designed to look at segregation of background mutations at the *SCA2* locus. Strain AY54 was mated with AY60A and AY60E. The AY60 strains were derived from AY53 parents by integration of the *sca1Δ::hisG* allele as previously described. Diploids were selected on SC-Ura-Leu medium and after sporulation tetrads were dissected and patched onto YPD. Because *sca1Δ::hisG* diploid strains do not sporulate, we introduced a SCA1 gene into the RPB1-containing plasmid vector pYERPIIpgkcyhsca. After several days growth, patches were replica plated to either SC-Leu or SC-Leu+5FOA plates. Only tetrads in which all four spores were Leu^- (having lost the *LEU2 RPB1* plasmid) are reported in Table 3.

RESULTS

Cloning and molecular characterization of the *SCA1* gene: Two recessive suppressor mutations were isolated by their ability to restore viability to strains carrying otherwise lethal CTD substitution mutations. Strain S80 was isolated as a suppressor of a mutant CTD containing alanine in position two, while strain S29 was isolated as a suppressor of a mutant CTD containing glutamic acid in position two. Complementation of these recessive suppressor mutations (MATERIALS AND METHODS) was used to identify a candidate suppressor gene. Two identical plasmids containing 10-kb inserts of yeast genomic DNA were able to complement suppression upon transformation of S80I, a derivative of the original suppressor strain with *rpb1-a2(18)* integrated at the *RPB1* locus. These plasmids were also introduced into S29I, which differs from the original suppressor S29 by integration of *rpb1-e2(15)*. Both plasmids were able to complement suppression in both strains.

Deletion analysis identified a 5.5-kb *Cla*I-*Nde*I fragment that is sufficient to complement the suppression in strains S80 or S29 (Figures 3 and 4). The sequence of this fragment (GenBank accession number U09176) revealed a 5.3-kb open reading frame, and internal deletions in this open reading frame eliminated the ability to complement S80 suppression. We have named this gene *SCA1* for suppressor of CTD alanine. Subsequent sequence comparison revealed that *SCA1* is identical to the yeast *SRB9* gene, which was isolated by its ability to complement a recessive mutation that suppresses the cold-sensitive phenotype of a CTD truncation mutation (HENGARTNER *et al.* 1995). The predicted molecular weight of the SCA1 protein is 159,749 D. Database searches reveal no significant similarity to previously identified proteins. The predicted amino acid sequence of the SCA1 protein contains several highly charged regions, asparagine-rich regions, and a polyglutamine stretch.

Physical mapping of *SCA1*: A 1.8-Kb *Eco*RI-*Nde*I probe containing the 3′ end of the *SCA1* gene and the 5′ end of the adjacent *APT2* gene (YURYEV and CORDEN 1994) was hybridized to an ordered set of phage clones containing yeast inserts (ATCC 76269). This probe hybridized to a single λ phage clone (ATCC 70580), which places the *SCA1* gene on the distal right arm of chromosome *IV*.

***SCA1* is a nonessential gene:** To determine whether *SCA1* is an essential gene, one of the *SCA1* alleles was disrupted in a YPH4/YPH47 diploid strain (see MATERIALS AND METHODS). One of the transformants was sporulated and 12 tetrads were dissected. In all 12 tetrads, all four spores were viable and the Ura marker segregated 2:2. By a different technique (MATERIALS AND METHODS) a *sca1Δ::hisG* allele was introduced into the haploid strain AY25. The resulting strain (AY52) has a slightly slow rate of growth with a division time of ~120 min (compared with 90 min for AY25) and cells are very clumpy. AY52 has no temperature or cold-sensitive phenotypes and is Ino^+.

Suppression of different CTD mutations: To establish a relationship between the original suppressor strains S80 and S29 and the *sca1* disruption strain AY52, we tested these strains for their ability to suppress different CTD mutations. Different *rpb1* alleles (WEST and CORDEN 1995) were introduced into S80, S29, and AY52 (MATERIALS AND METHODS). The resulting strains carry a chromosomal suppressor mutation and *RPB1* on a counterselectable *URA3* plasmid. To assess the ability of these strains to suppress different CTD mutations, we tested their ability to grow in the presence of 5FOA. An example of the selection is shown in Figure 5 and the complete results are shown in Table 2. The three recipient strains exhibit the same pattern of suppressibility indicating that S80, S29, and *sca1* are functionally related.

Do the S80 and S29 strains contain mutant alleles of *SCA1*? To address this question, we first marked the *SCA1* locus by introducing a *LEU2* gene between *SCA1*

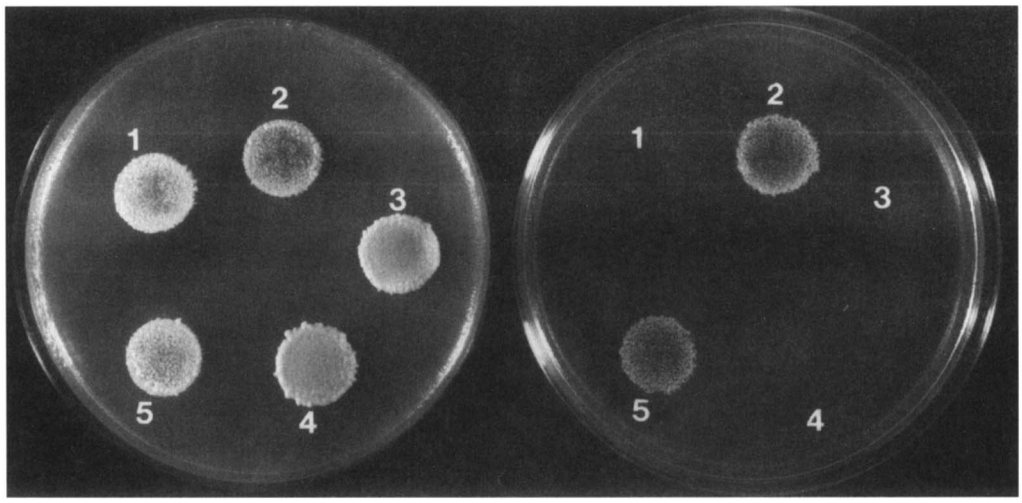


FIGURE 3.—*SCA1* complements S80 suppression. Strains were patched on an SC-leu-ura plate then replica-plated on YPD plates and after 3 days of growth replica-plated on an SC-leu plate (left) or sequentially on SC-leu + cycloheximide, followed by SC-leu + 5FOA and cycloheximide (right). (1) Strain S80I with YEpRPIIpgk transformed with pRS415 (*LEU2*, CEN) (SIKORSKI and HIETER 1988) carrying a 5.5-Kb *Cla*I-*Nde*I fragment containing the complete *SCA1* gene. (2) Strain S80I containing YEpRPI-Ipgk transformed with pRS415 (*LEU2*, CEN) alone. (3) Strain AY7 with YEpRPIIcyh and pY1A^2(18). This strain was mutagenized to isolate extragenic suppressors of *rpb1-a2(18)*. (4) Diploid strain S80/N418 heterozygous for the suppressor mutation. (5) Strain S80I carrying YEpRPIIpgk transformed with a pRS415 (*LEU2*, CEN) based plasmid carrying the *SCA1* gene with internal *Nsi*I deletion (Figure 4).

and the adjacent *APT2* gene in strains AY54A and AY54E. The resulting strains were mated to S80I and S29I and sporulated. Note that these crosses were homozygous for *SCA2-2*. Resulting haploid strains were tested for cosegregation of the Leu$^+$ and 5FOA^s (*SCA1*) phenotypes. In S29 crosses, 54 of 56 Leu$^+$ progeny were also 5FOA^s, indicating that S29 is a mutant allele of *SCA1*. In S80 crosses, 36 of 42 Leu$^+$ progeny were also 5FOA^s. This result could be interpreted in several ways. For example, S80 could be an allele of *SCA1* in which the mutation is some distance from the *LEU2* marker. Alternatively, the presence of a significant number of Leu$^+$ and 5FOA^r segregants could indicate involvement of other genes in S80 suppression. Further studies will be necessary to unequivocally determine the relationship between S80, S29, and *SCA1*.

Genetic analysis of *sca1Δ::hisG* strains: Further genetic analysis of *sca1* deletion strains indicated that suppression of CTD mutations requires a second gene we have designated *SCA2*. Table 3 presents the results of

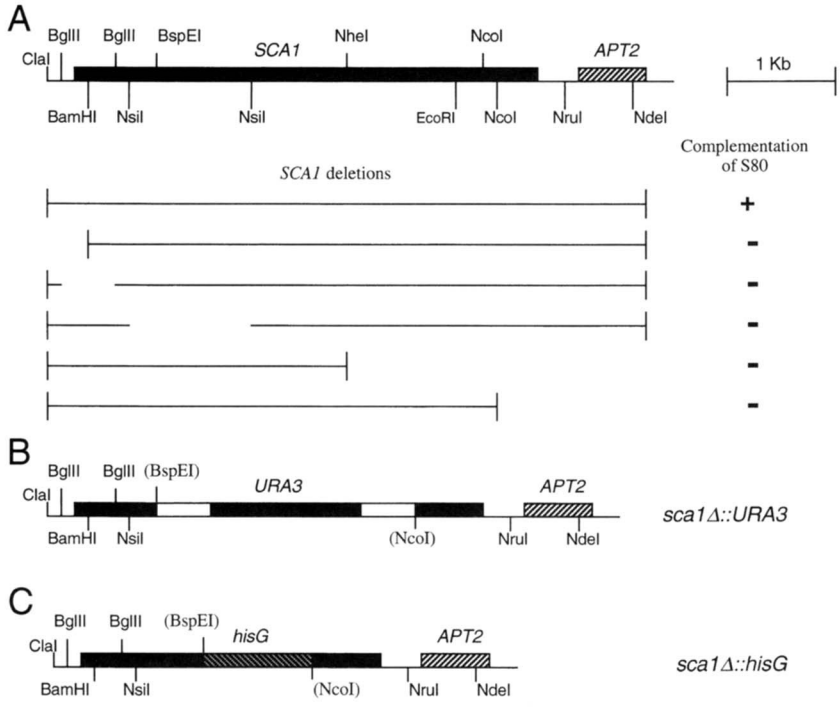


FIGURE 4.—*SCA1* maps. (A) Restriction map of the *SCA1-APT2* locus located on distal right arm of chromosome *IV*. Deletions used to map the position of essential *SCA1* sequences are shown below the map. (B) Map of the *sca1Δ::URA3* allele. (C) Map of *sca1Δ::hisG* allele present in AY52, AY54A, AY57-1 and AY57-5.

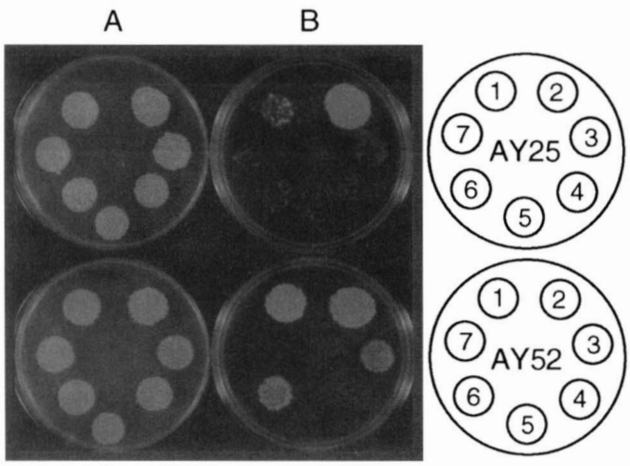


FIGURE 5.—Suppression by *SCA1* deletion. Strains carrying a deletion in the *SCA1* gene suppress *rpb1-WT7, rpb1-a2(18), rpb1-e2(15)* but not *rpb1-a5(15), rpb1-e5(18)* or *rpb1-WT0* mutations. Strain AY25 (*SCA1*$^+$) and the isogenic strain AY52 (*sca1*Δ*::hisG*) were transformed with CEN *LEU2* plasmids carrying different *RPB1* alleles. Leu$^+$ transformants were patched on an SC-ura-leu plate. After 3 days of growth patches were replica-plated onto YPD plates, and then onto either SC-leu plates (A); or sequentially to SC-leu + cycloheximide and then SC-leu with 5FOA + cycloheximide (B). Both strains have an *RPB1* gene on the doubly counterselectable RPIIpgk416 plasmid. (1) pY1WT(7) transformant. (2) Y1At (full length CTD; WEST and CORDEN 1995) transformant. (3) pY1A^2(18) transformant. (4) pY1E^5(18) transformant. (5) pY1ΔA (complete CTD deletion; WEST and CORDEN 1995) transformant. (6) pY1E^2(15) transformant. (7) pY1A^5(15) transformant. Note that the patch of AY25 transformed with pY1WT(7) (patch 1) has acquired revertants that have duplications in the *RPB1* gene region encoding CTD (data not shown).

three genetic crosses involving disrupted *SCA1* alleles. In the first crosses (1a and 1b), we attempted to associate the suppressor phenotype with the disrupted *SCA1* allele. While the number of informative tetrads is small, the failure of suppression of CTD mutations to segregate 2:2 (particularly in cross 1b) indicates the presence of an additional gene or genes in AY54 essential for *sca1*-mediated suppression.

In the second set of crosses shown in Table 3, we mated *SCA1* and *sca1*Δ*::hisG* strains both derived from the Z26 background. The progeny display 2:2 segregation of the suppressor phenotype indicating that *sca1*Δ*::hisG* is sufficient for suppression in the Z26-derived background.

In the third cross shown in Table 3, we created *sca1*Δ*::hisG* alleles in several N418-derived strains and mated these strains with a similarly disrupted Z26-derived strain. The progeny of these crosses show 2:2 segregation of the suppressor phenotype indicating that a single gene in the Z26 background is sufficient to allow *sca1* suppression of CTD mutations. The AY54A/AY60A diploid is 5FOA^s, indicating that the Z26-derived allele is recessive. Based on these crosses we have named this gene *SCA2* and have designated the Z26 allele *sca2-1* and the N418 allele *SCA2-2*.

Dominant SRB mutations suppress CTD mutations: The identity of *SCA1* and *SRB9* suggested that other *srb* mutations could suppress CTD substitution mutations. The dominant *SRB* mutations *SRB2-1, SRB4-1, SRB5-1* and *SRB6-1* (THOMPSON *et al.* 1993) were transformed with the CTD mutants shown in Table 2. Selection on 5FOA (not shown) demonstrated that these *SRB* mutants have the same suppression phenotype as the strains shown in Table 2.

Dominant effect of *rpb1-a5(15), rpb1-e5(18)* and *rpb1-WT0* mutations: Strains containing an *RPB1* gene on one plasmid and *rpb1-WT0* on another have slightly slowed growth, indicating that the *rpb1-WT0* mutation has a weak dominant phenotype (WEST and CORDEN 1995). To test other CTD mutations for dominance, we transformed mutant alleles into an S80I-derived strain carrying pYERPIIpgkcyh. Selection against the *RPB1*-containing plasmid shows that strain S80I cannot grow with *rpb1-a5(15), rpb1-e5(18)* or *rpb1-WT0* alleles in the absence of the wild-type *RPB1* gene. Thus, whereas cells suppressing the lethal effects of Ser-to-Ala substitutions at position two can grow with or without wild-type *RPB1*, the presence of a position five substitution or complete CTD deletion mutation has a dominant lethal effect. The dominance of position five mutants over position two mutants could not be similarly tested in the wild-type background but the slightly slower growth of WT0, E5, and A5 strains (not shown) suggests that weak dominance occurs in *SCA1* cells.

DISCUSSION

The CTD of RNA polymerase II plays an essential, though poorly understood, role in transcription. Phosphorylation of multiple sites within the CTD has been

TABLE 2

The S80, S29, and *sca1*Δ*::hisG* mutants exhibit similar patterns of suppression of CTD mutations

Suppressor strain	CTD mutation					
	WT(7)	A2(18)	E2(15)	A5(15)	E5(18)	WT(0)
S80	+	+	+	−	−	−
S29	+	+	+	−	−	−
AY52	+	+	+	−	−	−

+ indicates the ability of different CTD mutant alleles to support growth on 5FOA-cyclohexamide medium in different suppressor strains containing a *URA3 RPB1 CYH2* plasmid; −, no growth.

TABLE 3

sca2* is required for *sca1* suppression

Cross	Strains	Relevant genotypes	Full tetrads	Zero 5FOA^R spores	One 5FOA^R spore	Two 5FOA^R spores
1a	AY54A	*sca1::ΔhisG rpb1-a2(18) sca2-1*	3	1		2
	AY53A	*SCA1$^+$ rpb1-a2(18) SCA2-2*				
1b	AY54A	*sca1::ΔhisG rpb1-a2(18) sca2-1*	5		5	
	AY53E	*SCA1$^+$ rpb1-e2(15) SCA2-2*				
2a	AY56A	*SCA1$^+$ rpb1-a2(18) sca2-1*	1			1
	AY57-1	*sca1::ΔhisG rpb1-a2(18) sca2-1*				
2b	AY56E	*SCA1$^+$ rpb1-e2(15) sca2-1*	3			3
	AY57-1	*sca1::ΔhisG rpb1-a2(18) sca2-1*				
2c	AY56A	*SCA1$^+$ rpb1-a2(18) sca2-1*	6			6
	AY57-5	*sca1::ΔhisG rpb1-a2(18) sca2-1*				
2d	AY56E	*SCA1$^+$ rpb1-e2(15) sca2-1*	8		1	7
	AY57-5	*sca1::ΔhisG rpb1-a2(18) sca2-1*				
3a	AY54A	*sca1::ΔhisG rpb1-a2(18) sca2-1*	12		1	11
	AY60A	*sca1::ΔhisG rpb1-a2(18) SCA2-2*				
3b	AY54A	*sca1::ΔhisG rpb1-a2(18) sca2-1*	7		1	6
	AY60E	*sca1::ΔhisG rpb1-e2(15) SCA2-2*				

5FOA resistance indicates suppression of the mutant rpb1 allele.

temporally linked to the initiation of transcription, leading to the proposal that CTD modification is required to release RNA polymerase from the preinitiation complex (LAYBOURN and DAHMUS 1990; DAHMUS 1994). Studies with inhibitors of CTD kinase, however, suggest that CTD phosphorylation is not essential for transcription initiation *in vitro* (SERIZAWA *et al.* 1993). To examine the role of CTD phosphorylation *in vivo*, we have created and studied a set of mutant RNA polymerase genes in which phosphorylatable serine residues in every repeat of the CTD have been changed (WEST and CORDEN 1995). The lethal effect of alanine substitution at either position two or five is consistent with a need to phosphorylate these sites, although the possibility that altered CTD structure is responsible cannot be ruled out. To begin to address CTD phosphorylation genetically, we have isolated suppressors of CTD phosphorylation site mutations.

***SCA1* and the mutant CTD suppressor phenotype:** Our original suppressor strains (S80 and S29) were identified by their ability to suppress alanine or glutamate substitutions in position two of the consensus CTD heptapeptide repeat. Suppression in both of these strains is recessive; this allowed us to clone the wild-type *SCA1* gene by complementation. While we have not proved that the original suppressors contain mutant *sca1* alleles, *sca1Δ::hisG* cells display the same pattern of suppressibility as the original suppressor strains. Linkage of suppression complementation to a *LEU2* marker inserted adjacent to the *SCA1* locus in S29 suggests that S29 contains a mutant *sca1* allele. A similar test for linkage between S80 and *SCA1* was inconclusive leaving open the possibility that an additional mutation may be required for suppression by S80.

The *SCA1* mutants, like the *SRB* mutants, were isolated by suppression of a mutant CTD phenotype in a strain derived from Z26. The results of the matings shown in Table 3 indicate that an additional gene is required for *sca1* mutants to suppress the CTD mutant phenotype. We have named this gene *SCA2* and have shown that the allele present in Z26 (*sca2-1*) is recessive to the allele in N418 and is required for suppression by the *sca1Δ::hisG* suppressor. It will be of some interest to identify the *SCA2* gene and to determine whether the *sca2-1* allele is required for suppression by other SRB genes identified in Z26 (NONET and YOUNG 1989).

Serines in positions two and five of the consensus CTD repeat sequence are genetically distinct: *SCA1* suppressor mutants are able to suppress substitution mutations in the second position of the consensus CTD heptapeptide repeat but not mutations in position five. This result demonstrates that position two and five are involved in genetically distinct functions and that *SCA1* is involved in the function provided by position two. Position five suppressors have been isolated but have not been tested for suppression of position two mutations. A second indication that positions two and five are genetically distinct comes from analysis of the position five mutants in the *sca1* mutant background. In contrast to the case in a *SCA1* background, the position five mutants display a dominant negative lethal phenotype in *sca1* cells. Apparently the loss of function of *SCA1* that suppresses position two mutations changes the position five mutated RNA polymerase such that it now interferes with the function of the position two mutated enzyme.

Our genetic results imply that CTD function depends

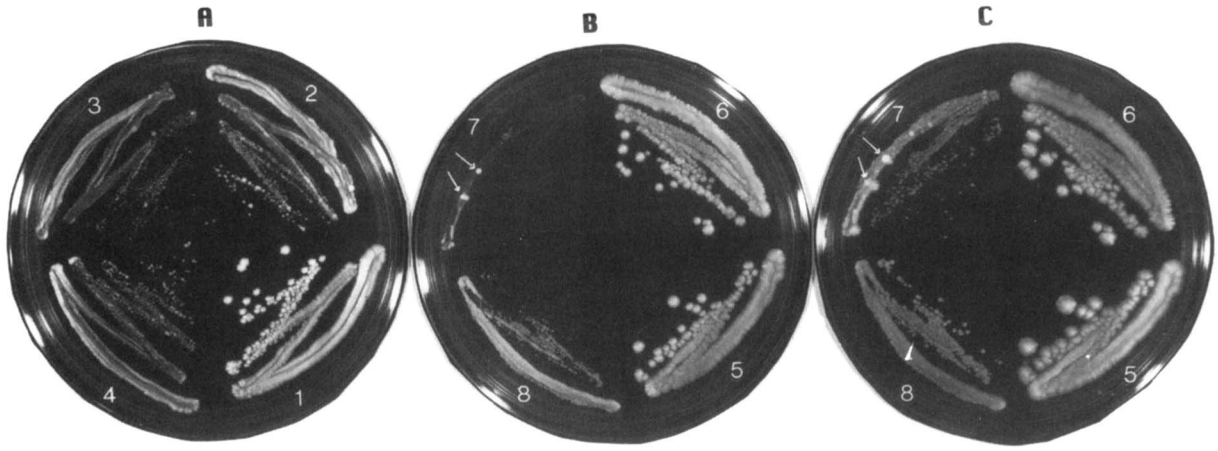


FIGURE 6.—Phenotypes of the S80, S29 and AY52 strains with different types of *RPB1* alleles with mutated CTD. (1) AY7 (*RPB1*$^+$); (2) S80 [*rpb1-a2(18)*]; (3) S80I [*rpb1-a2(18)*]; (4) S80I [*rpb1-a2(18); RPB1*$^+$]; (5) AY52 (*RPB1*$^+$); (6) AY52 (*rpb1-WT7*); (7) AY52 [*rpb1-a2(18)*]; (8) AY52 [*rpb1-e2(15)*]; (A and B) After 4 days of growth on YPD plate at 30°; (C) the same as B, but after 6 days of growth. Arrows show the faster growing revertants of the AY52 strain carrying Y1A^2(18) plasmid. Most of the *rpb1-a2(18)* suppressors isolated after mutagenesis acquire such revertants at high frequency. Also note that S80 strain and its derivatives grow as fast as these revertants and that introduction of the *RPB1* gene in S80I does not rescue its slow growth phenotype unlike AY52 strain.

on more than one type of phosphorylation; an implication supported by biochemical experiments. We earlier reported that Cdc2 kinase phosphorylates serine residues in both positions two and five of the consensus heptapeptide (ZHANG and CORDEN 1991). Other CTD kinases are specific for serine in position five. STONE and REINBERG (1992) identified an Aspergillus CTD kinase that phosphorylates serine at position five but not two. M. GEBARA and J. L. CORDEN (unpublished observations) have shown that a CTD kinase associated with yeast TFIIH is specific for position five. Finally, ROY *et al.* (1994) and M. GEBARA and J. L. CORDEN (unpublished data) have shown that the mammalian TFIIH-associated CTD kinase CAK (Cdc2 activating kinase) phosphorylates serine five but not serine two. These results provide a biochemical basis for our genetic observation that the serines in positions two and five are not equivalent and suggest that Sca1p may regulate phosphorylation at position five.

***SCA1* deletion suppresses CTD truncation mutations:** In addition to suppressing substitution mutations, *SCA1* mutants can also suppress the effects of CTD truncation. In a *sca1* background, the slow growth phenotype of *rpb1-WT8* was suppressed and the previously lethal *rpb1-WT7* was able to grow. The ability of a *sca1* mutation to suppress CTD truncation mutations suggests that truncation is similar to amino acid substitutions involving position two.

Several observations suggest, however, that CTD truncation and position two substitution are not completely equivalent. First, the suppressed truncation mutants grow faster than the substitutions (Figure 6). This might be expected if position two serves some important, yet almost nonessential function. In truncation mutations, some position two sites remain while the substitution mutations have permanently lost these sites. A second difference between the CTD truncation and position two substitution mutations concerns the generation of spontaneous revertants of the slow growth phenotype. Slow-growing CTD truncation mutants rapidly accumulate revertants by reduplication of the sequences encoding the heptapeptide repeats (Figure 5; NONET and YOUNG 1989). In contrast, we have never seen a rapidly growing revertant of a slow-growing CTD substitution mutant with duplicated mutant hetapeptide repeats. It is possible that the lost function of the substituted CTD cannot be compensated by an increase in the number of the nonconsensus repeats. Consistent with this observation we note that the A2(18) mutation has more position five sites that the sum of position two plus five sites in the *rpb1-WT8* truncation.

Other genes involved in CTD function: The ability of *SCA1* mutants to suppress CTD truncation is similar to the Srb phenotype reported by NONET and YOUNG (1989). Nine *SRB* genes have now been identified by YOUNG and colleagues (NONET and YOUNG 1989; THOMPSON *et al.* 1993; HENGARTNER *et al.* 1995; LIAO *et al.* 1995) and their protein products have been shown to form part of a high molecular weight complex that associates with a fraction of RNA polymerase II (THOMPSON *et al.* 1993) enabling pol II to respond to transcription activator proteins *in vitro* (reviewed in KOLESKE and YOUNG 1995). How the mutant SRB proteins function to enable transcription by truncated CTD mutants is unknown.

We have found that *SRB2-1, SRB4-1, SRB5-1* and *SRB6-1* mutations (NONET and YOUNG 1989) identified as suppressors of the CTD truncation mutation also can suppress lethal *rpb1-a2(18)* and *rpb1-e2(15)* alleles but not r*pb1-a5(15)* or *rpb1-e5(18)* alleles. Thus, it ap-

pears that CTD truncation mutation and CTD substitution at position two mutations are genetically interchangable. It is possible that there may be a different set of genes suppressing the CTD substitution at position five mutations.

Deletion of the *SIN1* gene also suppresses the cold-sensitive phenotype of a CTD truncation containing 11 heptapeptide repeats and restores viability to a lethal CTD truncation mutation containing only nine repeats (PETERSON *et al.* 1991). We have tested CY192 (*sin1* null) and its *SIN1*$^+$ counterpart CY190 for their ability to suppress our CTD mutations. Neither strain is able to suppress the CTD substitution mutations (data not shown). Surprisingly, both *sin1* and *SIN1*$^+$ strains allow growth of *rpb1-WT7*, a lethal truncation in our background. Apparently, the different strain background in CY190 contains a mutation that allows growth of our CTD truncation but not that of *rpb1D-103*, which contains 11 heptapeptide repeats (PETERSON *et al.* 1991). Thus, the nature of the CTD truncation mutation as well as the genetic background of the strain are important in suppression of CTD mutations.

The complexity of the holoenzyme provides an elaborate network of potential genetic interactions. In addition to the SRBs, holoenzyme contains other factors, such as Gal11p, Sug1p, Rgr1p, and Sin4p, that play multiple roles in transcription regulation (KOLESKE and YOUNG 1995; LI *et al.* 1995). Several additional holoenzyme components remain to be identified. H. RIENHOFF and C. JOHNSON in our lab identified the genes *GRR1* (FLICK and JOHNSTON 1990) and *TIF51A* (SCHNIER *et al.* 1991) as high copy suppressors of a mutant yeast RNA polymerase II containing a long CTD derived from the mouse RNA polymerase II gene (unpublished data). These proteins may also be holoenzyme components.

What role Sca1p (Srb9p) plays in holoenzyme function is not clear. The only holoenzyme components with a suggested function are Srb10p and Srb11p, which are a Cdk/cyclin pair implicated in CTD phosphorylation (LIAO *et al.* 1995). Alleles of *SRB10* and *SRB11* have also been identified in searches for genes involved in regulated transcription repression. *ARE1* (*SRB10*) is necessary for full repression of **a**-specific genes in α cells (WAHI and JOHNSON 1995). Both *SSN3* (*SRB10*) and *SSN8* (*SRB11*) are involved in glucose repression (KUCHIN *et al.* 1995).

SCA1 shares several properties with these genes. First, the *sca1* mutants we have studied are recessive like the *srb10* and *srb11* mutants reported by HENGARTNER *et al.* (1995). Second, like the *are1* mutants reported by WAHI and JOHNSON (1995), *sca1* mutant cells are clumpy and mate poorly. Finally, we have recently determined that *sca1* mutant cells are defective in glucose repression (J. CORDEN, unpublished data). These results indicate that Sca1p may be part of a holoenzyme subcomplex (with Srb10p and Srb11p) involved in transcription repression. The results presented here suggest that CTD phosphorylation is important in repression and indicate different roles for serines two and five in the consensus heptapeptide repeat. Taken together, the genetics of CTD suppression indicate a complex set of interactions among a number of different genes suggesting we are still far from understanding CTD function, even in *S. cerevisiae.*

We thank Dr. PHIL HIETER for providing the yeast genomic library and Dr. R. YOUNG for providing strains and exchanging the data. We are especially grateful to Dr. JEF BOEKE for helpful advise and comments on the manuscript. This work was supported by the Howard Hughes Medical Institute.

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Communicating editor: F. WINSTON