Underproduction of the Largest Subunit of RNA Polymerase II Causes Temperature Sensitivity, Slow Growth, and Inositol Auxotrophy in *Saccharomyces cerevisiae*

Jacques Archambault,[1,2] David B. Jansma[2] and James D. Friesen

Department of Genetics, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada and Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Manuscript received August 8, 1995
Accepted for publication December 1, 1995

ABSTRACT

In the yeast *Saccharomyces cerevisiae,* mutations in genes encoding subunits of RNA polymerase II (RNAPII) often give rise to a set of pleiotropic phenotypes that includes temperature sensitivity, slow growth and inositol auxotrophy. In this study, we show that these phenotypes can be brought about by a reduction in the intracellular concentration of RNAPII. Underproduction of RNAPII was achieved by expressing the gene (*RPO21*), encoding the largest subunit of the enzyme, from the *LEU2* promoter or a weaker derivative of it, two promoters that can be repressed by the addition of leucine to the growth medium. We found that cells that underproduced RPO21 were unable to derepress fully the expression of a reporter gene under the control of the *INO1* UAS. Our results indicate that temperature sensitivity, slow growth and inositol auxotrophy is a set of phenotypes that can be caused by lowering the steady-state amount of RNAPII; these results also lead to the prediction that some of the previously identified RNAPII mutations that confer this same set of phenotypes affect the assembly/stability of the enzyme. We propose a model to explain the hypersensitivity of *INO1* transcription to mutations that affect components of the RNAPII transcriptional machinery.

RNA polymerase II (RNAPII), the enzyme responsible for the transcription of all protein-encoding genes in eukaryotes, is a multisubunit enzyme whose structure has been conserved during evolution (reviewed in SAWADOGO and SENTENAC 1990; YOUNG 1991; ARCHAMBAULT and FRIESEN 1993). In the yeast *Saccharomyces cerevisiae,* RNAPII is made up of two large subunits that are similar to the two large subunits, β' and β, of *Escherichia coli* RNAP, as well as 10 smaller subunits, five of which also are components of RNAPI and RNAPIII.

Mutational studies aimed at identifying functional regions of RNAPII have yielded two broad categories of mutations thus far (reviewed in ARCHAMBAULT and FRIESEN 1993). One category comprises mutations that affect the assembly of the enzyme and/or its stability (HIMMELFARB *et al.* 1987; ARCHAMBAULT *et al.* 1990; KOLODZIEJ and YOUNG 1991). These mutations could affect either the stability of individual subunits or their ability to engage in critical protein-protein interactions required for the proper assembly of the enzyme or for the maintenance of its structure once assembled. The other category comprises mutations that affect specific aspects of the transcription cycle. Mutations have been isolated that affect the positioning of the transcription start site (HEKMATPANAH and YOUNG 1991; BERROTERAN *et al.* 1994; FURTER-GRAVES *et al.* 1994; HULL *et al.* 1995), response to transactivator proteins (ALLISON and INGLES 1989; ARNDT *et al.* 1989; SCAFE *et al.* 1990a) or the ability to interact with regulatory factors such as elongation factor TFIIS (ARCHAMBAULT *et al.* 1992b).

A major limitation of the mutational approach is that often it is difficult to determine whether the phenotypes imposed by a given mutation are a consequence of an assembly/stability defect, a transcription defect *per se,* or a combination of both. This is exemplified by the observation that inositol auxotrophy is often associated with mutations affecting RNAPII, regardless of the type of selection scheme that is used to isolate them. Mutations located throughout the genes encoding the two largest subunits (ARNDT *et al.* 1989; NONET and YOUNG 1989; SCAFE *et al.* 1990a–c; ARCHAMBAULT *et al.* 1992a; BERROTERAN *et al.* 1994), the sixth largest subunit (S. NOURAINI, J. ARCHAMBAULT and J. D. FRIESEN, unpublished data), or deletion of the gene encoding the fourth largest subunit of RNAPII (WOYCHIK and YOUNG 1989) give rise to inositol auxotrophy. It was first recognized by ARNDT *et al.* (1989) that the inositol auxotrophy can be explained by the finding that the gene *INO1,* encoding inositol-1-phosphate synthase, is not derepressed in mutant-polymerase cells starved for inositol. This is in contrast to many other genes, which are transcribed at normal levels in these mutant cells.

This paper is dedicated to the memory of Howard J. Himmelfarb.

Corresponding author: James D. Friesen, Department of Genetics, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario M5G 1X8, Canada. E-mail: jim@resunix.ri.sickkids.on.ca

[1] *Present address:* Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada, M5G 1L6.

[2] These authors contributed equally to this work.

Why do so many mutations that occur in many different regions of the enzyme affect transcription of *INO1* and what is the mechanism underlying this phenotype? We argued previously that a reduction, by any means, of the amount of assembled RNAPII would result in an Ino$^-$ phenotype (ARCHAMBAULT *et al.* 1992a). This suggestion was based largely on the observation that the Ino$^-$ and temperature-sensitive (ts) phenotypes imposed by a mutation, *rpo21-4,* in the gene encoding the largest subunit of RNAPII could be suppressed by overproducing the sixth largest subunit of the enzyme (ARCHAMBAULT *et al.* 1990, 1992a). This result and others (ARCHAMBAULT *et al.* 1990, 1992a) are consistent with the hypothesis that more RNAPII can be assembled in the suppressed strain than in the mutant strain and therefore that the Ino$^-$ and ts phenotypes are due to a decrease in the level of assembled RNAPII in the mutant strain. However, in these studies, as in other mutational studies, it is difficult to determine whether the mutation that affects the largest subunit has an effect only on the assembly/stability of the enzyme and not on transcription *per se.*

In the present study we sought to gain more direct evidence in support of the suggestion that a decrease in the level of assembled RNAPII is sufficient to confer inositol auxotrophy and temperature sensitivity. We constructed yeast strains in which the assembly of RNAPII was reduced by limiting the availability of one of its subunits, in this case the largest one. We found that cells that underproduced the largest subunit of RNAPII were inositol auxotrophs because they failed fully to induce transcription of *INO1* when starved for inositol. In addition, we found that growth of these cells was slow at temperatures ranging from 23° to 30° and was inhibited at high temperature. Our results indicate that a reduction in the steady-state amount of RNAPII is sufficient to cause temperature sensitivity, slow growth and inositol auxotrophy. This suggests that some of the previously identified mutations affecting RNAPII, which conferred this set of phenotypes, affect the assembly/stability of the enzyme. We propose a model to explain why transcription of *INO1* is particularly sensitive to mutations affecting the RNAPII transcriptional machinery.

MATERIALS AND METHODS

Strains and growth media: *S. cerevisiae* strains used in this study are listed in Table 1. Growth media and yeast manipulation were as described previously (SHERMAN *et al.* 1986). Yeast transformation was performed essentially as described (ITO *et al.* 1983). Repression of the *LEU2* promoter was accomplished by growing cells in medium containing leucine, isoleucine and threonine at the indicated concentrations.

Plasmids: All DNA manipulations were performed essentially as described (MANIATIS *et al.* 1982). Plasmid DJ20 contains a 7.0-kb *Hin*dIII DNA fragment encompassing *RPO21* cloned into the *Hin*dIII site of pFL39 (*TRP1 CEN6 ARS*) (BONNEAUD *et al.* 1991). Plasmid JA469 that carries the *pLEU2-RPO21* allele contains a 400-bp fragment encompassing the *LEU2* promoter and its 5′-untranslated region (5′-UT) cloned upstream of the *RPO21* open reading frame (ORF). For construction of this plasmid, the *LEU2* promoter and 5′-UT were amplified by PCR using plasmid JJ250 (JONES and PRAKASH 1990) as a template and two oligonucleotides. The downstream oligonucleotide was mutagenic in such a way as to create an *Nco*I restriction site (5′-CC**ATG**G-3′) at the translation-initiation ATG of *LEU2.* The amplified fragment was digested with *Eco*RI and *Nco*I, which cut the PCR fragment immediately upstream of the *LEU2* promoter (position −400) and at the initiator ATG, respectively; this fragment was cloned between the *Eco*RI and *Nco*I sites of plasmid JAY432 so as to replace the *GAL10* promoter fragment of this plasmid with the *LEU2* promoter. To construct plasmid JAY432, a 5.4-kb *Nco*I-*Hin*dIII *RPO21* fragment (from nucleotide +1 to +5415; in this numbering system the "A" residue of the translation-initiation ATG is at position +1) containing the entire *RPO21* ORF, which had been mutated previously so as to contain an *Nco*I site at the initiator ATG (ARCHAMBAULT *et al.* 1992a), was fused downstream of the *GAL10* promoter (carried on a *Eco*RI-*Nco*I fragment) (ARCHAMBAULT *et al.* 1992a). The resulting *GAL10-RPO21* fragment (*Eco*RI-*Hin*dIII) was inserted between the *Eco*RI and *Hin*dIII sites of pFL39. Plasmid JA443, which carries the *pLEU2Δ-RPO21* allele, was constructed by digestion of plasmid JA469 (*pLEU2-RPO21*) with *Ssp*I and *Nco*I, blunting of the ends with the Klenow fragment of DNA polymerase I, followed by religation. In this process the DNA sequences located between the *Ssp*I site in the promoter and the *Nco*I site in the ATG of *pLEU2-RPO21* were deleted. Versions of the plasmids carrying *pLEU2-RPO21* and *pLEU2Δ-RPO21,* respectively, were constructed that lack the translation-initiation codon of *RPO21* by digestion of these plasmids with *Nco*I (which cleaves at the ATG), treatment with S1 nuclease followed by religation. Plasmid JAY47 carries the same *pGAL-RPO21* fragment as plasmid JAY432 in addition to a 1.6-kb *Hin*dIII-*Eco*RI fragment downstream of *RPO21* cloned into the *Eco*RI site of plasmid INT2 (*URA3 CEN1 ARS1*) (PERCIVAL-SMITH and SEGALL 1986). Assays for promoter strength used plasmids based on pFL39, which were constructed so as to fuse either the promoters of *LEU2, LEU2Δ* or *RPO21* along with the first 662 nucleotides of the *RPO21* ORF in frame to the *lacZ* gene of *E. coli.* These fusion genes were constructed by replacing in pDJ20 (*RPO21*), pJA469 (*pLEU2-RPO21*) and pJA443 (*pLEU2Δ-RPO21*), respectively, the *Spe*I-*Hin*dIII fragment that encodes the C-terminal part of the *RPO21* ORF and 3′-UT by a *Xba*I-*Stu*I fragment (a *Hin*dIII linker was

TABLE 1

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

Strain[a]	Relevant genotype
W303-1a	*MAT***a** *can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1*
W303-1b	*MATα can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1*
AJ1	W303-1a with [YEp13; *LEU2*]
YF1733	W303-1b with *rpo21::ADE2* [pJAY47; *pGAL-RPO21 URA3*]
YF1960	YF1733 with *LEU2*
AJ2	YF1733 with [YEp13; *LEU2*]
YF1971	W303-1b with *pLEU2-RPO21 LEU2*
YF2122	W303-1b with *LEU2* [pFL39; *TRP1*]

[a] Strain W303-1a and W303-1b were obtained from R. ROTHSTEIN.

attached to the *Stu*I end) encoding *lacZ* from YEp356R (MYERS *et al.* 1986).

Transfer of the *pLEU2-RPO21* allele to the endogenous *RPO21* chromosomal location, was accomplished by creating plasmid DJ58 that carries a *RPO21* fragment (from −1583 to +5415) in which sequences between −722 and +1 were replaced by the *LEU2* promoter and 5′-UT. pDJ58 was constructed by inserting a *Hin*dIII-*Bst*E2 *RPO21* fragment (from −1583 to −722) upstream of the *LEU2* promoter in plasmid JA469 (*pLEU2-RPO21*).

Transfer of the *pLEU2-RPO21* allele to the chromosomal *RPO21* locus: A *Hin*dIII-*Bgl*II fragment from pDJ58 containing the *RPO21* upstream sequences (−1583 to −722), the *LEU2* promoter and the beginning of the *RPO21* ORF (+1 to +3408) was introduced into yeast strain YF1960 (*MATα rpo21::ADE2 LEU2* [pJAY47; *pGAL-RPO21*]). YF1960 was constructed in three steps. First, strain W303-1b was transformed with a single-copy episomal plasmid (pJAY47; *pGAL-RPO21 URA3*) in which *RPO21* is expressed from the *GAL10* promoter. Second, the chromosomal copy of *RPO21* was disrupted by the insertion of *ADE2* between the *Bst*E2 (−722) and *Spe*I (+662) sites of *RPO21* to create strain YF1733. Growth of this strain is maintained only on medium containing galactose since the maintenance plasmid expresses *RPO21* from the *GAL10* promoter. Third, strain YF1733 was made prototrophic for leucine by transformation with a *LEU2* fragment and selection for Leu^+ transformants to create strain YF1960. Following introduction of the *pLEU2-RPO21* fragment into strain YF1960, Ade^- transformants (pink colored) that were able to grow on glucose medium lacking leucine were selected to select for transformants in which the chromosomal *RPO21* allele was replaced by the *pLEU2-RPO21* allele. Replacement of *RPO21* by *pLEU2-RPO21* was confirmed by PCR. One transformant carrying the *pLEU2-RPO21* allele in the chromosome was then streaked on medium that lacked leucine and contained uracil to screen for cells that had lost the maintenance plasmid (pJAY47). One such Ura^- clone (YF1971, *MATα pLEU2-RPO21 LEU2*) was used in this study.

β-galactosidase assays: β-galactosidase activity was measured as described by MILLER (1972). Cells were grown in selective medium with the indicated concentration of leucine, isoleucine and threonine. For each measurement, β-galactosidase activity was determined on three independent cultures.

Immunoblot (Western blot) analysis: Whole-cell extracts were prepared from cultures grown to an optical density (600 nm) of 0.5 essentially as described previously (TYERS *et al.* 1992). Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and subjected to immunoblot analysis essentially as described (HARLOW and LANE 1989). The presence of the largest subunit of RNAPII (RPO21) was detected using the monoclonal antibody (mAb) 8WG16 directed against the RPO21 polypeptide (THOMPSON *et al.* 1989). As a loading control, the same blot was exposed to a rabbit polyclonal antiserum directed against the yeast BiP protein (kindly provided by Dr. D. WILLIAMS, University of Toronto). The secondary antibody was peroxidase-conjugated goat anti-mouse when using mAb 8WG16 or anti-rabbit when using anti-BiP antiserum. Detection was by the enhanced chemiluminescence method as described by the manufacturer (Amersham). Efforts to quantify the amount of *RPO21* polypeptide present in *pLEU2-RPO21* cells grown in the presence of leucine relative to the level detected in wild-type cells were made by immunoblotting serial dilutions of whole-cell extracts and comparing multiple exposures.

RESULTS

Expression of the largest subunit of RNAPII from the *LEU2* promoter: To test the hypothesis that a reduction in the level of RNAPII could confer temperature sensitivity, slow growth and inositol auxotrophy, we set out to construct yeast strains in which the level of expression of the largest subunit of RNAPII (the *RPO21* gene product) would be limiting for enzyme assembly. This can be accomplished by fusing the ORF and downstream sequences of *RPO21* to a heterologous promoter of lesser strength than that of *RPO21*. We first tried the promoter of *LEU2*, a gene involved in leucine biosynthesis (ANDREADIS *et al.* 1984), because it has been characterized extensively and its activity can be repressed by the addition of leucine to the growth medium (ANDREADIS *et al.* 1984; TU and CASADABAN 1990). Maximal repression of the *LEU2* promoter can be achieved by supplementing the growth medium with isoleucine and threonine in addition to leucine (TU and CASADABAN 1990). This regulation of *LEU2* transcription is mediated through a well defined upstream activating sequence (UAS) that binds the *LEU3* gene product (FRIDEN and SCHIMMEL 1988; TU and CASADABAN 1990). A series of gene fusions was constructed (Figure 1). We first fused the promoter and 5′-untranslated sequence of *LEU2* upstream of the *RPO21* ORF (see MATERIALS AND METHODS); we named this allele *pLEU2-RPO21*. A second allele, similar to *pLEU2-RPO21* but lacking sequences located between the *LEU2* UAS and translation-initiation codon, was also created (see MATERIALS AND METHODS). This allele, which we named *pLEU2Δ-RPO21*, lacks a DNA element required for maximal expression of *LEU2* but not for its regulation by *LEU3* (TU and CASADABAN 1990). It was shown previously that a *LEU2* promoter that lacks this element is weaker than the wild-type *LEU2* promoter but is still regulated by leucine (TU and CASADABAN 1990).

Strength of the pLEU2 and pLEU2Δ promoters: We compared the strength of the pLEU2 and pLEU2Δ promoters relative to that of *RPO21* by fusing these three promoters, along with their 5′-untranslated regions and the first 662 nucleotides of the *RPO21* ORF, in frame to the *lacZ* gene of *E. coli*. These fusion genes were introduced into yeast strain AJ1 (W303-1a [YEp13, *LEU2*]) and the amount of β-galactosidase activity was measured (Figure 2). In medium lacking leucine, the condition under which activity of the *LEU2* promoter is maximal, the *LEU2* promoter directed expression of 13.4 units of β-galactosidase, a level of activity similar to that measured in strains that express *lacZ* from the *RPO21* promoter. However, in contrast to the expression of *RPO21-lacZ*, that of *pLEU2-lacZ* was repressed ~40-fold by the addition of leucine, isoleucine and threonine to the growth medium. The magnitude of this regulation is similar to that reported previously for *LEU2* (TU and CASADABAN 1990). As expected, the pLEU2Δ promoter directed the expression of lower levels of β-galactosidase activity in medium lacking leucine but still retained the ability to be repressed by leucine. From these results, we anticipated that the tran-

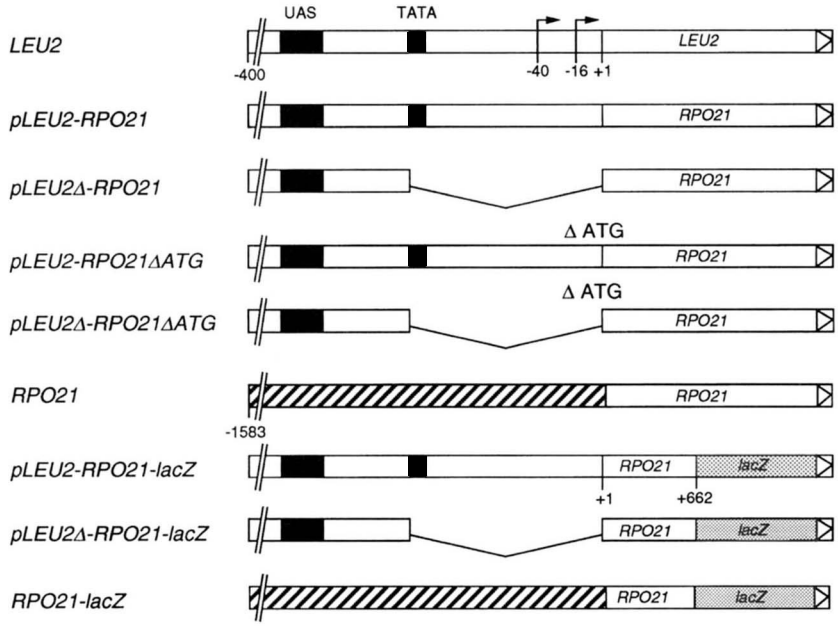

FIGURE 1.—Structure of the fusion genes used in this study. The top line represents the structure of the *LEU2* gene that comprises the promoter and 5′-UT from nucleotide −400 to +1 (+1 being the position of the first nucleotide of the initiator ATG) fused to the *LEU2* ORF. The two arrows indicate the position of the transcription start sites mapped previously (ANDREADIS *et al.* 1984). The two black boxes indicate the location of the UAS and a TATA element that are required for maximal expression of *LEU2* (TU and CASADABAN 1990). The next four lines represent the structure of the fusion genes in which the *RPO21* ORF was fused either to the intact *LEU2* promoter and its 5′-UT (plasmid JA469) or to a deletion-derivative that lacks the "TATA" element because of a deletion of nucleotides −120 to −1 (plasmid JA443). "ΔATG" indicates alleles in which the initiator ATG was deleted. The sixth line represent the structure of the *RPO21* gene that comprises the promoter and 5′-UT from position −1583 to +1 (hatched box) fused to the *RPO21* ORF (plasmid DJ20). The seventh, eighth and ninth lines represent the structure of the *lacZ* fusions that were derived from *pLEU2-RPO21*, *pLEU2Δ-RPO21* and *RPO21*, respectively.

scription of *RPO21* from the *LEU2* promoter would be near wild type (*i.e.*, the level obtained with the *RPO21* promoter) in medium lacking leucine. A significant decrease in the level of transcription of *RPO21* could be achieved by addition of leucine to the growth medium

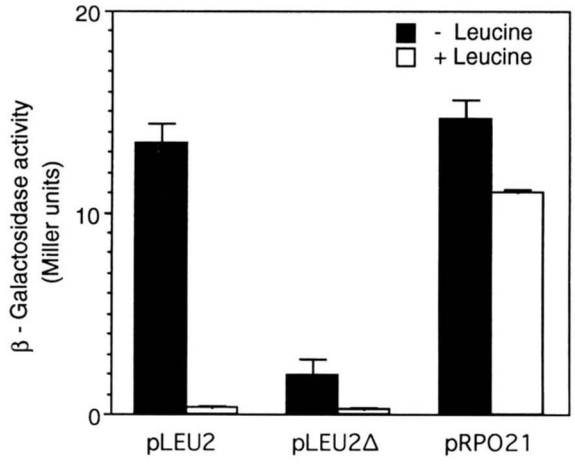

FIGURE 2.—Strength of the pLEU2, pLEU2Δ and pRPO21 promoters. Levels of β-galactosidase activity expressed in Miller units were determined in strain AJ1 expressing a *RPO21-lacZ* fusion protein from either the pLEU2, pLEU2Δ or pRPO21 promoter on a single-copy replicating vector. Cells were grown either in the absence (filled bars) or presence (open bars) of 2.0 mM leucine, isoleucine and threonine.

of cells expressing *RPO21* from the *LEU2* promoter or by expressing *RPO21* from the *LEU2*Δ promoter.

Ability of *pLEU2-RPO21* and *pLEU2Δ-RPO21* to support growth of yeast cells: We next tested the ability of the *pLEU2-RPO21* and *pLEU2Δ-RPO21* alleles to support cell growth by complementing a *RPO21* null allele (*rpo21::ADE2*). As a control, a similar plasmid containing the entire *RPO21* gene expressed from its endogenous promoter was also used in these experiments. These alleles, carried on a single-copy replicating plasmid (pFL39; *TRP1 CEN6 ARS*), were introduced into yeast strain AJ2 (*rpo21::ADE2* [pJAY47; *URA3 pGAL-RPO21*][YEp13; *LEU2*]) by selection for tryptophan prototrophy on medium containing galactose (to allow for expression of *pGAL-RPO21* from the maintenance plasmid) and lacking leucine (to derepress the pLEU2 and pLEU2Δ promoters). We obtained strains that lacked the maintenance plasmid by growing these transformants for several generations in glucose medium containing uracil and lacking leucine and subsequently by screening for uracil-auxotrophy on glucose medium at 23°. As can be seen in Figure 3, Ura$^-$ cells carrying the *pLEU2-RPO21* allele were able to grow as well as cells carrying a wild-type *RPO21* gene on solid medium lacking leucine. This finding was consistent with our previous observation (see above) that the strength of the *LEU2* promoter is similar to that of *RPO21* when

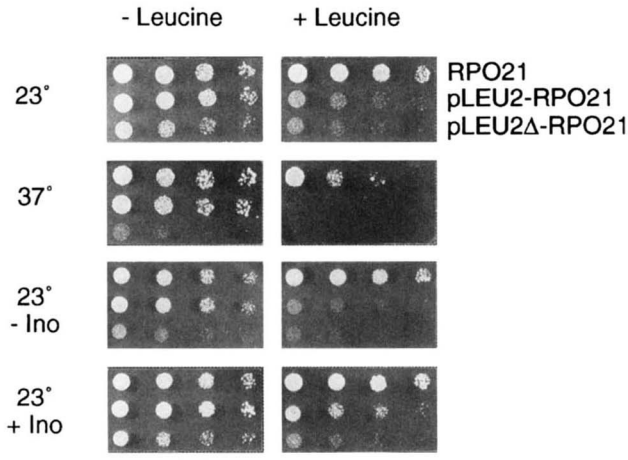

FIGURE 3.—Phenotypes of cells expressing *RPO21* from either the pLEU2, pLEU2Δ or RPO21 promoter. Plasmids carrying the *pLEU2-RPO21*, *pLEU2Δ-RPO21*, or *RPO21* allele were introduced into yeast strain AJ2 (*MATα rpo21::ADE2* [pJAY47; *pGAL-RPO21*][YEp13; *LEU2*]). Transformed strains were screened for loss of the maintenance plasmid (pJAY47) and were tested for their ability to support growth on solid medium containing glucose at 23° and 37° and on glucose medium that either lacked (−Ino) or contained 10 μM inositol (+Ino) at 23°. Repression of the *LEU2* promoter was accomplished by adding 2.0 mM leucine, isoleucine and threonine to the growth medium. Serial dilutions of the cell culture were spotted on the plates; these contained 10,000, 2000, 400 and 80 cells, respectively.

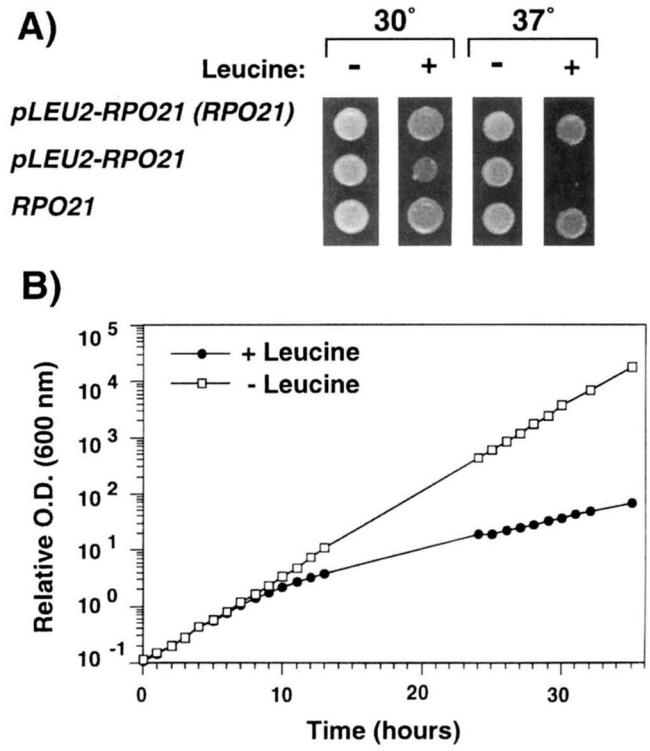

FIGURE 4.—Effect of leucine on the growth of yeast cells in which the chromosomal *RPO21* promoter was replaced by the *LEU2* promoter. (A) A yeast strain was constructed in which the *RPO21* locus was replaced by the *pLEU2-RPO21* allele (see MATERIALS AND METHODS). This strain was then transformed with either a single-copy plasmid (pDJ20) carrying wild-type *RPO21* or with a control plasmid (pFL39). In the figure, these transformed cells are designated *pLEU2-RPO21 (RPO21)* and *pLEU2-RPO21*, respectively. These transformed strains, along with a wild-type strain (in which the chromosomal copy of *RPO21* is wild type, strain YF2122), were tested for their ability to grow on solid medium containing glucose at 30° and 37° in the presence (+leucine) or absence (−leucine) of 2 mM leucine, isoleucine and threonine. (B) Portions of a culture of *pLEU2-RPO21* cells grown in liquid medium at 30° were inoculated at time zero in medium lacking (−leucine) or containing (+leucine) 0.5 mM leucine, isoleucine and threonine. The cells were diluted into fresh medium, when appropriate, to ensure continued logarithmic growth. Growth was determined by absorbance at 600 nm.

leucine is absent from the growth medium. However, when leucine was added to the medium or when *RPO21* was transcribed from the weaker pLEU2Δ promoter, cell growth was impaired. We also showed that deleting the translation-initiation codon in *pLEU2-RPO21* and *pLEU2Δ-RPO21* (see MATERIALS AND METHODS) abolished their ability to support growth, indicating that complementation of *rpo21::ADE2* requires that the translation of the fusion-gene mRNAs be initiated at the correct AUG codon (data not shown). These results indicated that a substantial decrease in the level of transcription of *RPO21* cannot be tolerated without reducing the growth rate.

Cells that underproduce the RPO21 polypeptide are temperature-sensitive for growth and auxotrophic for inositol: We next tested cells that express *RPO21* from the pLEU2 or pLEU2Δ promoters for their ability to grow at high temperature (37°) or on medium lacking inositol (at 23°). We found that cells expressing *RPO21* from the *LEU2* promoter grew as well as wild-type cells at high temperature and on medium lacking inositol under conditions where the pLEU2 promoter was derepressed (*i.e.*, when leucine was absent from the growth medium) (Figure 3). This was not surprising since our analysis of the strength of the *RPO21* and *LEU2* promoters suggested that, under these growth conditions, *RPO21* is expressed at similar levels in both strains. However, when leucine, isoleucine and threonine were added to the growth medium, *pLEU2-RPO21* cells grew extremely poorly at high temperature or at 23° on medium lacking inositol (Figure 3). Addition of 10 μM inositol to the growth medium relieved the Ino^- phenotype (Figure 3). Similar results were observed for cells expressing *RPO21* from the pLEU2Δ promoter. These results indicated that underproduction of the largest subunit of RNAPII is sufficient to confer thermosensitivity and inositol auxotrophy.

Transfer of the *pLEU2-RPO21* allele to the *RPO21* chromosomal location: A yeast strain was constructed in which the endogenous *RPO21* gene was replaced by the *pLEU2-RPO21* allele (see MATERIALS AND METHODS). This strain grew as well as a wild-type strain at 30° and 37° when grown in the absence of leucine, isoleucine and threonine (Figure 4A). In contrast, this strain grew

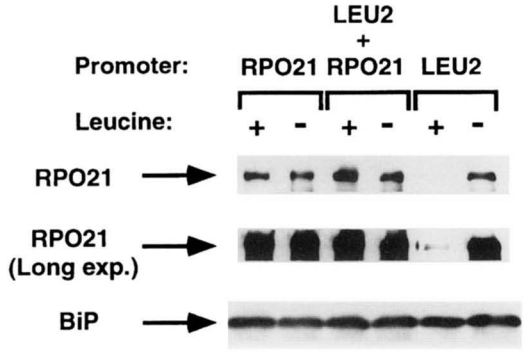

FIGURE 5.—Amount of RPO21 protein in strains carrying the *pLEU2-RPO21* allele. The RPO21 protein was detected by immunoblot analysis using the mAb 8WG16 antibody in total cellular extracts prepared from cultures of *pLEU2-RPO21* cells (pLEU2) or from the same strain carrying a wild-type *RPO21* gene on a single copy plasmid (pDJ20) (pLEU2 + pRPO21) or from a wild-type strain (pRPO21). Cells were grown either in the presence (+) or absence (−) of leucine, isoleucine and threonine. The amount of RPO21 protein present in *pLEU2-RPO21* cells grown in the presence of leucine was detected only after a longer exposure of the autoradiogram (RPO21 long exposure). As a control, the amount of the BiP protein was also detected in these extracts using a rabbit polyclonal antiserum directed against yeast BiP.

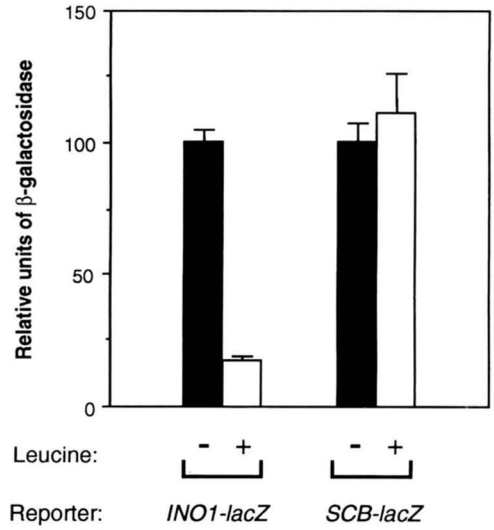

FIGURE 6.—Expression of *INO1-lacZ* and *SCB-lacZ* fusion genes in *pLEU2-RPO21* cells. Relative β-galactosidase activity were determined for *pLEU2-RPO21* cells expressing either an *INO1-lacZ* or a *SCB-lacZ* fusion gene. Cells were grown in the absence of inositol and in the presence (+) or absence (−) of 2.0 mM leucine, isoleucine and threonine. For each reporter gene, the level of β-galactosidase activity measured for cells grown in the absence of leucine, isoleucine and threonine was assigned a value of 100.

slowly at 30° and was temperature-sensitive at 37° when leucine, isoleucine and threonine were present in the growth medium (Figure 4A). Both, the slow-growth and temperature-sensitive phenotypes could be complemented by introducing a wild-type copy of *RPO21* into this *pLEU2-RPO21* strain. These results are consistent with those obtained in Figure 3. This *pLEU2-RPO21* strain was then used to measure the difference in growth rate between cells grown in the absence or presence of leucine at 30°. As can be seen in Figure 4B, *pLEU2-RPO21* cells grown in the absence of leucine had a doubling time of 2 hr, similar to that of wild-type cells (data not shown). Addition of leucine, isoleucine and threonine to the medium increased the doubling time to 5.5 hr. This doubling time was reached ~10–15 hr after the addition of leucine, isoleucine and threonine to the medium and presumably represents the time required to reduce the cellular content of RNAPII synthesized before the addition of these amino acids (time zero).

We verified by immunoblot analysis that the addition of leucine, isoleucine and threonine to the growth medium reduced the amount of *RPO21* gene product in these cells. As can be seen in Figure 5, when grown in the absence of leucine, *pLEU2-RPO21* cells had a similar amount of RPO21 polypeptide as wild-type cells. In contrast, *pLEU2-RPO21* cells grown in the presence of leucine, isoleucine and threonine had significantly less RPO21 protein than wild-type cells. We obtained a gross estimate of this difference by comparing the level of RPO21 to the amount of BiP protein (NICHOLSON *et al.* 1990) present in these extracts (see MATERIALS AND METHODS). We found that *pLEU2-RPO21* cells had ~10-fold less RPO21 polypeptide than wild-type cells. However, this could be an underestimate of the difference, since a suboptimal amount of RPO21 could indirectly reduce expression of BiP. As a control, we also showed that *pLEU2-RPO21* cells that had been transformed with a single-copy plasmid expressing wild-type *RPO21* did not show a reduction in the steady-state level of RPO21 polypeptide when grown under repressing conditions (Figure 5) (pLEU2 + pRPO21).

***INO1* expression is reduced in cells that underproduce RPO21:** We determined the ability of *pLEU2-RPO21* cells to induce transcription of *INO1* when starved for inositol, by measuring β-galactosidase activity in cells carrying an *INO1-lacZ* fusion gene. This fusion gene carries the *INO1* UAS placed upstream of the *CYC1* TATA-box and transcriptional-initiation sites (pJH359) (LOPES *et al.* 1991). A similar reporter gene (pBA259) (ANDREWS and HERSKOWITZ 1989) carrying four SCB binding sites (which bind the transcription factor SWI4/SWI6) instead of the *INO1* UAS was used as a control in these experiments. As can be seen in Figure 6, *pLEU2-RPO21* cells showed a sixfold reduction in β-galactosidase activity produced from the *INO1-lacZ* reporter gene when grown in the presence of leucine, isoleucine and threonine. In contrast, transcription of the *SCB-lacZ* gene, which is also driven by the *CYC1* promoter but under the control of a different UAS, was unaffected by the addition of these amino acids to the growth medium. These results indicated that the inositol auxotrophy of *pLEU2-RPO21* cells, which are grown in the presence of leucine, isoleucine and threonine, is caused by a reduction in the level of *INO1* expression. The inositol auxo-

trophy and reduction in *INO1* expression were not detected in *pLEU2-RPO21* cells that had been transformed with a plasmid bearing a wild-type copy of *RPO21* (data not shown), indicating that underproduction of RPO21 is sufficient to bring about these phenotypes. Furthermore, since the *INO1-lacZ* fusion used in this study contains only the *INO1* UAS and not its promoter, the failure to induce expression of *INO1-lacZ* must be due to an inability to activate its transcription in response to the signals originating from the *INO1* UAS. A previous study also indicated that the failure of some RNAPII mutants to transcribe *INO1* is the result of an inability to respond to the *INO1* UAS (Scafe *et al.* 1990a).

The magnitude (sixfold) of the reduction in the transcription of *INO1-lacZ* detected in cells that underproduce *RPO21* is similar to that brought about by other mutations that affect components of the transcriptional machinery, such as those that truncate the carboxy-terminal domain (CTD) of RNAPII, the *rpb1-1* mutation, or a deletion of the *SRB2* gene. These last three mutations reduce expression of *INO1-lacZ* approximately fourfold (Koleske *et al.* 1992).

DISCUSSION

The results described in this study indicate that an ~10-fold reduction in the steady-state amount of RPO21 is not a lethal event but rather is sufficient to confer phenotypes often associated with mutations affecting RNAPII, such as temperature sensitivity, slow growth and inositol auxotrophy. Since the only known function of RPO21 is as a component of RNAPII, we infer that the phenotypes caused by underproduction of RPO21 are a consequence of a reduction in the intracellular amount of RNAPII. Because we did not measure directly the extent by which RNAPII is reduced in cells that underproduce RPO21, we cannot rule out the possibility that a 10-fold reduction in the level of RPO21 does not lead to a concomitant 10-fold reduction in the level of RNAPII. However, it is unlikely that a substantial reduction in the amount of RPO21 would cause only a marginal reduction in the level of RNAPII since RPO21 is not produced in excess in wild-type yeast cells (Kolodziej and Young 1991). The finding that *pLEU2-RPO21* cells grown under repressing conditions produce ~10-fold less RPO21 protein than wild-type cells (Figure 5), even though the pLEU2 promoter is ~40 times weaker than the *RPO21* promoter (Figure 2), may indicate that a compensatory mechanism exists that maintains the required amount of RPO21 protein when transcription of the gene is reduced. Such a compensatory mechanism has been described previously in mammalian cell lines (Somers *et al.* 1975; Guialis *et al.* 1977, 1979) and in *Caenorhabditis elegans* (Dalley *et al.* 1993). Recently, we have obtained evidence for the existence of a similar mechanism in yeast (D. B. Jansma and J. D. Friesen, unpublished observations).

Our finding that reducing the steady-state level of RNAPII is sufficient to confer temperature sensitivity, slow growth and inositol auxotrophy leads to the prediction that some of the previously isolated RNAPII mutations that confer this same set of phenotypes may have affected the assembly/stability of the enzyme. Our results also provide an explanation as to why many mutations in different subunits and in different locations of RNAPII impose a similar set of phenotypes. Our results do not exclude the possibility that these previously identified RNAPII mutations may also have affected other aspects of RNAPII function in addition to affecting its assembly/stability. It is also worth noting that temperature sensitivity, slow growth and inositol auxotrophy can be caused by RNAPII mutations that do not affect the assembly of the enzyme. One such example is mutations that truncate the CTD of the largest subunit of RNAPII (Nonet *et al.* 1987; Allison *et al.* 1988). The similarities between CTD-truncation mutations and mutations that reduce the level of RNAPII, such as those described in this study, are discussed below.

Regulation of *INO1* transcription: Our results indicate that a decrease in the intracellular level of RNAPII affects transcription from the *CYC1* promoter when it is placed under the control of the *INO1* UAS but not when controlled by SCB elements (which bind the transcription factor SWI4/SWI6) (Andrews and Herskowitz 1989). How is transcription regulated by the *INO1* UAS and why is this regulation altered in cells that underproduce RNAPII? It was shown previously that transcription of *INO1* is controlled by three regulatory genes, *INO2, INO4* and *OPI1* (Figure 7) (reviewed in Carman and Henry 1989). The *INO2* and *INO4* gene products are positive activators of *INO1* expression and form a complex that binds a DNA element that is present in two copies in the *INO1* UAS (Hirsh and Henry 1986; Lopes *et al.* 1991; Ambroziak and Henry 1994). These same DNA sequences are also required for the negative regulation of *INO1* expression by the *OPI1* gene (Ashburner and Lopes 1995a). It has been suggested that the *OPI1* protein represses transcription by antagonizing the ability of the INO2/INO4 complex to activate transcription in a manner similar to that of GAL80 on the GAL4 transactivator protein (Ashburner and Lopes 1995a). Interestingly, the promoter of *INO2* also contains a binding site for the INO2/INO4 complex and is also subject to the positive and negative regulation of *INO2, INO4* and *OPI1* (Ashburner and Lopes 1995a).

Transcription of *INO1* is also subject to the regulatory effect of chromatin and proteins involved in remodeling chromatin. Mutations in *SWI1(ADR6), SWI2(SNF2), SWI3, SNF5* and *SNF6,* which encode proteins that are part of a large complex (Peterson *et al.* 1994; Coté *et al.* 1994) required to overcome the negative regulatory effect of chromatin at certain promoters (Hirschhorn *et al.* 1992; reviewed in Peterson and Tamkun 1995), affect the expression of many unrelated genes, includ-

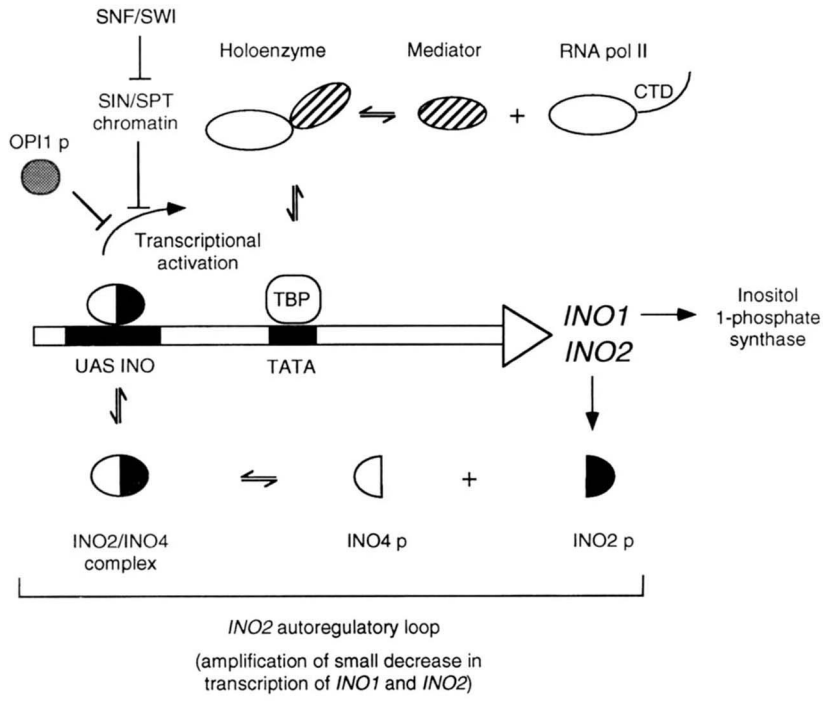

FIGURE 7.—Model for the transcription of *INO1* and *INO2*. This model emphasizes the role of certain proteins (discussed in the text) involved in regulating transcription-initiation by RNAPII at the *INO1* and *INO2* promoters. Transcription from these two promoters requires two DNA elements, the UAS INO and the TATA box, which are bound by the INO2/INO4 complex and by the general initiation factor TBP, respectively. Transcription from the *INO1* and *INO2* promoters requires formation of the RNAPII holoenzyme, which is depicted as an association between the mediator complex and the CTD of RNAPII. Initiation of transcription by RNAPII requires that the holoenzyme be recruited at the promoter, at least in part by an association with TBP, in response to the transcriptional activation signal of the INO2/INO4 complex. The activity of this complex is regulated negatively by the *OPI1* protein. Transcriptional activation by the INO2/INO4 complex is also subject to the negative regulation of chromatin, which may prevent it or TBP, from binding to DNA. This negative effect of chromatin is alleviated by the SNF/SWI complex. Wild-type-level expression of *INO1* and *INO2* also requires that the *INO2* protein be synthesized in sufficient amount and that it associates with the *INO4* protein. Since the levels of the *INO2* protein are limiting for *INO1* and *INO2* transcription, small variations in the intracellular concentration of *INO2* protein are amplified by the *INO2* autoregulatory loop. In this model, the hypersensitivity of *INO1* transcription to mutations affecting components of the transcriptional machinery is explained by the amplification of small transcriptional defects by the *INO2* autoregulatory loop.

ing *INO1* (PETERSON and HERSKOWITZ 1992; reviewed in WINSTON and CARLSON 1992) (Figure 7). Mutations that were isolated previously as suppressors of mutations affecting the SWI/SNF complex such as mutations in *SIN2* encoding histone H3, *SIN1(SPT2)* encoding an HMG1-like protein (KRUGER and HERSKOWITZ 1991) or *SIN3* (HUDAK *et al.* 1994), also affect expression of *INO1*. In contrast to the *SWI/SNF* genes, *SIN1*, *SIN2* and *SIN3* are required for repression of *INO1* when inositol is present in the growth medium.

Why is expression of *INO1* particularly sensitive to mutations affecting the transcriptional machinery?: The fact that transcription of *INO1* is determined by the level of transcription of the positive regulator *INO2* (ASHBURNER and LOPES 1995b) and that *INO2* is under the control of an autoregulatory loop (ASHBURNER and LOPES 1995a) could explain why transcription of *INO1* is particularly sensitive to a decrease in the levels of RNAPII. In this case, a small reduction in the ability of the transcription machinery to respond to the regulatory signal from the *INO2* UAS would result in decreased expression of *INO2* that in turn would lead to a much larger reduction in the transcription of *INO1* and *INO2* itself. This amplification of slight transcriptional defects would certainly be accentuated by the fact that the levels of *INO2* transcription (and presumably of *INO2* protein) appear to be rate-limiting for transcription of *INO1* and that the *INO2* promoter is the weakest yeast promoter characterized thus far (ASHBURNER and LOPES 1995a). In this model therefore, the sensitivity of *INO1* expression to a decrease in the level of RNAPII, or to any other type of mutation affecting components of the transcriptional machinery, is explained by the fact that a slight defect in transcription of *INO1* and *INO2* is amplified by the *INO2* autoregulatory loop (Figure 7). In general, it may be that the transcription of genes whose regulatory proteins are limiting for their expression and are under the control of an autoregulatory loop are more sensitive to mutations that affect components of the transcriptional machinery.

Underproduction of RNAPII compared to truncation of the CTD: Transcription of *INO1* also requires a set of proteins encoded by the suppressor of RNAPII (*B*) genes (*SRB*) that were identified in a search for suppres-

sors of mutations that shorten the CTD of the largest subunit of RNAPII (NONET and YOUNG 1989; THOMPSON *et al.* 1993) (Figure 7). Several of the *SRB* polypeptides (SRB2, 4–11) are components of a multisubunit complex termed "the mediator," which also contains other global regulators of transcription such as SUG1, GAL11 and the general initiation factor TFIIF (KIM *et al.* 1994). The mediator can also be purified as part of a much larger complex containing RNAPII (KIM *et al.* 1994) and, under certain purification conditions, TFIIB and TFIIH (THOMPSON *et al.* 1993; KOLESKE and YOUNG 1994). This large complex, termed the "RNAPII holoenzyme" (reviewed in KOLESKE and YOUNG 1995) can respond to transcriptional-activator (KIM *et al.* 1994) and repressor (KUCHIN *et al.* 1995; WAHI and JOHNSON 1995) proteins. The RNAPII holoenzyme is thought to be the form of RNAPII that is recruited at the promoter of most yeast genes (THOMPSON and YOUNG 1995) at least in part through interactions with the TATA-box binding protein (TBP) (KOLESKE *et al.* 1992; THOMPSON *et al.* 1993) and with activator proteins (HENGARTNER *et al.* 1995).

In this context, it is worth considering why yeast cells carrying mutations that shorten the CTD have similar phenotypes as cells underproducing RNAPII, namely temperature sensitivity, slow growth and inositol auxotrophy. Mutations that truncate the CTD are unlikely to affect the steady-state level of the enzyme; this suggestion is supported by the findings that mutations that truncate the CTD do not reduce the stability of the largest subunit (NONET *et al.* 1987; ALLISON *et al.* 1988) but rather affect the ability of RNAPII to respond to the regulatory signals of certain UASs (including the *INO1* UAS) (ALLISON and INGLES 1989; SCAFE *et al.* 1990a). However, because the CTD participates in the assembly of the RNAPII holoenzyme, it is possible that mutations that shorten the CTD affect the ability of RNAPII to interact with the mediator complex. In support of this hypothesis is the fact that the mediator can be dissociated from RNAPII using a monoclonal antibody directed against the CTD (KIM *et al.* 1994). Therefore, the phenotypes imposed by mutations that shorten the CTD may result from less RNAPII holoenzyme being assembled in these mutant cells. Similarly, underproduction of the largest subunit of RNAPII, as was done in this study, would also lead to a reduction in the amount of RNAPII holoenzyme, in this case not by affecting an interaction with the mediator complex, but by reducing the amount of RNAPII available to assemble into the holoenzyme. Although speculative, these similarities provide an explanation as to why truncation of the CTD and underproduction of RNAPII impose similar phenotypes. Finally, since the holoenzyme is recruited to promoters, at least in part through an association with TBP (KOLESKE *et al.* 1992; THOMPSON *et al.* 1993), it is not surprising that mutations in TBP also cause an Ino$^-$ phenotype (ARNDT *et al.* 1995).

With these ideas in mind, we tested whether a dominant mutation in *SRB2* (*SRB2-1*), which was isolated on the basis of its ability to suppress the phenotypes of CTD-truncation mutations, would suppress the phenotypes of cells that underproduce RPO21. We found that *SRB2-1* was unable to suppress the slow-growth phenotype of cells that underproduce RNAPII (data not shown). This result was, perhaps, not surprising since *SRB2-1* suppresses specifically CTD-truncation mutations and not any other mutations in *RPO21*, some of which might affect the assembly/stability of the enzyme since they confer inositol auxotrophy. Similarly, we found that the phenotypes of cells that underproduce RNAPII were not suppressed by lengthening the CTD or by overproducing the sixth largest subunit of RNAPII (RPO26) (data not shown). Lengthening the CTD was shown previously to suppress weakly the effects of mutations that reduced the activity of the transcriptional activator protein GAL4 (ALLISON and INGLES 1989). Overexpression of RPO26 was shown to suppress, in an allele-specific manner, the inositol auxotrophy, temperature-sensitivity and slow-growth phenotypes imposed by the *rpo21-4* mutation, a mutation that is thought to affect the assembly/stability of RNAPII (ARCHAMBAULT *et al.* 1990, 1992a). These results suggested that in those previous studies suppression by *SRB2-1*, or by lengthening of the CTD or by overexpression of *RPO26*, was not brought about by bypassing the requirement for a threshold level of RNAPII.

Underproduction of RNAPIII: A finding of this work and of previous studies of RNAPII mutants is that the transcription of specific genes such as *INO1* is affected to a greater extent than that of other genes such as *SCB-lacZ* by alterations in the structure or levels of RNAPII. This differential effect on gene-expression is not unique to RNAPII. In the case of RNAPIII, it was found that mutations affecting the largest subunit (GUDENUS *et al.* 1988) or some of the smaller subunits, RPC82 (CHIANNILKULCHAI *et al.* 1992), RPC53 (MANN *et al.* 1992), RPC34 (STETTLER *et al.* 1992) and RPC31 (MOSRIN *et al.* 1990), lead to a reduction in the amount of tRNAs, while that of 5S RNA was relatively unchanged. It was suggested that RNAPIII either has a lower affinity for the initiation complexes assembled at tRNA genes than for those assembled at the 5S RNA gene or that the enzyme is more stable in the nucleolus (where 5S RNA is synthesized) than in the nucleoplasm (where tRNAs are synthesized).

We thank Dr. DAVID WILLIAMS for the gift of anti-BiP antiserum and Dr. BRENDA ANDREWS, Dr. SUSAN HENRY and Dr. RICHARD YOUNG for providing us with plasmids. We also thank Dr. JACK GREENBLATT, Dr. JOHN LOPES, Dr. JACQUELINE SEGALL, and SHAHRZAD NOURAINI for critical reading of the manuscript. This work was supported by a grant and a studentship from the Medical Research Council of Canada to J.D.F. and J.A., respectively.

LITERATURE CITED

ALLISON, L. A., and C. J. INGLES, 1989 Mutations in RNA polymerase II enhance or suppress mutations in *GAL4*. Proc. Natl. Acad. Sci. USA **86:** 2794–2798.

ALLISON, L. A., J. K. WONG, V. D. FITZPATRICK, M. MOYLE and C. J. INGLES, 1988 The C-terminal domain of the largest subunit of RNA polymerase II of *Saccharomyces cerevisiae, Drosophila melanogaster,* and mammals: a conserved structure with an essential function. Mol. Cell. Biol. **8:** 321–329.

AMBROZIAK, J., and S. A. HENRY, 1994 *INO2* and *INO4* gene products, positive regulators of phospholipid biosynthesis in *Saccharomyces cerevisiae,* form a complex that binds to the *INO1* promoter. J. Biol. Chem. **269:** 15344–15349.

ANDREADIS, A., Y.-P. HSU, M. HERMODSON, G. KOHLHAW and P. SCHIMMEL, 1984 Yeast *LEU2.* Repression of mRNA levels by leucine and primary structure of the gene product. J. Biol. Chem. **259:** 8059–8062.

ANDREWS, B. J., and I. HERSKOWITZ, 1989 Identification of a DNA binding factor involved in cell-cycle control of the yeast *HO* gene. Cell **57:** 21–29.

ARCHAMBAULT, J., and J. D. FRIESEN, 1993 Genetics of eukaryotic RNA polymerases I, II, and III. Microbiol. Rev. **57:** 703–724.

ARCHAMBAULT, J., K. T. SCHAPPERT and J. D. FRIESEN, 1990 A suppressor of an RNA polymerase II mutation of *Saccharomyces cerevisiae* encodes a subunit common to RNA polymerases I, II, and III. Mol. Cell. Biol. **10:** 6123–6131.

ARCHAMBAULT, J., M. A. DREBOT, J. C. STONE and J. D. FRIESEN, 1992a Isolation and phenotypic analysis of conditional-lethal, linker-insertion mutations in the gene encoding the largest subunit of RNA polymerase II in *Saccharomyces cerevisiae.* Mol. Gen. Genet. **232:** 408–414.

ARCHAMBAULT, J., F. LACROUTE, A. RUET and J. D. FRIESEN, 1992b Genetic interaction between transcription elongation factor TFIIS and RNA polymerase II. Mol. Cell. Biol. **12:** 4142–4152.

ARNDT, K. M., S. RICUPERO-HOVASSE and F. WINSTON, 1995 TBP mutants defective in activated transcription *in vivo.* EMBO J. **14:** 1490–1497.

ARNDT, K. T., C. A. STYLES and G. R. FINK, 1989 A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell **56:** 527–537.

ASHBURNER, B. P., and J. M. LOPES, 1995a Autoregulated expression of the yeast *INO2* and *INO4* helix-loop-helix activator genes effects cooperative regulation on their target genes. Mol. Cell. Biol. **15:** 1709–1715.

ASHBURNER, B. P., and J. M. LOPES, 1995b Regulation of yeast phospholipid biosynthetic gene expression in response to inositol involves two superimposed mechanisms. Proc. Natl. Acad. Sci. USA **92:** 9722–9726.

BERROTERAN, R. W., D. E. WARE and M. HAMPSEY, 1994 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. Mol. Cell. Biol. **14:** 226–237.

BONNEAUD, N., O. OZIER-KALOGEROPOULOS, G. Y. LI, M. LABOUESSE, L. MINVIELLE-SEBASTIA *et al.,* 1991 A family of low and high copy replicative, integrative and single-stranded *S. cerevisiae/E. coli* shuttle vectors. Yeast **7:** 609–615.

CARMAN, G. M., and S. A. HENRY, 1989 Phospholipid biosynthesis in yeast. Annu. Rev. Biochem. **58:** 635–669.

CHIANNILKULCHAI, N., R. STALDER, M. RIVA, C. CARLES, M. WERNER *et al.,* 1992 *RPC82* encodes the highly conserved, third-largest subunit of RNA polymerase C (III) from *Saccharomyces cerevisiae.* Mol. Cell. Biol. **12:** 4433–4440.

COTÉ, J., J. QUINN, J. L. WORKMAN and C. L. PETERSON, 1994 Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science **265:** 53–60.

DALLEY, B. K., T. M. ROGALSKI, G. E. TULLIS, D. L. RIDDLE and M. GOLOMB, 1993 Post-transcriptional regulation of RNA polymerase II levels in *Caenorhabditis elegans.* Genetics **133:** 237–245.

FRIDEN, P., and P. SCHIMMEL, 1988 LEU3 of *Saccharomyces cerevisiae* activates multiple genes for branched-chain amino acid biosynthesis by binding to a common decanucleotide core sequence. Mol. Cell. Biol. **8:** 2690–2697.

FURTER-GRAVES, E. M., B. D. HALL and R. FURTER, 1994 Role of a small RNA pol II subunit in TATA to transcription start site spacing. Nucleic Acids Res. **22:** 4932–4936.

GUDENUS, R., S. MARIOTTE, A. MOENNE, A. RUET, S. MEMET *et al.,* 1988 Conditional mutants of *RPC160,* the gene encoding the largest subunit of RNA polymerase C in *Saccharomyces cerevisiae.* Genetics **119:** 517–526

GUIALIS, A., B. G. BEATTY, C. J. INGLES and M. M. CRERAR, 1977 Regulation of RNA polymerase II activity in α-amanitin-resistant CHO hybrid cells. Cell **10:** 53–60.

GUIALIS, A., K. E. MORRISON and C. J. INGLES, 1979 Regulated synthesis of RNA polymerase II polypeptides in Chinese hamster ovary cell lines. J. Biol. Chem. **254:** 4171–4176.

HARLOW, E., and D. LANE, 1988 *Antibodies: A Laboratory Manual.* Cold Spring Laboratory Press, Cold Spring Harbor, NY.

HEKMATPANAH, D. S., and R. A. YOUNG, 1991 Mutations in a conserved region of RNA polymerase II influence the accuracy of mRNA start site selection. Mol. Cell. Biol. **11:** 5781–5791.

HENGARTNER, C. J., C. M. THOMPSON, J. ZHANG, D. M. CHAO, S.-M. LIAO *et al.,* 1995 Association of an activator with an RNA polymerase II holoenzyme. Genes Dev. **9:** 897–910.

HIMMELFARB, H. J., E. M. SIMPSON and J. D. FRIESEN, 1987 Isolation and characterization of temperature-sensitive RNA polymerase II mutants of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **7:** 2155–2164.

HIRSH, J. P., and S. A. HENRY, 1986 Expression of the *Saccharomyces cerevisiae* inositol-1-phosphate synthase (*INO1*) gene is regulated by factors that affect phospholipid synthesis. Mol. Cell. Biol. **6:** 3320–3328.

HIRSCHHORN, J. N., S. A. BROWN, C. D. CLARK and F. WINSTON, 1992 Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. **6:** 2288–2298.

HUDAK K. A., J. M. LOPES and S. A. HENRY, 1994 A pleiotropic phospholipid biosynthetic regulatory mutation in *Saccharomyces cerevisiae* is allelic to *sin3* (*sdi1, ume4, rpd1*). Genetics **136:** 475–483.

HULL, M. W., K. MCKUNE and N. A. WOYCHIK, 1995 RNA polymerase II subunit RPB9 is required for accurate start site selection. Genes Dev. **9:** 481–490.

ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JONES, J. S., and L. PRAKASH, 1990 Yeast *Saccharomyces cerevisiae* selectable markers in pUC18 polylinkers. Yeast **6:** 363–366

KIM, Y.-J., S. BJÖRKLUND, Y. LI, M. H. SAYRE and R. D. KORNBERG, 1994 A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:** 599–608.

KOLESKE, A. J., and R. A. YOUNG, 1994 An RNA polymerase II holoenzyme responsive to activators. Nature **368:** 466–469.

KOLESKE, A. J., and R. A. YOUNG, 1995 The RNA polymerase II holoenzyme and its implications for gene regulation. Trends Biochem. Sci. **20:** 113–116.

KOLESKE, A. J., S. BURATOWSKI, M. NONET and R. A. YOUNG, 1992 A novel transcription factor reveals a functional link between the RNA polymerase II CTD and TFIID. Cell **69:** 883–894.

KOLODZIEJ, P. A., and R. A. YOUNG, 1991 Mutations in the three largest subunits of yeast RNA polymerase II that affect enzyme assembly. Mol. Cell. Biol. **11:** 4669–4678.

KRUGER, W., and I. HERSKOWITZ, 1991 A negative regulator of *HO* transcription, SIN1 (SPT2), is a nonspecific DNA-binding protein related to HMG1. Mol. Cell. Biol. **11:** 4135–4146.

KUCHIN, S., P. YEGHIAYAN and M. CARLSON, 1995 Cyclin-dependent protein kinase and cyclin homologs SSN3 and SSN8 contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA **92:** 4006–4010.

LOPES, J. M., J. P. HIRSCH, P. A. CHORGO, K. L. SCHULZE and S. A. HENRY, 1991 Analysis of sequences in the *INO1* promoter that are involved in its regulation by phospholipid precursors. Nucleic Acids Res. **19:** 1687–1693.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

MANN, C., J.-Y. MICOUIN, N. CHIANNILKULCHAI, I. TREICH, J.-M. BUHLER *et al.,* 1992 *RPC53* encodes a subunit of *Saccharomyces cerevisiae* RNA polymerase C (III) whose inactivation leads to a predominantly G1 arrest. Mol. Cell. Biol. **12:** 4314–4326.

MILLER, J., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbour, NY.

MOSRIN, C., M. RIVA, M. BELTRAME, E. CASSAR, A. SENTENAC *et al.,* 1990 The *RPC31* gene of *Saccharomyces cerevisiae* encodes a subunit of RNA polymerase C (III) with an acidic tail. Mol. Cell. Biol. **10:** 4737–4743.

MYERS, A. M., A. TZAGOLOFF, D. M. KINNEY and C. J. LUSTY, 1986

Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene **45:** 299–310.

NICHOLSON, R. C., D. B. WILLIAMS and L. A. MORAN, 1990 An essential member of the HSP70 gene family of *Saccharomyces cerevisiae* is homologous to immunoglobulin heavy chain binding protein. Proc. Natl. Acad. Sci. USA **86:** 1159–1163.

NONET, M., and R. A. YOUNG, 1989 Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of *Saccharomyces cerevisiae* RNA polymerase II. Genetics **123:** 715–724.

NONET, M., D. SWEETSER and R. A. YOUNG, 1987 Functional redundancy and structural polymorphism in the large subunit of RNA polymerase II. Cell **50:** 909–915.

PERCIVAL-SMITH, A., and J. SEGALL, 1986 Characterization and mutational analysis of a cluster of three genes expressed preferentially during sporulation of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **6:** 2443–2451.

PETERSON, C. L., and I. HERSKOWITZ, 1992 Characterization of the yeast *SWI1, SWI2,* and *SWI3* genes, which encode a global activator of transcription. Cell **68:** 573–583.

PETERSON, C. L., and J. W. TAMKUN, 1995 The SWI-SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. **20:** 143–146.

PETERSON, C. L., A. DINGWALL and M. P. SCOTT, 1994 Five *SWI/SNF* gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91:** 2905–2908.

SAWADOGO, M., and A. SENTENAC, 1990 RNA polymerase B(II) and general transcription factors. Annu. Rev. Biochem. **59:** 711–754.

SCAFE, C., D. CHAO, J. LOPES, J. P. HIRSCH, S. HENRY *et al.,* 1990a RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. Nature **347:** 491–494.

SCAFE, C., C. MARTIN, M. NONET, S. PODOS, S. OKAMURA *et al.,* 1990b Conditional mutations occur predominantly in highly conserved residues of RNA polymerase II subunits. Mol. Cell. Biol. **10:** 1270–1275.

SCAFE, C., M. NONET and R. A. YOUNG, 1990c RNA polymerase II mutants defective in transcription of a subset of genes. Mol. Cell. Biol. **10:** 1010–1016.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SOMERS, D. G., M. L. PEARSON and C. J. INGLES, 1975 Isolation and characterization of an α-amanitin-resistant rat myoblast mutant cell line possessing α-amanitin-resistant RNA polymerase II. J. Biol. Chem. **250:** 4825–4831.

STETTLER, S., S. MARIOTTE, M. RIVA, A. SENTENAC and P. THURIAUX, 1992 An essential and specific subunit of RNA polymerase III (C) is encoded by gene *RPC34* in *Saccharomyces cerevisiae.* J. Biol. Chem. **267:** 21390–21395.

THOMPSON, C. M., and R. A. YOUNG, 1995 General requirement for RNA polymerase II holoenzyme *in vivo.* Proc. Natl. Acad. Sci. USA **92:** 4587–4590.

THOMPSON, C. M., A. J. KOLESKE, D. M. CHAO and R. A. YOUNG, 1993 A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:** 1361–1375.

THOMPSON, N. E., T. H. STEINBERG, D. B. ARONSON and R. R. BURGESS, 1989 Inhibition of *in vivo* and *in vitro* transcription by monoclonal antibodies prepared against wheat germ RNA polymerase II that react with the heptapeptide repeat of eukaryotic RNA polymerase II. J. Biol. Chem. **264:** 11511–11520.

TU, H., and M. J. CASADABAN, 1990 The upstream activating sequence for L-leucine gene regulation in *Saccharomyces cerevisiae.* Nucleic Acids Res. **18:** 3923–3931.

TYERS, M., G. TOKIWA, R. NASH and B. FUTCHER, 1992 The Cln3-Cdc28 kinase complex of *S. cerevisiae* is regulated by proteolysis and phosphorylation. EMBO J. **11:** 1773–1784.

WAHI, M., and A. D. JOHNSON, 1995 Identification of genes required for α2 repression in *Saccharomyces cerevisiae.* Genetics **140:** 79–90.

WINSTON, F., and M. CARLSON, 1992 Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:** 387–391.

WOYCHIK, N. A., and R. A. YOUNG, 1989 RNA polymerase II *RPB4* is essential for high- and low-temperature yeast cell growth. Mol. Cell. Biol. **9:** 2854–2859.

YOUNG, R. A., 1991 RNA polymerase II. Annu. Rev. Biochem. **60:** 689–715.

Communicating editor: F. WINSTON