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The *sua8* Suppressors of *Saccharomyces cerevisiae* Encode Replacements of Conserved Residues within the Largest Subunit of RNA Polymerase II and Affect Transcription Start Site Selection Similarly to *sua7* (TFIIB) Mutations

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Mutations in the *Saccharomyces cerevisiae sua8* gene were found to be suppressors of an aberrant ATG translation initiation codon in the leader region of the *cyc1* gene. Analysis of *cyc1* transcripts from *sua8* mutants revealed that suppression is a consequence of diminished transcription initiation at the normal start sites in favor of initiation at downstream sites, including a site between the aberrant and normal ATG start codons. This effect is not *cyc1* gene specific since initiation at other genes, including *ADH1*, *CYC7*, and *HIS4*, was similarly affected, although initiation at *HIS3* and *SPT15* was unaffected. The *SUA8* gene was cloned and partially sequenced, revealing identity to *RPB1*, which encodes the largest subunit of RNA polymerase II. The *sua8* suppressors are the result of single amino acid replacements of highly conserved residues. Three replacements were found either within or immediately preceding homology block D, and a fourth was found adjacent to homology block H, indicating that these regions play a role in defining start sites in vivo. Nearly identical effects on start site selection were observed for *sua7* suppressors, which encode altered forms of TFIIB. Synthetic lethality was associated with double *sua7 sua8* suppressor mutations, and recessive *sua7* mutants failed to fully complement recessive *sua8* mutants in heterozygous diploids (nonallelic noncomplementation). These data indicate that the largest subunit of RNA polymerase II and TFIIB are important determinants of transcription start site selection in *S. cerevisiae* and suggest that this function might be conferred by interaction between these two proteins.

Transcription initiation by RNA polymerase II (RNA pol II) is mediated by the ordered assembly of a preinitiation complex that encompasses the initiation site (17, 83). In addition to RNA pol II, formation of the preinitiation complex involves several general transcription factors, including TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIJ (reviewed in references 24 and 91). Complex assembly is initiated by TFIID, which includes TBP, the TATA-binding protein. TFIIB then binds to form a TATA-TFIID-TFIIB complex that is a prerequisite for RNA pol II binding. In DNase I protection experiments, TFIIB covers the initiator region, suggesting a role in start site selection (17). RNA pol II, in association with TFIIF, then binds the complex, followed by TFIIE, TFIIH, and TFIIJ. This model was developed by using primarily the adenovirus major late promoter and was thought to reflect initiation at all or most eukaryotic class II promoters. Recently, however, transcription from a supercoiled immunoglobulin heavy-chain gene was reconstituted by using only TBP, TFIIB, and RNA pol II, establishing that these three components are sufficient for accurate initiation, at least under certain conditions (58).

Although many of the components of the preinitiation complex have been defined and a general model for transcription initiation has been developed, the mechanisms by which RNA pol II selects transcription start sites remains poorly understood. In higher eukaryotes, transcription generally occurs at a fixed distance from the TATA promoter element (9, 25), with no clear preference for specific DNA sequences (14). This finding implies that start sites are defined simply by spacing from TATA. However, there is at least one example of a functional TATA box located downstream of the initiation site (21), and in some cases, the correct start sites are retained after elimination of the TATA box (7, 87). Moreover, many promoters lack an obvious TATA box. Housekeeping genes, for example, often contain a GC-rich region in lieu of a TATA box. Initiation from this class of promoter generally occurs at multiple, dispersed sites and is dependent on the transcription factor Sp1 (15, 43). Other promoters contain neither a TATA box nor a GC-rich sequence. Such promoters are often developmentally regulated and contain either single or multiple, clustered start sites (e.g., see reference 78).

Studies designed to explain how initiation occurs in the absence of a TATA box have identified initiators that function to direct start site selection (4, 6, 35, 48, 78). Initiator elements are functionally similar to TATA elements in that either can be sufficient to direct accurate start site selection (56). Whereas TATA directs initiation ~30 bp downstream, the initiator encompasses the start site. In the absence of a TATA box, initiators are still dependent on TBP (79), a finding that is in accord with the TBP requirement for initiation by all three classes of RNA polymerase (26, 72). A recent study suggests that the initiator functions by directing TBP to the −30 region irrespective of sequence (92). Sequence analysis has failed to define a single consensus initiator, and several distinct initiator-binding proteins have been identified (20, 48, 65, 73).

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TABLE 1. Yeast strains used in this study

Strain[a]	Genotype
T15	*MATα CYC1⁺ cyc7-67 leu2-3,112 ura3-52 cyh2*
T16	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2*
YMH75	*MATa cyc1-5000 cyc7-67 ura3-52 leu2-3,112 his3-Δ1 trp1-289 can1-100*
YDW546	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua7-1*
YMH71-9C	*MATa cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1*
YIP363	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua7-3*
YRB78	*MATa cyc1-5000 cyc7-67 ura3-52 his1-1 can1-100 sua8-1*
YDW383	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua8-1*
YDW438	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua8-2*
YDW575	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua8-3*
YDW5112	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua8-4*
RS420[b]	*MATa ura3-52 leu2-3,112 his3 his4* (or *his7*) *trp1 rpb1-1*
B-6088[c]	*MATa cyc1-1 cyc7-67 ura3-52 his5-2 lys2*
B-7056[c]	*MATα cyc1-706::CYH2 cyc7-67 ura3-52 leu2-3,112 cyh2*

[a] Except where indicated, all strains were constructed in our laboratory. Strains T15 and T16 are isogenic; the YDW and YIP strains were isolated as suppressors of the *cyc1-5000* mutation in T16 (see Materials and Methods). YDW546 and YIP363 were described previously (62).
[b] From M.-S. Lee and W. T. Garrard (University of Texas Health Sciences Center, Dallas). The *rpb1-1* allele is defined in references 54 and 70.
[c] From F. Sherman (University of Rochester).

A fundamental distinction between transcription initiation by RNA pol II in higher eukaryotes and in the yeast *Saccharomyces cerevisiae* is that initiation in yeast cells generally occurs at multiple sites at variable distances from TATA. Experiments designed to determine the relationship between TATA and initiation sites at the *CYC1* (32, 44), *CYC7* (36), *HIS3* (22), *HIS4* (51), and *PHO5* (66) genes clearly established that the TATA element defines the window within which initiation can occur, but that specific sequences within the window define the actual start sites. Mutational analysis and survey of initiation site sequences revealed an initiation sequence, defined as Py-A-A-Pu (36) or Py-A-A/T-(Pu) (29). It is not known whether this sequence binds a specific initiation factor or whether it is the sequence preferred by RNA pol II. TATA-less promoters have also been described for *S. cerevisiae* with initiation generally occurring at multiple, dispersed sites (e.g., references 50 and 90). In either case, the mechanism of start site selection and the components of the transcriptional apparatus required for initiation are not well defined.

Genetic methods have been especially successful in identifying factors that affect transcription initiation in yeast cells. A large group of genes, designated *spt*, were found as suppressors of Ty or δ insertion mutations in the 5′ noncoding region of the *HIS4* or *LYS2* gene (27, 85; reviewed in reference 84). The *spt* suppressors function at the transcriptional level by altering the relative use of the Ty promoter and the adjacent *HIS4* or *LYS2* promoter. Two classes of *SPT* genes have been identified. One class encodes general transcription factors or related proteins; the other encodes histones or related proteins (84). Another class of genes, designated *sit*, was identified as suppressors that restored *HIS4* expression in the absence of the transcriptional activators encoded by the *GCN4*, *BAS1*, and *BAS2* genes (3). Suppression of histidine auxotrophy by *sit1* to *sit4* occurs at the transcriptional level by restoring initiation at the normal *HIS4* start site, as well as at several downstream sites that seem to be TATA independent. The *SIT1* and *SIT2* genes are identical to *RPB1* and *RPB2*, the genes encoding, respectively, the largest and second-largest subunits of RNA pol II (3). Defects in the two largest subunits of RNA pol II were also identified in a screen for *rpb1* and *rpb2* mutations that conferred *spt* phenotypes (37). In addition to effects on the relative use of adjacent promoter elements, these *rpb1* mutations conferred a modest effect on start site selection at the *HIS4* wild-type gene. In a selection scheme designed to uncover genes that affect specifically transcription start site selection, a gene designated *SHI* was found (28). The *shi* mutation shifted start sites upstream, closer to the TATA box. The product of the *SHI* gene has not been reported.

In our laboratory, we are characterizing revertants of a *cyc1* mutant that contains an aberrant ATG translation start codon (uATG) that is upstream of and out of frame with the normal start codon. Strains carrying this allele, designated *cyc1-5000*, express <5% of the normal amount of iso-1-cytochrome *c* as a result of translation of the upstream, rather than normal, open reading frame (34). Eight different genes, designated *sua1* to *sua8*, have been found to be suppressors of *cyc1-5000* (50, 61, 62). The mechanism of suppression by the *sua1* to *sua6* suppressors is unknown, although none affects transcription start site selection (50, 61). In contrast, the *sua7* suppressors have a dramatic effect on start sites, nearly eliminating initiation at the normal *cyc1* start sites in favor of downstream sites, including a major site between the uATG and normal ATG start codon (62). The *SUA7* gene was isolated and identified as TFIIB/factor e (62, 82), establishing that TFIIB is a determinant of start site selection in vivo. Here we report that *sua8* suppressors are functionally related to *sua7* suppressors, exerting nearly identical effects on start site selection at *cyc1* and other genes, and that *sua8* alleles encode altered forms of the largest subunit of RNA pol II.

MATERIALS AND METHODS

Yeast strains, media, and nomenclature. The strains used in this study are listed in Table 1. Standard methods were used for strain crosses, diploid selection, sporulation, and tetrad dissections (75). YPD and synthetic complete (SC) media are defined by Sherman (74). SC/Lat medium contains 2% lactate rather than glucose as the carbon source. Ino medium contains 2% glucose as the carbon source and either lacks inositol (−Ino) or contains 10 mg of inositol per liter (+Ino). Phenotypes are defined as follows: Csm^- and Tsm^-, either no growth or distinctly impaired growth on YPD medium at 16 and 37°C, respectively; Ino^-, distinctly impaired growth on −Ino medium relative to +Ino medium;

and Cyc$^-$, <5% of the normal amount of iso-1-cytochrome *c*, defined by low-temperature, whole-cell spectroscopy (76).

Isolation and genetic analysis of *sua8* mutants. Strain T16 was exposed to the mutagen diepoxybutane under conditions described previously (33), and Cyc$^+$ revertants were identified according to a scheme designed specifically to uncover *trans*-acting suppressors of the uATG of *cyc1-5000* (34). Revertants were scored for iso-1-cytochrome *c* content (Cyc phenotype) and for pleiotropic phenotypes (Csm$^-$, Tsm$^-$, and Ino$^-$) potentially associated with the suppressors. Strains were analyzed by standard yeast genetic methods (75) to determine dominance or recessiveness, single-gene mutations, cosegregation of the suppressor and pleiotropic phenotypes, and complementation grouping relative to previously defined *sua* suppressors.

Recombinant DNA techniques. Recombinant plasmids were constructed and screened according to standard procedures (68). Plasmid DNA was isolated from *Escherichia coli* (12) and from *S. cerevisiae* (40) as described previously. Transformations of *E. coli* and *S. cerevisiae* were done by the calcium chloride (45) and lithium acetate (30, 41) methods, respectively.

Isolation and characterization of the *SUA8* gene. Strain YDW383 (*sua8-1*) was transformed by using a YCp50 yeast genomic library (63). Four Tsm$^+$ colonies were obtained from a total of ~16,000 Ura$^+$ transformants. Each Tsm$^+$ transformant contained <5% of the normal amount of iso-1-cytochrome *c* (Cyc$^-$), consistent with complementation of the *sua8* suppressor. When cured of plasmid DNA on 5-fluoro-orotic acid medium (13), both the Tsm$^-$ and Cyc$^+$ phenotypes were restored for all four transformants, indicating that the Tsm$^+$ and Cyc$^-$ phenotypes were conferred by plasmid DNA rather than by strain reversion. Plasmid DNA was recovered from each transformant and reintroduced into strain YDW383 as well as into the other three *sua8* mutants. In each case, the transformants were phenotypically Cyc$^-$ and either Tsm$^+$ or Csm$^+$, confirming that each plasmid fully complemented all four *sua8* mutants. One of these plasmids, designated pM229, was chosen for further analysis. Southern blot hybridizations of restriction-digested, genomic DNA and of size-fractionated, intact yeast chromosomes revealed that the complementing DNA had been derived intact from chromosome IV (data not shown).

Sequence analysis of *SUA8*. Partial DNA sequence of the *SUA8*-complementing region was obtained by using the Sequenase kit (United States Biochemical Corp.) and the M13 universal primer. Plasmid pM243 was linearized at the unique *Bam*HI and *Sac*I sites within the multiple cloning region, and a nested set of deletions was generated from the *Bam*HI site by using exonuclease III and S1 nuclease (Erase-a-Base Kit; Promega, Inc.). Single-stranded template DNA was prepared from selected clones by using the VCS M13 helper bacteriophage (Stratagene Cloning Systems) in the presence of kanamycin as described elsewhere (68). Sequence analysis revealed that *SUA8* is allelic to *RPB1*. To confirm that *RPB1* is indeed the wild-type allele of *sua8*, strains T16 (SUA8$^+$ Tsm$^+$), YDW383 (*sua8-1* Tsm$^-$), and YDW438 (*sua8-2* Tsm$^-$) were crossed with strain RS420 (*rpb1-1* Tsm$^-$ [54]). Whereas T16 complemented *rpb1-1*, the two independent *sua8* mutants failed to complement, defined by retention of the Tsm$^-$ phenotype in the diploids.

Retrieval and sequence analysis of *sua8* suppressor alleles. The *sua8* alleles were retrieved by gap repair (64), using either linearized or gapped derivatives of plasmid pM243 (see Fig. 2). The alleles were retrieved as follows: *sua8-1* and *sua8-2* by using pM243En, *sua8-3* by using pM243T/Pf, and *sua8-4* by using pM243En/Sn. Linearized and gapped DNAs were purified in a 0.7% agarose gel, extracted with GeneClean (Bio 101, Inc.), and used to transform strains YDW383, YDW438, YDW575, and YDW5112 by the method of Gietz et al. (30). Ninety-six Ura$^+$ transformants of each strain were transferred to uracil-lacking master plates and replica printed to the appropriate media for scoring Csm, Tsm, and Ino phenotypes; the Cyc phenotype was scored by assaying iso-1-cytochrome *c* content in whole cells (76). Ura$^+$ transformants that retained the *sua8* mutant phenotypes were considered to be potential clones. Plasmid DNA was recovered from eight potential clones of each allele, checked for *sua8* integrity by restriction analysis, and subsequently reintroduced into the respective *sua8* mutants, scoring for retention of all phenotypes. Single-stranded template DNA was prepared as described above for pM243 and sequenced by using a collection of 22 *RPB1*-specific primers (most were a generous gift from R. A. Young; all others were synthesized by Oligos, etc., Inc.). Mutations encoding only a single amino acid replacement were detected for each *sua8* allele. (A total of seven sequence polymorphisms were also found; all occurred at the wobble position and were neutral with respect to the protein sequence.) To confirm that the identified mutations conferred all of the *sua8* phenotypes, either the *Tth*111I-*Pfl*MI (*sua8-1* to *sua8-3*) or the *Pfl*MI-*Bsi*WI (*sua8-4*) restriction fragment of pM243 was replaced with the corresponding fragment that contained the *sua8* mutation(s). The resulting plasmids were introduced into the respective *sua8* mutants, and retention of the *sua8* phenotypes was confirmed.

RNA procedures. Strains were grown in 50-ml YPD cultures to an optical density at 600 nm of 1.0. Total RNA was isolated by the method of McNeil and Smith (46). Northern (RNA) analysis was performed by size fractionating 15 μg of total RNA in a 1.5% formaldehyde–agarose gel and transferring the material to Magna Nylon membranes (Micron Separations, Inc.). Prehybridization and hybridization reactions were performed under the conditions recommended by the supplier. *CYC1* and *ACT1* probes were [α-^{32}P]UTP-labeled RNA transcripts derived from the 0.6-kb *Eco*RI-*Hin*dIII *CYC1* or 1.4-kb *Bam*HI-*Hin*dIII *ACT1* DNA fragment contained in the vector pGEM3 (Promega, Inc.). Relative *CYC1* mRNA levels were determined by normalization to the *ACT1* mRNA level, using an LKB UltroScan XL scanning densitometer.

Transcription start sites were mapped by primer extension analysis as described previously (62). Extension reactions were done with avian myeloblastosis virus reverse transcriptase (Promega, Inc.) and [γ-^{32}P]ATP-labeled oligonucleotide primers. The *CYC1*-specific primer oIP13 (GTCT TGAAAAGTGTAGCACC, 53 to 34) was used both as a primer to generate the DNA sequencing ladder and for primer extension reactions. The primers oIP87 (CTTTGG AACTGGAATATC, 84 to 67), oRB93 (CTAGCTCACGA GATAGCG, 125 to 108), oRB100 (CAAGTGAAACGTAT TCCTTC, 73 to 54), oRB101 (CGTACTTTCTTTAGCCA TG, +18 to −1), and oRB102 (CTTTAAACGTTCCTCAT CGG, 24 to 5) were used to map initiation sites at the *ADH1*, *HIS3*, *HIS4*, *CYC7*, and *SPT15* genes, respectively. The cDNA products were analyzed on an 8% polyacrylamide DNA sequencing gel.

RESULTS

Isolation and genetic analysis of *sua8* mutants. Strain T16 expresses <5% of the normal amount of iso-1-cytochrome *c*

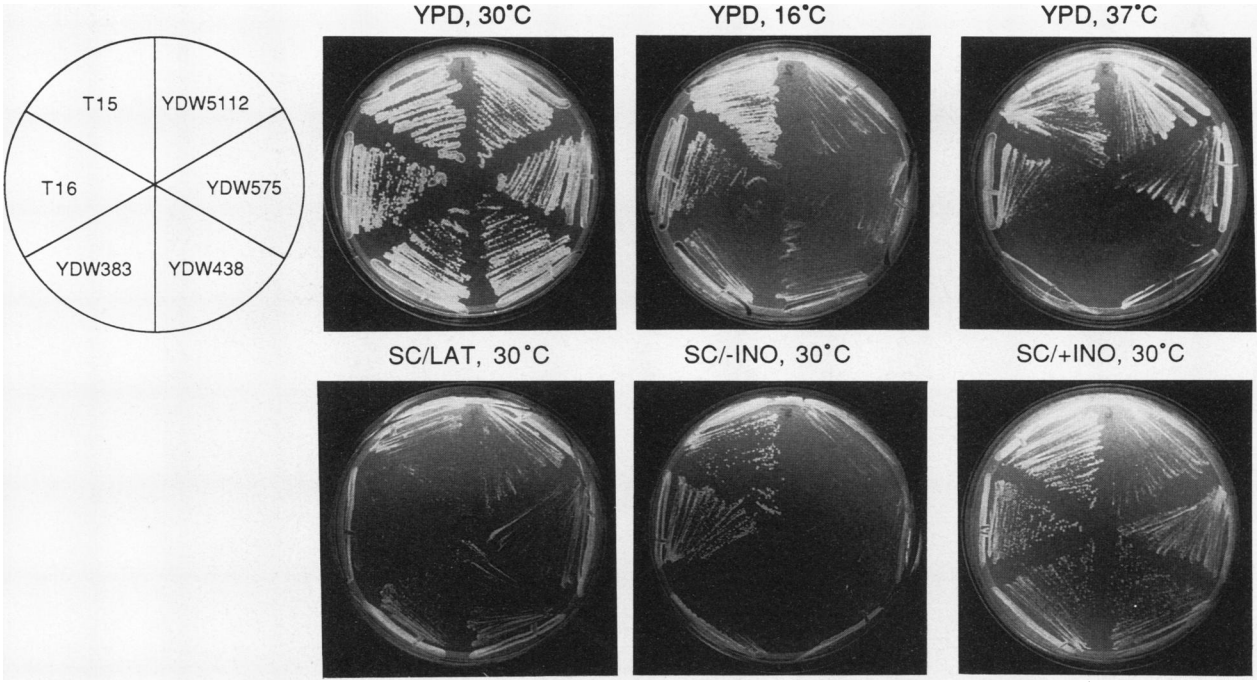

FIG. 1. Phenotypes associated with *sua8* suppressor mutants. The Cyc, Csm, Tsm, and Ino phenotypes were scored on the following media: Csm and Tsm, YPD at 16 and 37°C, respectively; Cyc, SC/Lat; Ino, −Ino or +Ino. The genotypes for strains T15, T16, YDW383, YDW438, YDW575, and YDW5112 are listed in Table 1.

(Cyc$^-$ phenotype) as the result of an aberrant upstream open reading frame (uORF) located in the leader region of the *cyc1* gene. Cyc$^+$ revertants of T16 were isolated by a technique designed to uncover specifically *trans*-acting suppressors of the *cyc1-5000* uORF (34). Genetic analysis of 15 of these revertants identified four independent strains, YDW383, YDW438, YDW575, and YDW5112 (Table 1), that are the result of recessive mutations within the same complementation group, designated *sua8*; each expresses 20 to 35% of the normal amount of iso-1-cytochrome *c*. All four strains exhibit pleiotropic phenotypes that cosegregate with the suppressors. Strains YDW383 and YDW438 are heat lethal (Tsm$^-$ at 37°C), and all four strains are cold sensitive (Csm$^-$ at 16°C) (Fig. 1). Also, all four strains grow poorly on −Ino medium (Ino$^-$), a phenotype often associated with mutations in the general transcriptional machinery (3, 70, 71) and attributed to the extreme sensitivity of *INO1* expression to transcriptional perturbations (53).

The *sua8* suppressors are allelic to *RPB1*. The *SUA8* wild-type gene was isolated from a yeast genomic library by complementation of the Tsm$^-$ phenotype conferred by the *sua8-1* allele of strain YDW383. Restriction and complementation analysis was used to delimit *SUA8* to a 7.1-kb *Hin*dIII DNA fragment (Fig. 2). DNA sequences were determined for three regions of this fragment, indicated by the arrows. Sequence comparisons with the GenBank and EMBL data libraries revealed identity to *RPB1* (*RPO21*), the gene encoding the largest subunit of RNA pol II (2, 54; reviewed in reference 89). The *sua8* mutants and an *rpb1-1* mutant failed to cross-complement, confirming that *SUA8* and *RPB1* are allelic.

Mechanism of suppression of the *cyc1-5000* uATG by *sua8*. The identity of the *SUA8* gene implied that suppression of the *cyc1-5000* defect occurs at the transcriptional level. Northern analysis indicated that the steady-state level of the

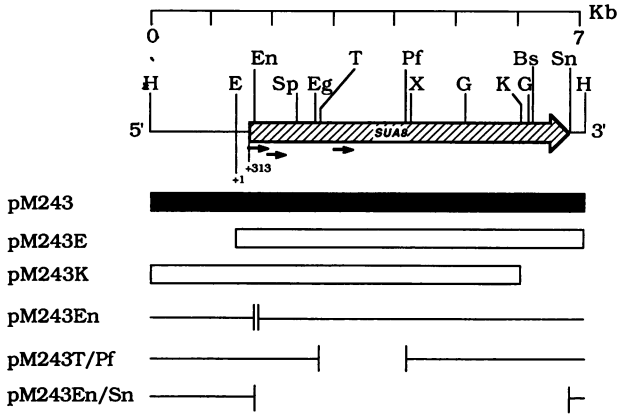

FIG. 2. *SUA8* plasmids. A partial restriction map of the *SUA8* locus is shown. All plasmids are derivatives of pM243, which was constructed by transferring the 7.1-kb *Hin*dIII fragment encompassing the entire *SUA8* (*RPB1*) gene from the original clone, pM229, to plasmid pRS316, a low-copy-number *URA3* vector (77). Complementation of the Cyc$^+$ and Tsm$^-$ phenotypes associated with the *sua8-1* suppressor is denoted by the solid bar; open bars denote failure to complement. Plasmids denoted by lines were used to retrieve the mutated regions of the *sua8* suppressor alleles by gap repair (see Materials and Methods). Plasmid pM243En was linearized at the *Eco*NI site (position 482); pM243T/Pf was deleted of the *Tth*111I-*Pfl*MI fragment (positions 1399 to 2763); plasmid pM243En/Sn was deleted of the *Eco*NI-*Sna*BI fragment (positions 482 to 5512). The numbering system is consistent with that of Allison et al. (2), which assigns +1 to the *Eco*RI site and +313 to the A of the ATG translation start codon. Arrows indicate regions of the *SUA8* gene that were sequenced, in each case representing >250 nucleotides. Two nucleotides differed from the published *RPB1* sequence (2) (AGG→AGA and CTA→CTG at codons 36 and 509, respectively), both of which are neutral with respect to the protein sequence. Restriction site abbreviations: Bs, *Bsi*WI; E, *Eco*RI; Eg, *Eag*I; En, *Eco*NI; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; Pf, *Pfl*MI; Sn, *Sna*BI; Sp, *Spe*I; T, *Tth*111I; X, *Xho*I.

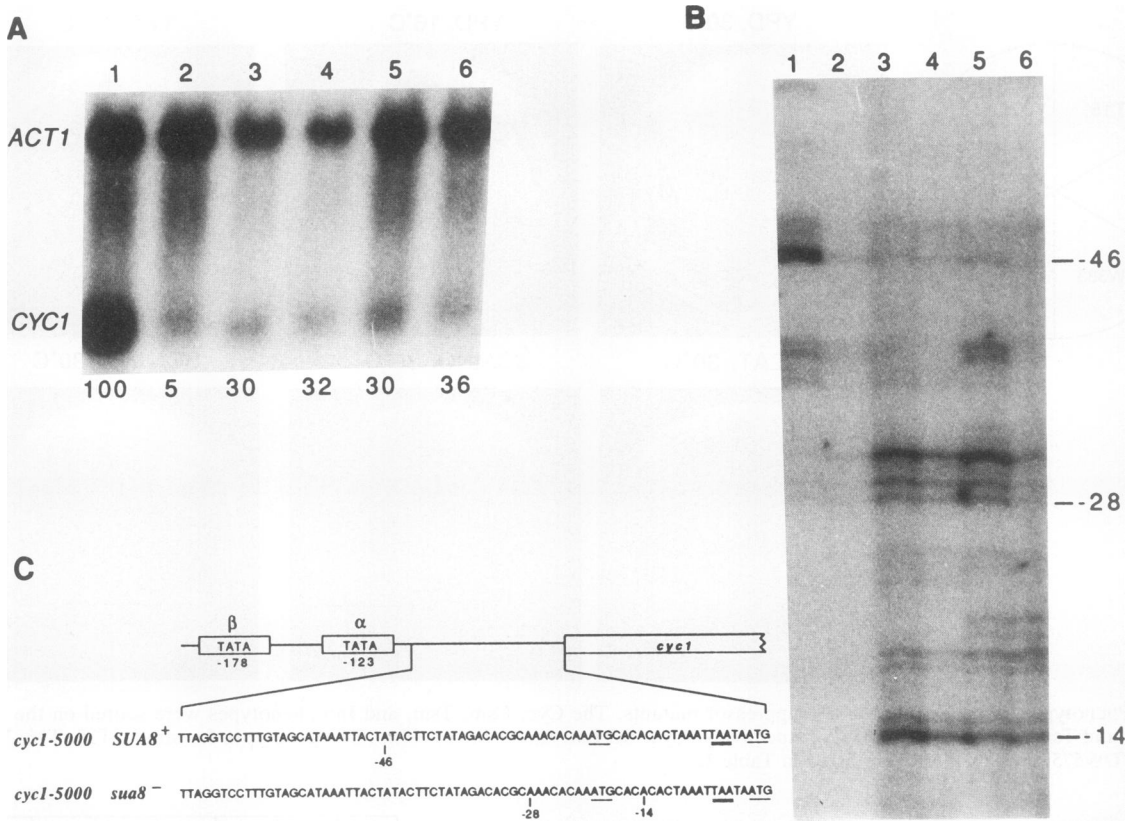

FIG. 3. *CYC1* transcript analyses in normal and *sua8* suppressor strains. (A) Northern blot analysis of total RNA, using *CYC1* and *ACT1* RNA probes. The strains for lanes 1 to 6 are T15 (*CYC1 SUA8*), T16 (*cyc1-5000 SUA8*), YDW383 (*cyc1-5000 sua8-1*), YDW438 (*cyc1-5000 sua8-2*), YDW575 (*cyc1-5000 sua8-3*), and YDW5112 (*cyc1-5000 sua8-4*), respectively. Relative *CYC1* mRNA levels are presented at the bottom of each lane and were determined by densitometric scanning (LKB UltroScan XL), using *ACT1* mRNA levels as an internal standard. (B) Primer extension analysis of *cyc1* transcription start sites. The strains for lanes 1 to 6 are the same as for panel A. The major initiation site in strains T15 and T16 is indicated at position −46. (The faint bands seen for T16 do not reflect sample underloading but are due to the low steady-state level of the *cyc1-5000* transcript; previous results clearly established that *cyc1* transcription initiation sites are identical in strains T15 and T16 [62].) The major downstream start sites that are enhanced in the *sua8* mutants are indicated at positions −28 and −14 (+1 corresponds to the A of the normal ATG start codon). (C) Schematic diagram depicting the structure and partial DNA sequence of the *cyc1-5000* promoter/leader region, including the α and β TATA boxes (44), the major initiation site at position −46 in the *SUA8*$^+$ strains, the downstream sites in the −28 region and at position −14 that are enhanced in the *sua8* mutants, and the uATG (in-frame stop codon is double underlined) and normal ATG at positions −20 and +1, respectively.

cyc1 transcript in the *cyc1-5000 SUA8* strain (T16) is about 5% of the level found in the isogenic *CYC1 SUA8* strain (T15), whereas in the four *cyc1-5000 sua8* strains, these levels are enhanced to 30 to 36% of normal (Fig. 3A). No obvious differences in transcript length were apparent. These transcript levels correlate approximately with the iso-1-cytochrome *c* levels in each strain and presumably reflect altered transcript stabilities resulting from ribosomal dissociation following translation of the *cyc1-5000* uORF, a premise consistent with the effects of nonsense mutations on the stabilities of *PGK1*, *URA1*, and *URA3* transcripts (16).

Transcription start sites at *cyc1-5000* were determined by primer extension. The major initiation sites reported previously (32, 44, 47) were found for strain T15 (as well as for T16). However, initiation at these sites, including the major site at position −46, was dramatically diminished in each of the *sua8* strains, with enhanced initiation at downstream sites, most notably in the −28 region and at position −14 (Fig. 3B). This initiation pattern accounts for the elevated levels of iso-1-cytochrome *c* in the suppressor strains, since transcripts initiating at −14 exclude the uATG start codon at position −20 (Fig. 3C). Primer extension results identical to that depicted for strain T16 (*cyc1-5000 SUA8*$^+$; Fig. 3B, lane 2) were obtained for each of the four suppressor strains that had been transformed with the *SUA8*$^+$ plasmid pM243 (data not shown), thereby confirming that the altered start sites observed in lanes 3 to 6 are solely a consequence of the *sua8* suppressor mutations. These results demonstrate that the largest subunit of RNA pol II can play a significant role in transcription start site selection in *S. cerevisiae*.

Effect of *sua8* suppressors on start site selection at other class II genes. To determine whether *sua8* mutants have a general effect on transcription start site selection, initiation patterns at other genes were determined (Fig. 4). Initially, we chose *ADH1* for this analysis because start sites at this locus have been accurately mapped (8) and because mutations at other loci have been shown to affect *ADH1* start sites (28, 62). The major upstream initiation site at position −37 of *ADH1*, as well as flanking minor sites, were markedly diminished in the *sua8-3* and *sua8-1* mutants in favor of initiation at discrete sites further downstream (compare lanes 1 and 2 with lane 3). Moreover, the *sua8* effect on

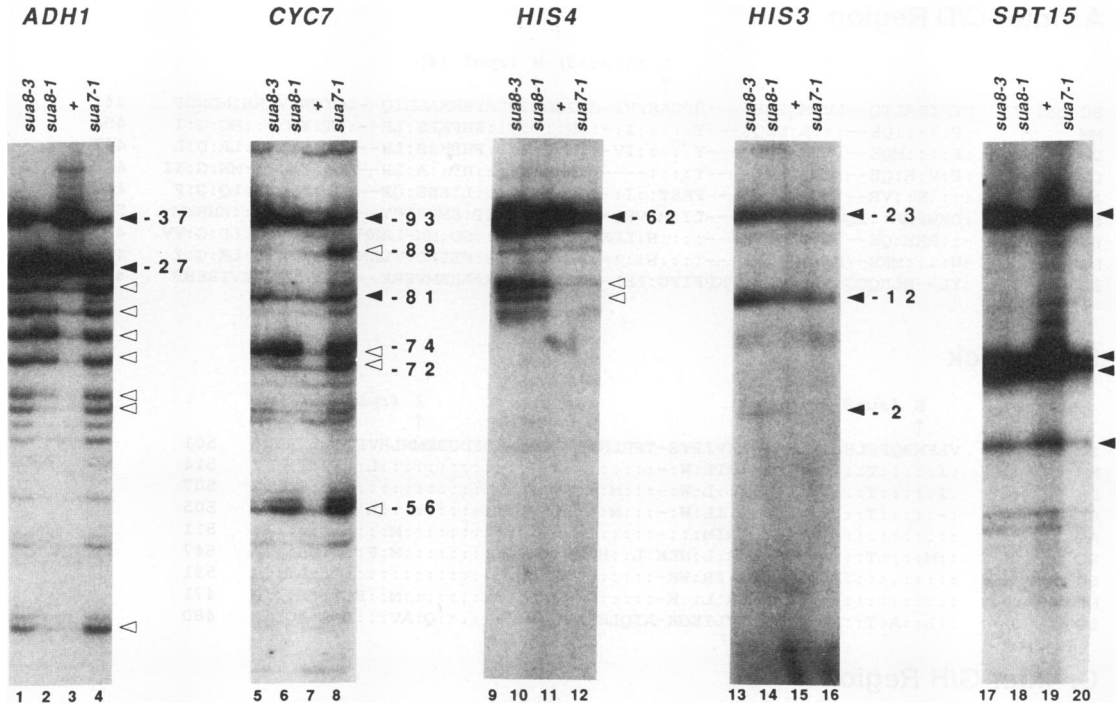

FIG. 4. Transcription start sites at the *ADH1*, *CYC7*, *HIS4*, *HIS3*, and *SPT15* genes in *SUA*$^+$, *sua8*, and *sua7* strains. The strains used in these experiments are T16 (*SUA*$^+$), YDW383 (*sua8-1*), YDW575 (*sua8-3*), and YDW546 (*sua7-1*) (see Table 1 for complete genotypes). The three *sua* mutants are derivatives of strain T16. Transcription start sites were mapped by primer extension as described in Materials and Methods. The principal start sites found in the wild-type strain are indicated by filled arrowheads; downstream start sites enhanced in the suppressor strains are indicated by open arrowheads. Whereas Healy et al. (36) found position −89 to be the principal start site at *CYC7* and −93 to be a minor site, in our strains −93 is the principal start site and −89 is a minor site. For each gene, start sites were defined by using sequenced *CYC1* DNA as size markers. All indicated start sites are relative to the ATG translation start codon of the respective genes; accordingly, −23, −12, and −2 at *HIS3* correspond, respectively, to +1, +12, and +22 defined by Struhl (81).

ADH1 is very similar to that of the *sua7-1* suppressor (lane 4) (62). This result establishes that the *sua8* suppressors are not *cyc1* specific and that *sua7* and *sua8* suppressors can exert nearly identical effects on transcription start site selection.

Despite the multiple pleiotropic phenotypes conferred by the *sua8* suppressors (Fig. 1) and the marked effects on initiation at the *CYC1* and *ADH1* loci (Fig. 3 and 4), the growth rates of the *sua8* mutants are not significantly impaired on rich medium at normal temperature (Fig. 1). This finding suggested that the *sua8* suppressors do not exert start site effects at all class II genes. We investigated this by mapping start sites at additional genes, including *CYC7*, *HIS4*, *HIS3*, and *SPT15*. As described above for *ADH1*, initiation sites were determined for two different *sua8* mutants, *sua8-3* and *sua8-1*, for the *SUA*$^+$ parent strain, and for the *sua7-1* mutant (Fig. 4). All three suppressors exhibited reproducible start site effects at *CYC7*, including, most prominently, enhanced initiation at position −74 in all three *sua* mutants (Fig. 4; compare lane 7 with lanes 5, 6, and 8) and positions −89, −72, and −56 in the *sua7* mutant (lane 8). However, unlike *cyc1* and *ADH1*, initiation at the principal start site at position −93 was not diminished in the *sua* mutants (compare lanes 5, 6, and 8 with lane 7). A downstream shift in initiation at *HIS4* was also observed for the two *sua8* mutants; however, *HIS4* initiation was unaffected by *sua7-1* (compare lanes 9 and 10 with lane 12). All three suppressors are essentially without effect on initiation at the *HIS3* and *SPT15* genes (lanes 13 to 20). Thus, the *sua8* suppressors can exert variable effects on start site selection, ranging from no effect to dramatic effects. Furthermore, some genes are affected identically by *sua7* and *sua8* suppressors, whereas others are differentially affected.

The *sua8* suppressor mutations. To determine the structural alterations in the largest RNA pol II subunit that confer the suppressor phenotypes, the *sua8* alleles were cloned by gap repair (see Materials and Methods) and sequenced. In each case, the entire coding region, with the exception of ~600 bp at the 3′ end, was sequenced by using a collection of *RPB1*-specific primers. Results are presented in Table 2.

TABLE 2. Summary of *sua8* mutations and associated phenotypes

Allele	Phenotype[a]				Mutation[b]	Amino acid replacement
	Cyc (%)	Csm	Tsm	Ino		
sua8-1	30	−/+	−	−	A_{1646}→G	Asn-445→Ser
sua8-2	25	−/+	−	−	A_{1646}→G	Asn-445→Ser
sua8-3	20	−/+	+	−/+	G_{1519}C→CG	Ala-402→Arg
sua8-4	35	−/+	+	−/+	G_{4478}→T	Gly-1388→Val

[a] The Cyc phenotype refers to iso-1-cytochrome *c* content, assayed by low-temperature, whole-cell spectroscopy (76). The indicated levels compare to <5% of normal in the parent strain T16 (*cyc1-5000 SUA8*$^+$). The other phenotypes refer to growth (+), distinctly impaired growth (−/+), or no growth (−) on rich (YPD) medium at 16°C (Csm) or 37°C (Tsm) or on −Ino synthetic medium at 30°C (Ino).

[b] The nucleotide numbering system is consistent with that of Allison et al. (2), such that the A of the ATG start codon is designated +313.

A. Inter-C/D Region

```
                                        R (sua8-3)  H (rpb1-14)
                                          ↑           ↑
SC pol II  PYNIDRLTQ--LVRNGPNE----HPGAKYVI-RDSGDRIDLRYSKRAGDIQ--LQYGWKVERHIMDNDP    441
MM         :F:::::QE--:::R:NSQ----Y:::::I:-::N:::::::FHPKPS:LH--::T:Y:::::MC:G:I    454
DM         :F::::MQE--:::R:NSQ----Y:::::IV-::N:E:::::FHPKSS:LH--::C:Y:::::LR:D:L    447
CE         :F:V:K:QE--::NR:DTQ----Y:::::-----EN:A:V::::HP::A:LH--::P:YR::::MK:G:II   446
AT         ::::E::VR--::FIS-------FSET::I:-::D:Q:S::::L:KSS:QH--:EL:Y:::::LQ:G:F    451
SC pol I   :DKWPGA::IQNEDGSLVS----LI:MSVEQ-:KALANQL:TP:SNVSTHT----LNK::Y:::KNR:V    586
SC pol III -::RHK:QE--:IV::::V----:::::N:LLK:NEDA:RN:::GD:MK-LAKN::I:DV::::LE:G:VV   471
MT         -W::::MKE--HIE:::DV----:::::N:::-:PD:RK:RA-:NETKDVVLEN:KP:YI::::LK:G:I    411
EC         :YL--RLHQCG:PKKMALELFKPFIYG:LEL-:GLATTI--KAAKKMVERE--EAVVWDILDEVIREHP    420
```

B. D Block

```
                S (sua8-1;sua8-2)                                  I (rpb1-17)
                  ↑                                                  ↑
SC pol II   VLFNRQPSLHKMSMMAHRVKVIPYS-TFRLNLSVTSPYNADFDGDEMNLHVPQSEETRAEL   501
MM          :I:::::T:::::::G:::RIL:W:-:::::::T:::::::::::::::L:::L:::::I   514
DM          :I:::::T:::::::G:::::L:W:-:::M:::C:::::::::::::::::::M:::::V   507
CE          :-:::::T:::::::G::::IL:W:-:::M:::::::::::::::::::::L:::L:::::I   505
AT          ::::::::::::::I:G::IRIM:::-:::::::::::::::::::::::M:::::F:::::V   511
SC pol I    ::M::::T:::A:::G:K:R:L:NEK:L::HYAN:GA:::::::::::M:F::N:NA:::A   647
SC pol III  ::::::::::RL:ILS:YA:IR:WR-:::::EC:CT:::::::::::::::::T::A:::A   531
MT          ::::::::::RM:::::E:R:L::K-:::::::C:CP:::::::::::M::F:T::S:::A   471
EC          ::L::A:T::RLGIQAFEPVLIEGK-AIQLHPLVCAA:::::::Q:AV:::LTL:AQL:A   480
```

C. Inter-G/H Region

```
                N (rpb1-15)                                                V (sua8-4)
                  ↑                                                          ↑
SC pol II   IDPTRIYTNSFIDIMEVLGIEAGRAALYKEVYNVIASDGSYVNYRHMALLVDVMTTQGGLTSVTRHGFNRSNT   1394
MM          V::V:TTS:DIVE:FT::::::V:K::ER:L:H::SF:::::::::L:::C:T::CR:H:MAI::::V::QD:   1424
DM          V::I:TSS:DICE:FQ::::::V:KSVE::MNA:LQFY:L::::::L:::C::::AK:H:MAI::::I::QD:   1416
CE          :::V:TTS:DICE:F:::::::V:K:IER:MD:::SF:::::::::L:::C::::AK:H:MAYS:::I::QEV   1416
AT          V::K:TTS:HL:E:I::::::::V:R::LD:LRV::SF:::::::::L:I:C:T::YR:H:MAI::::I::ND:   1454
SC pol III  :G-S:TT::HVLEVFS::::::A:YSIIR:INYTMSNH:MS:DP::IQ::G::::YK:EVLGI::F:LSKMRD   1381
MT          V:KV:TT::DIHE:ET::::::A:N:IIH:AKRTMEEQ:LT:DI::IM:VA:M::AD:SVK:IG:::ISGEKA   1261
```

FIG. 5. The *sua8* suppressor mutations encode replacements of evolutionarily conserved residues. (A) The alignment of the regions between homology blocks C and D is shown for the largest subunit of the RNA pol II of *S. cerevisiae* (SC pol II) (2), *Mus musculus* (MM) (1), *Drosophila melanogaster* (DM) (42), *Caenorhabditis elegans* (CD) (11), and *A. thaliana* (AT) (52); the largest subunits of RNA pol I and III of *S. cerevisiae* (SC pol I [49] and SC pol III [2]); the largest subunit of RNA polymerase of the archaebacterium *Methanobacterium thermoautotrophicum* (MT) (10); and the β′ subunit of *E. coli* (EC) (57). (B) The indicated sequences are contiguous with the sequences shown in panel A and comprise the highly conserved homology block D. (C) The indicated sequences immediately precede homology block H. In all three panels, the sequence of the largest subunit of *S. cerevisiae* RNA pol II is shown. Residues that are identical with this sequence are indicated by colons; gaps in the alignments are depicted as dashes. The alignments were constructed by using the CLUSTAL program of PC/GENE. The RNA pol I and *E. coli* sequences are not included in panel C because of limited similarity to RNA pol II in the region preceding block H. The *sua8-1* and *sua8-2* alleles are identical, encoding an N→S replacement at position 445, which is invariant among all of the RNA polymerases; the *sua8-3* allele encodes an A→R replacement at position 402, which is highly conserved; and the *sua8-4* allele encodes a G→V replacement at invariant position 1388. The *rpb1-14*, *rpb1-17*, and *rpb1-15* replacements were reported by Scafe et al. (70).

The *sua8-1* and *sua8-2* suppressors, although of independent origin, were the result of a single A-to-G transition at position 1646, encoding an asparagine-to-serine replacement at amino acid position 445. The *sua8-3* suppressor is the result of a tandem double-base-pair GC-to-CG substitution at position 1519, encoding an alanine-to-arginine replacement at position 402. Position 445 lies within homology block D, and position 402 is between blocks C and D (Fig. 5; summarized in Fig. 6). Although the sequence between homology blocks C and D exhibits considerable variability, Ala-402 is conserved, differing only in *Arabidopsis thaliana* among the RNA pol II subunits. Asn-445 is invariant, not only among the eukaryotic proteins, including yeast RNA pol I and pol III, but also in the archaebacterial RNA polymerase and in the β′ subunit of prokaryotic RNA polymerase. The *sua8-4* suppressor was the result of a single G-to-T substitution at position 4478, encoding a glycine-to-valine replacement at amino acid position 1388. Gly-1388 is also evolutionarily invariant, located within a conserved region immediately preceding homology block H (Fig. 5 and 6). These results define the regions within and adjacent to homology block D and adjacent to block H as important determinants of transcription start site selection.

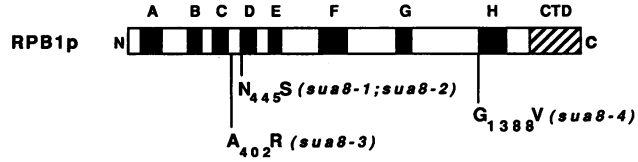

FIG. 6. Schematic representation of the *sua8* suppressor replacements within the largest subunit of RNA pol II (RPB1p). Homology blocks A to H, depicting regions of sequence similarity among eukaryotic RNA polymerases, are shown, along with the CTD heptapeptide repeat at the C terminus. The sequence contexts of the *sua8*-encoded amino acid replacements are shown in Fig. 5.

TABLE 3. Nonallelic noncomplementation between *sua7* and *sua8* mutants

Strain	Relevant genotype[a]	% Iso-1-cytochrome *c* content[b]
T15	*CYC1*$^+$	100
T16	*cyc1-5000*	<5
YMH75	*cyc1-5000*	<5
YDW546	*cyc1-5000 sua7-1*	30
YMH71-9C	*cyc1-5000 sua7-1*	30
YIP363	*cyc1-5000 sua7-3*	25
YDW383	*cyc1-5000 sua8-1*	30
YRB78	*cyc1-5000 sua8-1*	25
YDW575	*cyc1-5000 sua8-3*	20
YDW5112	*cyc1-5000 sua8-4*	35
T16 × YMH75	*cyc1-5000/cyc1-5000*	<5
YDW546 × YMH75	*cyc1-5000/cyc1-5000 SUA7/sua7-1*	<5
YIP363 × YMH75	*cyc1-5000/cyc1-5000 SUA7/sua7-3*	<5
YDW383 × YMH75	*cyc1-5000/cyc1-5000 SUA8/sua8-1*	<5
YDW575 × YMH75	*cyc1-5000/cyc1-5000 SUA8/sua8-3*	<5
YDW5112 × YMH75	*cyc1-5000/cyc1-5000 SUA8/sua8-4*	<5
YDW546 × YRB78	*cyc1-5000/cyc1-5000 SUA7/sua7-1 SUA8/sua8-1*	15
YIP363 × YRB78	*cyc1-5000/cyc1-5000 SUA7/sua7-3 SUA8/sua8-1*	15
YMH71-9C × YDW383	*cyc1-5000/cyc1-5000 SUA7/sua7-1 SUA8/sua8-1*	20
YMH71-9C × YDW575	*cyc1-5000/cyc1-5000 SUA7/sua7-1 SUA8/sua8-3*	15
YMH71-9C × YDW5112	*cyc1-5000/cyc1-5000 SUA7/sua7-1 SUA8/sua8-4*	20

[a] Complete genotypes are listed in Table 1.
[b] Assayed by low-temperature, whole-cell spectroscopy (76).

Genetic evidence that yeast TFIIB interacts with the largest subunit of RNA pol II. The *sua7* and *sua8* suppressors exert nearly identical effects on transcription start site selection at *cyc1*, *ADH1*, and *CYC7* (Fig. 3 and 4). Furthermore, all *sua7* and *sua8* mutants are cold-sensitive, a phenotype often associated with defects in components of multisubunit complexes (e.g., references 67 and 88). This observation suggests that TFIIB and the largest subunit of RNA pol II functionally interact to define transcription start sites. This premise was tested genetically by constructing haploid *sua7-1 sua8-1* double mutants. Strain YDW383 (*sua8-1* Tsm$^-$ Csm$^-$), harboring a plasmid carrying the *SUA8*$^+$ gene (pM243; Fig. 2), was crossed with strain YMH71-9C (*sua7-1* Csm$^-$), and the resulting diploid was sporulated and dissected. Among 32 progeny, 6 failed to grow when cured of plasmid DNA, indicating that viability of these 6 segregants is dependent on the plasmid-borne *SUA8*$^+$ gene and suggesting that they are *sua7 sua8* double mutants. The genotypes for two of these plasmid-dependent segregants were confirmed by analyzing the meiotic progeny from crosses with *SUA7*$^+$ *SUA8*$^+$ strains (either B-6088 and B-7056; Table 1). When cured of plasmid DNA, all asci producing four viable spores yielded 2:2 segregation of Tsm$^-$: Tsm$^+$ and 4:0 segregation of Csm$^-$:Csm$^+$, demonstrating that the plasmid-dependent strains were indeed *sua7 sua8* double mutants. Thus, *sua7-1 sua8-1* double-suppressor mutations result in synthetic lethality, suggesting interaction between TFIIB and the largest RNA pol II subunit.

Additional evidence supports this conclusion. When *sua7* or *sua8* suppressor strains were backcrossed to a *SUA*$^+$ strain of opposite mating type, all suppressor phenotypes, including Csm$^-$, Tsm$^-$, Ino$^-$, and Cyc$^+$, were lost, establishing that the suppressor mutations are fully recessive. However, when *sua7* mutants were crossed with *sua8* mutants, the resulting heterozygous diploids (*sua7/SUA7 sua8/SUA8*) exhibited intermediate phenotypes with respect to Cyc, displaying 15 to 20% of the normal amount of iso-1-cytochrome *c*, compared with 20 to 35% in the *sua7* and *sua8* haploid mutants and <5% in the diploids derived from the backcrosses (Table 3). This phenomenon is known as nonallelic (or unlinked) noncomplementation, defined by retention of phenotypes associated with recessive mutations in two different genes in a diploid that contains a wild-type allele of both genes (reference 80 and references therein). Nonallelic noncomplementation is thought to result from mutations in interacting gene products, possibly because interaction between the altered proteins generates a toxic product (80). Taken together, the similar effects of *sua7* and *sua8* suppressors on start site selection, the Csm$^-$ phenotype of all *sua7* and *sua8* mutants, the synthetic lethality conferred by double *sua7 sua8* mutants, and nonallelic noncomplementation in *sua7/SUA7 sua8/SUA8* diploids suggest an interaction between TFIIB and the largest subunit of RNA pol II during transcription initiation.

DISCUSSION

We have used a genetic selection based on suppression of an aberrant ATG codon (uATG) in the leader region of the yeast *cyc1* gene to identify genes that affect transcription start site selection. These suppressors, designated *sua*, do not necessarily affect transcription initiation; indeed, the *sua1* to *sua6* suppressors have no discernible effect on start site selection (50, 61). However, the *sua8* suppressors, as well as *sua7* suppressors (62), have a pronounced effect on start site selection, in each case shifting initiation downstream of normal. This effect readily accounts for the observed increase in iso-1-cytochrome *c* levels, since the downstream shift includes a start site between the uATG and the normal ATG start codon. *SUA7* encodes TFIIB/factor e (62, 82), and as presented in this report, *SUA8* is identical to *RPB1*, which encodes the largest subunit of RNA pol II (2). Thus, these two components of the general transcriptional apparatus are determinants of start site selection in *S. cerevisiae*.

Transcript mapping experiments have revealed several

notable characteristics of the *sua8* suppressors. First, the effect on start site selection is not *cyc1* specific. Rather, a marked effect on initiation was also found at the *ADH1*, *CYC7*, and *HIS4* genes (Fig. 4). Second, transcription at these genes is always shifted downstream of normal, indicating that the suppressors do not simply diminish start site specificity, allowing promiscuous initiation, but cause a directed shift. Third, the downstream sites are not new, but in every case can be seen as minor start sites in an *SUA8*$^+$ strain, sometimes visible only upon overexposure of autoradiograms (Fig. 3 and 4 and data not shown). Therefore, the *sua8* suppressors seem to affect the position, but not the specificity, of the initiator region. Fourth, despite the pronounced effect on initiation at some genes, the *sua8* suppressors are without effect at other genes. This is unlikely to be a consequence of differential transcriptional regulatory mechanisms, since start sites were determined in each case under noninducing conditions. Also, *HIS3* and *HIS4*, which are similarly regulated (38), are differentially affected by *sua8* (Fig. 4). The simplest interpretation of these results is that the suppressors shift the window within which initiation can occur downstream of normal. If acceptable start sites are available, a downstream shift occurs; otherwise, initiation occurs at the normal sites. Although plausible, this explanation does not account for two observations. One is that a downstream shift does not occur at *HIS3*, even though a minor downstream site is available at position −2 (81) (Fig. 4). The other is that the *sua8* suppressors shift initiation at *HIS4* (Fig. 4, lanes 9 and 10), establishing the presence of acceptable downstream sites, yet these sites are not used in the *sua7* mutant (Fig. 4, lane 12), even though *sua7* and *sua8* confer nearly identical downstream shifts at *cyc1* and *ADH1* (Fig. 3 and 4) (62).

The overall similarities between the *sua7* and *sua8* suppressors are striking. With the exception of the differential effects on *HIS4*, both sets of suppressors exert nearly identical effects on start site selection at all other genes tested. Also, the *sua7* and *sua8* suppressors confer similar pleiotropic phenotypes: all *sua7* and *sua8* suppressors are cold sensitive (Fig. 1) (62), and all exhibit at least partial inositol auxotrophy (Fig. 1) (60). Moreover, double *sua7 sua8* mutants are inviable (synthetic lethality), and recessive *sua7* mutants fail to fully complement recessive *sua8* mutants as heterozygous diploids (nonallelic noncomplementation). These results suggest that RNA pol II might interact with TFIIB through its largest subunit and that this interaction might comprise, at least in part, a functional domain of the preinitiation complex that is responsible for transcription start site selection. This premise is consistent with biochemical results suggesting that TFIIB forms a bridge between TFIID and RNA pol II, perhaps measuring the distance between the TATA box and the initiation site (17). Furthermore, recent structure-function studies show that TFIIB is composed of distinct domains that bind TBP and recruit RNA pol II/TFIIF to the preinitiation complex (5, 19, 31, 39).

Sequence comparisons among the largest subunit of eukaryotic RNA polymerases have identified eight regions of significant structural similarity, designated homology blocks A to H, as well as a heptapeptide repeat at the C terminus (CTD) (reviewed in reference 89). Truncations of the CTD repeat diminished the response of RNA pol II to a subset of upstream activating sequence elements (59, 69), establishing a role for the CTD in communication between the gene-specific activators and the basal transcriptional machinery. A functionally distinct class of *rpb1* mutants has been isolated on the basis of the ability to alter relative transcription ratios at adjacent δ insertion and *HIS4* promoter elements (37). These alleles, designated *rpb1-501* and *rpb1-502*, are the result of single amino acid replacements within homology block G and cause a subtle upstream shift in start site selection at the *HIS4* wild-type gene. A third class of *rpb1* mutants, designated *sit1*, was isolated on the basis of the ability to restore *HIS4* transcription in the absence of the gene-specific transcriptional activators encoded by *GCN4*, *BAS1*, and *BAS2* (3); *sit1* mutations have not been defined.

Additional *rpb1* mutations have been defined in a screen for conditional mutants using mutagenized *RPB1* DNA in a plasmid shuffle system (54, 70). These mutations occurred primarily at highly conserved positions and include two replacements within or adjacent to homology block D. The *rpb1-14* allele encodes an arginine-to-histidine replacement at position 412 between homology blocks C and D; the *rpb1-17* allele encodes a methionine-to-isoleucine replacement at position 487 within homology block D (70). These replacements compare to replacements at conserved positions 402 and 445 for the *sua8* suppressors (Fig. 5). Positions 402 and 412 are highly conserved and positions 445 and 487 are invariant among all forms of RNA polymerase (Fig. 5). The *rpb1-15* allele encodes an isoleucine-to-asparagine replacement at position 1327, which precedes homology block H (70); this compares to the *sua8-4* replacement at position 1388 (Fig. 5). Like the *sua8-1* and *sua8-2* mutants, *rpb1-14* and *rpb1-17* mutants are Csm$^-$ Tsm$^-$, and *rpb1-17* is also Ino$^-$; *sua8-4* and *rpb1-15* mutants are Csm$^-$ Ino$^-$, and neither is Tsm$^-$. The structural and phenotypic similarities between these *rpb1* mutants and the *sua8* suppressors suggest that *rpb1-14*, *rpb1-17*, and *rpb1-15* might also affect transcription start site selection, a result that would be consistent with the evidence presented in this report supporting a functional role for these regions of the largest subunit of RNA pol II in start site selection.

The three nuclear RNA polymerase systems share significant structural characteristics. Five subunits are shared, and the three largest subunits and a small subunit are related in size and sequence among RNA pol I, II, and III (86). Also, TBP is a requirement in all three systems (26, 72), and a TFIIB-related protein functions in RNA pol III transcription (18, 23). Since the *sua8* suppressors affect start site selection and encode replacements of residues that are conserved among the three RNA polymerases, perhaps the equivalent positions, and possibly other conserved residues within or adjacent to homology blocks D and H, are involved in start site selection by RNA pol I and III.

The multiple pleiotropic phenotypes conferred by the *sua8* mutations can potentially be exploited as a means to identify other components of the general transcriptional apparatus. Suppressors of *sua8* mutants can conveniently be selected as revertants of the temperature-sensitive phenotypes conferred by *sua8* and can subsequently be identified genetically. Suppressors of cold sensitivity might be particularly revealing, since this phenotype is often associated with defects in components of multisubunit systems and therefore potentially suppressible by compensatory mutations in interacting components (67, 88). Possible effects of these suppressors on start site selection can be inferred by scoring iso-1-cytochrome *c* content in the *cyc1-5000* background. This analysis might yield other *RPB* genes, thereby identifying RNA pol II subunits that potentially interact directly with the largest subunit, or *SUA7*, providing additional genetic evidence for a TFIIB-RNA pol II interaction. Alter-

natively, novel components of the transcriptional machinery might be found.

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