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RNA Polymerase II Mutants Defective in Transcription of a Subset of Genes

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Saccharomyces cerevisiae **RNA polymerase II conditional mutants that selectively disrupt the synthesis of specific mRNAs were isolated. At the permissive temperature, several of the mutants were inositol auxotrophs as a result of inadequate induction of *INO1* transcription. The transcriptional defects exhibited by one of these Ino⁻ mutants (*rpb2-2*) were further investigated. The induction of *GAL10* and *HIS4* transcription in *rpb2-2* strains was similar to that of wild-type strains, in contrast to the lack of induction of *INO1* transcription. When shifted to the nonpermissive temperature, cells containing *rpb2-2* continued to accumulate some mRNAs but not others. Together, these results indicate that transcription of specific genes can be disrupted by RNA polymerase II mutations. The *rpb2-2* allele alters an amino acid residue that occurs in a highly conserved segment of the RPB2 protein and that is shared by homologous subunits in other species.**

The regulation of eucaryotic gene expression occurs primarily at the level of selective transcription initiation by RNA polymerase II. Specific promoter sequences, transcriptional activating protein factors, and RNA polymerase all play a role in transcription initiation. Transcriptional activating proteins bind specifically to *cis*-acting promoter elements such as enhancer sequences or the TATA box (12, 21). The most thoroughly studied transcriptional activating proteins have separate domains for DNA-binding and transcriptional activation (reviewed in references 13 and 22). The mechanism(s) by which these proteins activate transcription initiation by RNA polymerase II is unclear.

RNA polymerase II is composed of 9 to 14 polypeptides of 10 to 220 kilodaltons that are substantially conserved in size and structure throughout eucaryotes (31). The yeast nuclear RNA polymerases are among the best studied of the eucaryotic polymerases. Yeast RNA polymerase II contains 10 polypeptides, 7 of which are unique to the enzyme and 3 of which are shared with the other two polymerases. Genes encoding seven of the yeast RNA polymerase II subunits have been isolated and characterized (17, 18, 29, 35–37; N. A. Woychik, S.-M. Liao, P. Kolodziej, and R. A. Young, Genes Dev., in press). The sequences of the two largest subunits, *RPB1* and *RPB2* (1, 35), reveal that the RPB1 and RPB2 proteins are similar to the *Escherichia coli* RNA polymerase subunits β′ (*RpoC*) and β (*RpoB*), respectively. Biochemical and genetic evidence indicates that the RNA polymerase β subunit interacts with the σ subunit, which is essential for selective transcription initiation in *E. coli* (9, 26, 32, 38). It is not clear how components of the eucaryotic RNA polymerase interact with transcription factors.

To obtain clues to the functions of the *RPB1* and *RPB2* subunits, we have isolated and investigated RNA polymerase mutants that affect mRNA synthesis. We previously described a temperature-sensitive *RPB1* mutant that rapidly ceases all mRNA synthesis upon transfer to the nonpermissive temperature (27). Here we describe *RPB2* mutants that are defective in the transcription of specific genes.

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MATERIALS AND METHODS

Yeast strains and media. Yeast strains are shown in Table 1. Yeast RNA polymerase nomenclature and media are described by Nonet et al. (27). The *rpb2Δ297::HIS3* allele consists of replacement of the *Xba*I-*Mlu*I 4.95-kilobase (kb) fragment, containing the entire promoter, coding region, and terminator of *RPB2* (35), with a *Xba*I-*Bss*HII fragment that contains the *HIS3* gene from pRB328 (19). The *RPB2::pRP291* (*URA3 HIS3*) allele is described below. Integration of the *rpb2-2* allele was accomplished by selecting for 5-fluoro-orotic acid (5-FOA)-resistant segregants of strain Z112, followed by screening for a His⁻ phenotype. All integration events were confirmed by Southern blots.

Plasmids. Plasmid pRP29 consists of the 6.25-kb *Kpn*I genomic *RPB2* fragment (35) inserted into pFBI14 (3). pRP212, a *URA3 RPB2 CEN* shuttle plasmid, was constructed by inserting a 6.0-kb *Pvu*II-*Sal*I *RPB2* genomic fragment into a YCp50 DNA fragment produced by digestion with *Eco*RI, blunting by filling in of the 5′ overhang, and subsequent digestion with *Sal*I. pRP214, a *LEU2 RPB2 CEN* shuttle plasmid, was constructed by inserting the *LEU2* gene (*Hpa*I-*Sal*I fragment from YIp33) into *Sma*I- and *Sal*I-digested pRP212. pYS201 consists of the *Pvu*II-*Pvu*I genomic fragment of *RPB2* inserted into YIp5 that had been digested with *Eco*RI, filled in, and digested with *Pvu*I. pRP291 was constructed by removing three *Bgl*II fragments internal to the *RPB2* insert of pYS201 and replacing them with the *HIS3* gene on a 1.7-kb *Bam*HI fragment from pRB328. pRP19 and pJEF648 were described by Nonet et al. (27). pTM258, a gift of I. Chiu (Whitehead Institute), consists of a 3.1-kb *Hin*dIII genomic fragment encoding the *PGK1* gene inserted into pBR322 (see reference 15 for restriction map). pBC16 consists of a *Xho*I-*Sal*I genomic fragment encoding the *DED1* gene (see reference 34 for restriction map) inserted into pUC8. pJH308, a gift of S. Henry (Carnegie-Mellon University), contains the *INO1* gene (14). pNN76, a gift of M. Johnston (Washington University), contains the *GAL10* gene (33). pDH34 and pBC2 are pGEM plasmids that contain *ACT1* and a portion of *HIS4*, respectively, and were gifts of D. Hekmatpanah (Whitehead Institute).

TABLE 1. Yeast strains

Strain	Genotype	Source
DB1827	*MATα ura3-52 leu2-3,112 his3Δ200*	D. Botstein
Z24	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP212 (*URA3 RPB2*)]	This study
Z96	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP214 (*LEU2 RPB2*)]	This study
Z97	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-1L (*LEU2 rpb2-1*)]	This study
Z98	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-2L (*LEU2 rpb2-2*)]	This study
Z99	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-3L (*LEU2 rpb2-3*)]	This study
Z100	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-4L (*LEU2 rpb2-4*)]	This study
Z101	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-5L (*LEU2 rpb2-5*)]	This study
Z102	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-6L (*LEU2 rpb2-6*)]	This study
Z103	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-7L (*LEU2 rpb2-7*)]	This study
Z104	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-8L (*LEU2 rpb2-8*)]	This study
Z105	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-9L (*LEU2 rpb2-9*)]	This study
Z106	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-10L (*LEU2 rpb2-10*)]	This study
Z107	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-11L (*LEU2 rpb2-11*)]	This study
Z108	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-12L (*LEU2 rpb2-12*)]	This study
Z109	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2-2*	This study
Z110	*MATa ura3-52 his3Δ200 lys2-801 ade2-101 RPB2*::pRP291 (*URA3 HIS3*)	This study
F763	*MATα ura3-52 trp1Δ1*	G. Fink
Z111	*MATα ura3-52 leu2-3,112 his3Δ200 rpb1-1 ade2*	This study
Z112	*MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297 ::HIS3* [pRP2-2U (*URA3 rpb2-2*)]	This study

Chromosomal position of *RPB2*. The *RPB2* gene was physically mapped to chromosome XV by orthogonal-field-alternation gel electrophoresis of yeast chromosomes as described by Carle and Olson (5). The Southern blot was probed with pRP29 DNA (data not shown). To more precisely map the *RPB2* locus on chromosome XV by using classic meiotic techniques, *RPB2* was tagged with the *HIS3* and *URA3* genes by integrating pRP291 (described above) at the *RPB2* locus; this allele is designated *RPB2*::pRP291 (*HIS3 URA3*). Initial crosses demonstrated that *RPB2* was linked to *his3* and *ade2* but not closely linked to *pet17*, *arg1*, *arg8*, or *prt1*. Eighty-three tetrads of F763 × N319 were used to position *RPB2* between *his3* and *ade2* on chromosome XV. In examining the linkage between *RPB2*::pRP291 and *ade2*, we found 63 parental ditypes (PD), 20 tetratypes (TT), and 0 nonparental ditypes (NPD), indicating an interval of 12 centimorgans. Between *RPB2*::pRP291 and *his3* there occurred 41 PD, 42 TT, and 0 NPD, which indicates a linkage distance of 25.3 centimorgans. In the same cross, we observed 28 PD, 55 TT, and 0 NPD between *ade2* and *his3*. In some tetrads, unambiguous scoring of markers required looping out the integrated pRP291 DNA by selecting against the *URA3* gene on medium containing 5-FOA and subsequent scoring of the Ura$^-$ derivative of the original spore.

Isolation of *RPB2* mutants. The yeast strain Z24, which contains a deletion of the chromosomal *RPB2* gene complemented by a wild-type *RPB2* gene on a *URA3 CEN* plasmid (pRP212), was separately transformed with three independent pools of the mutagenized *LEU2* plasmid pRP214. pRP214 was mutagenized with hydroxylamine as described in Nonet et al. (27). Then 3,000 *Leu*$^+$ transformants were patched onto plates containing 5-FOA to select against the *URA3*-containing plasmid (4). Forty-five of the transformants failed to grow on 5-FOA, indicating that the mutagenesis resulted in the inactivation of the essential *RPB2* gene on 1.5% of the *LEU2* plasmids. The surviving clones (which had lost the *URA3* plasmid bearing a wild-type copy of *RPB2*) were then assayed for temperature sensitivity, cold sensitivity, and auxotrophy by spot testing to yeast extract-peptone-dextrose plates at 12, 15, 24, 36, and 38°C and to minimal medium plates supplemented with uracil at 24°C. Thirty temperature-sensitive clones that failed to grow at 36 or 38°C were isolated, as were 21 cold-sensitive clones that failed to grow at 15 or 12°C. Ten clones that were both temperature and cold sensitive were also obtained. In addition, many of the conditional mutants were auxotrophic. To determine whether the mutant phenotypes were due to mutations in *RPB2* or to unlinked mutations induced by transformation (20), we isolated the pRP214 plasmids containing the putative mutations by the smash-and-grab protocol of Hoffman and Winston (16). These 61 plasmids were then reassayed in the original Z24 strain, using the plasmid shuffle procedure. Twelve recessive, plasmid-linked *RPB2* mutants were isolated in this manner. Since mutations in *RPB1* and *RPB2* that produced inositol auxotrophs had been isolated (2), we tested our auxotrophs for inositol requirement. All five of the auxotrophic *RPB2* mutants isolated in our plasmid shuffle procedure required inositol for growth.

In vitro RNA polymerase assays. Crude extracts were prepared and used in the in vitro assays for RNA polymerase essentially as described by Nonet et al. (27). RNA polymerase I and III activities were measured together. A 6- to 15-μg sample of protein was added to each transcription reaction. The RNA polymerase assays were demonstrated to be linear throughout the range of protein concentrations used in the assays.

Northern (RNA) blot analysis. For the experiment shown in Fig. 6, total cellular RNA was isolated from the indicated strains grown at 24°C and at the indicated times after a shift to 36°C. A 10-ml sample of cells at an optical density at 600 nm of 0.5 was pelleted for 5 min at 3,000 × *g*, washed with ice-cold H_2O, and suspended in 200 μl of RNA extraction buffer (0.5 M NaCl, 0.2 M Tris [pH 8.0], 10 mM EDTA, 1% sodium dodecyl sulfate). An equal volume of phenol-chloroform-isoamyl alcohol (24:24:1) and acid-washed glass beads was added, and the mixture was vortexed 15 times for 15 s each time, with 15-s intervals on ice. The aqueous phase was extracted two more times with phenol-chloroform-isoamyl alcohol (24:24:1), and the nucleic acids were precipitated with 2.5 volumes of 100% ethyl alcohol. Denaturing gel electrophoresis, transfer to nitrocellulose, and hybridizations were performed as described by Nonet et al. (27). Radiolabeled probes were made by independently nick translating intact plasmids (described above) containing the yeast genes *RPB1*, *DED1*, *LYS2*, and *PGK1*.

For the analysis of *INO1* transcription, wild-type and three mutant strains (*rpb2-2*, *rpb2-6*, and *rpb2-10*) were grown overnight at 24°C in minimal medium supplemented with leucine and 400 μM inositol. When the cultures reached mid-log phase, a portion of each was reserved for RNA preparation as described above. The remaining cells were washed twice and then suspended in medium lacking inositol. RNA was prepared from cells after 1 and 3 h of growth

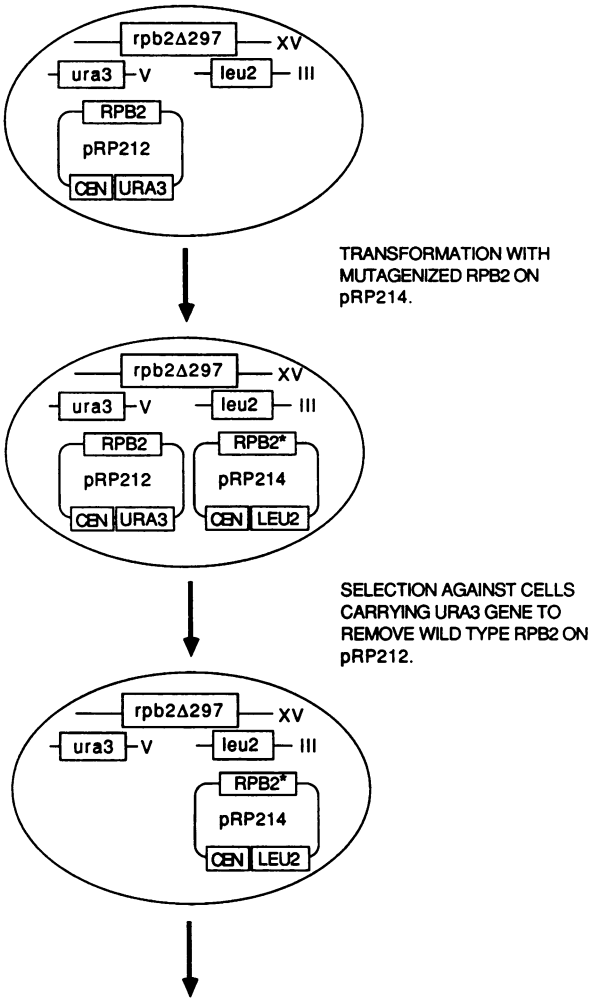


FIG. 1. Plasmid shuffle strategy for obtaining *RPB2* mutants. Plasmid pRP214 was mutagenized with hydroxylamine, amplified in *E. coli* HB101, and used to transform *S. cerevisiae* Z24 (Table 1). Transformants were plated on medium containing 5-FOA to select for loss of the wild-type *RPB2* plasmid pRP212 and were screened subsequently for conditional and auxotrophic phenotypes. Plasmids from putative *rpb2* mutants were isolated and individually retested by the plasmid shuffle procedure to determine whether the mutations were plasmid linked.

in inositol-free medium and subjected to Northern blot analysis as described above. Nick-translated plasmids pJH308 and pRP19 were used as probes.

For the analysis of *HIS4* transcription, wild-type and mutant cells were grown overnight in minimal medium supplemented with uracil. The cultures were diluted and grown for 4 h to an optical density at 600 nm of 2. A 5-ml sample of cells was treated for RNA preparation as described above. To the remaining culture was added 3-aminotriazole (to 10 mM); these cells were incubated for an additional 3 h, and RNA was isolated. A Northern blot prepared from these samples was probed with a labeled *HIS4* RNA probe. The blot was subsequently reprobed with a labeled *ACT1* DNA probe.

For the *GAL10* induction experiment, wild-type and mutant cells were grown in synthetic complete medium containing 2% raffinose to minimize catabolite repression. At an optical density at 600 nm of 1, 10 ml of cells was treated for RNA preparation as described above. Cells were shifted to the same medium containing 5% galactose to induce *GAL* gene expression. After an additional 3 h of incubation, cells were harvested for RNA as described above. A Northern blot prepared from these samples was probed with a labeled *GAL10* DNA probe. The blot was subsequently reprobed with a labeled *ACT1* DNA probe.

RESULTS AND DISCUSSION

Isolation of *RPB2* mutants. *RPB2*, a single-copy gene that is essential for yeast cell growth, encodes the 140-kilodalton subunit of RNA polymerase II (35, 37). The gene maps to chromosome XV, between *his3* and *ade2* (see Materials and Methods for details). To obtain clues to the function of the subunit, we used a plasmid shuffle method to screen efficiently for *RPB2* mutants with conditional and auxotrophic phenotypes (Fig. 1). The plasmid shuffle method uses a yeast strain in which a centromere-containing plasmid carrying *RPB2* complements a deletion of the chromosomal gene. The procedure involves replacing the plasmid containing the wild-type copy of the gene with a similar plasmid that has been mutagenized in vitro. This approach allowed us to concentrate the mutagenesis on the plasmid-borne gene, to screen for several different mutant phenotypes simultaneously, and to rapidly recover the mutations on plasmids for further analysis.

A screen of 3,000 yeast colonies bearing mutagenized *RPB2* plasmids yielded 12 recessive mutants with conditional or auxotrophic phenotypes. The growth phenotypes of these mutants on plates are shown in Fig. 2. Most of the

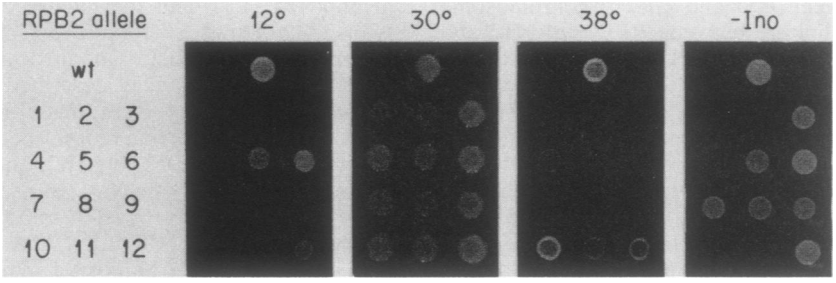


FIG. 2. Growth phenotypes of the *RPB2* mutants. The growth phenotypes of the *RPB2* mutants were assayed by using a spot test procedure. Single colonies were dispersed in 100 μl of sterile H_2O in the individual wells of micro-dilution plates, and equal volumes were transferred with a 48-prong apparatus to agar plates preincubated at the growth temperature. Isogenic strains carrying the wild-type *RPB2* allele (Z96) and the mutants *rpb2-1* to *rpb2-12* (Z97 to Z108, respectively) were used.

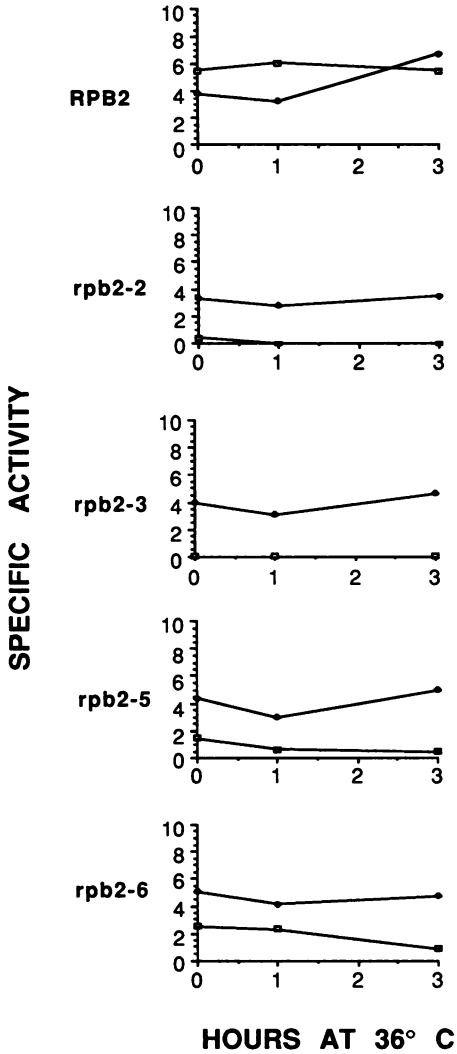


FIG. 3. RNA polymerase activities in mutant crude extracts. Extracts were prepared from wild-type (Z96) and mutant (Z98, Z99, Z101, and Z102) cells at the indicated times after a shift to 36°C and were assayed for RNA polymerase activities as described in Materials and Methods. Specific activity is expressed in units of 10^3 cpm/12 μg of protein. Symbols: □, RNA polymerase II activity; ●, sum of RNA polymerase I and RNA polymerase III activities (the contributions of RNA polymerases I and III are roughly equal [27]).

mutants exhibit a unique set of phenotypes, indicating that the 12 mutants contain different *RPB2* alleles. Eight of the mutants are both temperature sensitive and cold sensitive, one is exclusively cold sensitive, and three are exclusively temperature sensitive. The observation of Arndt et al. (2) that some RNA polymerase mutants exhibit inositol auxotrophy led us to examine the inositol phenotype of the *RPB2* mutants. Five of the *RPB2* conditional mutants are auxotrophic for inositol.

***RPB2* mutants are defective in RNA polymerase II activity in vitro.** To determine whether the *RPB2* mutations affect one or more of the three classes of RNA polymerase, crude extracts were prepared from a wild-type strain and four of the mutant strains and were assayed for RNA polymerase I, II, and III activity in vitro (Fig. 3). Extracts were prepared from cells grown at 24°C and from cells 1 and 3 h after a shift to the nonpermissive temperature, 36°C. In each case, the mutant extracts had reduced levels of RNA polymerase II

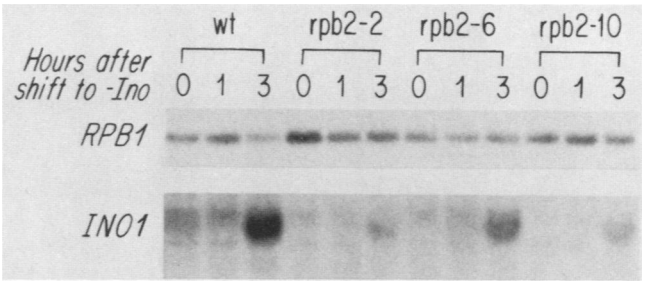


FIG. 4. Defective *INO1* transcription in *RPB2* mutants. *INO1* transcription in wild-type (Z96), *rpb2-2* (Z98), *rpb2-6* (Z102), and *rpb2-10* (Z106) strains was induced by inositol starvation. RNA was isolated just before or after a shift to inositol-free medium and was analyzed by Northern blot. The *INO1* probe used here hybridizes to an inducible 1.8-kb *INO1* transcript. *RPB1* mRNA was probed as a control.

but not RNA polymerase I and III activities. Two of the mutants, *rpb2-2* and *rpb2-3*, had no detectable RNA polymerase II activity, whereas the others, *rpb2-5* and *rpb2-6*, had detectable but low levels of RNA polymerase II activity.

The *RPB2* gene was originally isolated by using antibodies directed against the second largest subunit of yeast RNA polymerase II (37). The sequence of the gene predicted a 138,750-dalton protein with substantial sequence similarity to the β subunit of *E. coli* RNA polymerase (35), supporting the notion that *RPB2* encodes an RNA polymerase subunit but not eliminating the possibility that it encodes a homologous subunit of RNA polymerase I or III. The fact that extracts prepared from specific *RPB2* mutants lack RNA polymerase II activity but exhibit normal levels of RNA polymerase I and III activities in vitro indicates that *RPB2* encodes a genuine component of RNA polymerase II.

Some *RPB2* mutants exhibit gene-specific transcription defects at *INO1*. Five of the *RPB2* mutants exhibited a requirement for exogenous inositol independent of the growth temperature. None of the *RPB2* mutants acquired other nutritional requirements. To further investigate the inositol requirement, we examined the transcription of the *INO1* gene in wild-type and mutant cells. The *INO1* gene encodes inositol-1-phosphate synthase, the enzyme that catalyzes the first step in the conversion of glucose-6-phosphate to inositol. Low levels of inositol stimulate *INO1* transcription in wild-type cells (14). A lack of *INO1* transcription under conditions of inositol starvation might, therefore, account for the Ino^- phenotype of the *RPB2* mutants.

The levels of *INO1* mRNA were examined in wild-type cells and in several *RPB2* mutant cells (*rpb2-2*, *rpb2-6*, and *rpb2-10*) before and after a shift to inositol-free medium. RNA was prepared from cells before the medium shift and from cells starved for inositol for 1 and 3 h, subjected to electrophoresis, and transferred to nitrocellulose. The Northern blot was probed with *RPB1* DNA as an internal control and with *INO1* DNA (Fig. 4). The *INO1* transcript was induced to a high level in the wild-type strain and to a moderate level in the Ino^+ temperature-sensitive mutant *rpb2-6*. In contrast, the transcript was induced to much lower levels in the Ino^- mutants *rpb2-2* and *rpb2-10*. The reduced transcription of the *INO1* gene is probably sufficient to account for the inositol auxotrophy of these mutants.

To determine whether the inability to induce *INO1* transcription reflects a general defect in transcriptional induction, we investigated the regulation of *HIS4* and *GAL10* RNA synthesis in *rpb2-2* mutant and in wild-type cells (see

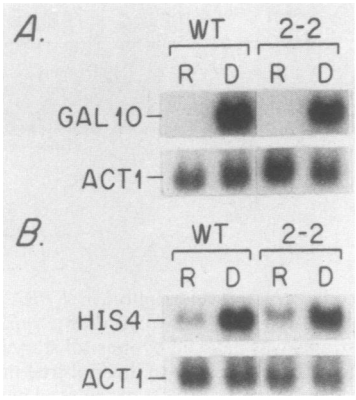


FIG. 5. Normal regulation of *HIS4* and *GAL10* in *rpb2-2* mutant cells. Northern analysis of mRNAs from wild-type and mutant cells was carried out as described in Materials and Methods. (A) *GAL10* transcription in wild-type and *rpb2-2* mutant cells, examined just before (R [repressed]) or 3 h after (D [derepressed]) exposure to galactose; (B) *HIS4* transcription in wild-type and *rpb2-2* mutant cells, examined just before (R) or 3 h after (D) exposure to 3-aminotriazole. *ACT1* mRNA was probed as an internal control in both experiments.

Materials and Methods). The derepression of transcription of both *HIS4* and *GAL10* was normal in the mutant strain (Fig. 5).

Five of the twelve *RPB2* mutants studied here and a similar proportion of *RPB1* mutants (30a) require inositol for growth. Transcription of the *INO1* gene or of a gene that regulates *INO1* appeared to be very sensitive to perturbations in the two largest RNA polymerase II subunits. This sensitivity may be a consequence of unusual requirements for activation of *INO1* transcription. For example, interactions between RNA polymerase II and transcription factors that act at the *INO1* promoter may be more easily disrupted than at other promoters.

Gene-specific defects in mRNA synthesis. We previously described an RNA polymerase II mutant, *rpb1-1*, in which all mRNA synthesis shuts down immediately after a temperature shift, and the rate of depletion of mRNAs appears to reflect their half-life values (27). To determine whether this is also the case for *RPB2* temperature-sensitive mutants, *rpb2-2* cells were selected for further study. The rates of depletion of specific mRNAs in *rpb2-2* cells were compared with those in the *rpb1-1* strain at the nonpermissive temperature. RNA was isolated from cells grown at 24°C and from cells that had been shifted to 36°C for 30 min, 1 h, and 3 h. A Northern blot of these samples was then probed with labeled *RPB1*, *LYS2*, *DED1*, and *PGK1* DNA fragments (Fig. 6).

The results revealed that after the temperature shift, *RPB1* and *LYS2* mRNAs continued to accumulate at approximately wild-type levels in *rpb2-2* cells but were depleted in *rpb1-1* cells. In contrast, *DED1* and *PGK1* mRNAs were depleted at similar rates in *rpb2-2* and *rpb1-1* cells, suggesting that they were transcribed poorly or not at all at the nonpermissive temperature in the *rpb2-2* mutant. These data suggest that the synthesis of some mRNAs continues at approximately normal rates, whereas synthesis of others stops or occurs at reduced rates in *rpb2-2* mutants at the nonpermissive temperature.

The *rpb2-2* mutation alters a highly conserved amino acid residue. To determine the exact nature of the *rpb2-2* mutation, plasmid DNA containing this allele was isolated and sequenced by using a set of oligonucleotide primers that span the entire *RPB2* gene. The *rpb2-2* mutation changes nucleotide 4233 (35) from a G to an A, altering a single amino acid residue located in a region at the carboxyl terminus of *RPB2* that is conserved among procaryotes and eucaryotes (Fig. 7). The mutation causes a substitution of an aspartic acid residue for a highly conserved glycine.

Selective transcriptional defects in *RPB2* mutants: possible mechanisms and effects. Our results indicate that some conditional mutations in *RPB2* exhibit gene-specific transcriptional defects. It is possible that some of the differential accumulation of mRNAs observed in the *rpb2-2* mutant at the nonpermissive temperature is due to differential rates of mRNA degradation, but this cannot account for the *INO1* transcriptional defects observed in the inositol auxotrophs. The inability to induce *INO1* transcription in the inositol auxotrophs, and the depletion of some mRNA species whereas others continue to accumulate at the nonpermissive temperature in *RPB2* conditional mutants, both appear to be due to an inability of RNA polymerase II to transcribe specific genes.

The sequence similarity between the *RPB2* protein and the *E. coli* RNA polymerase β subunit raises the possibility that these two proteins have similar functions. The σ subunit, which is essential for proper transcription initiation, proba-

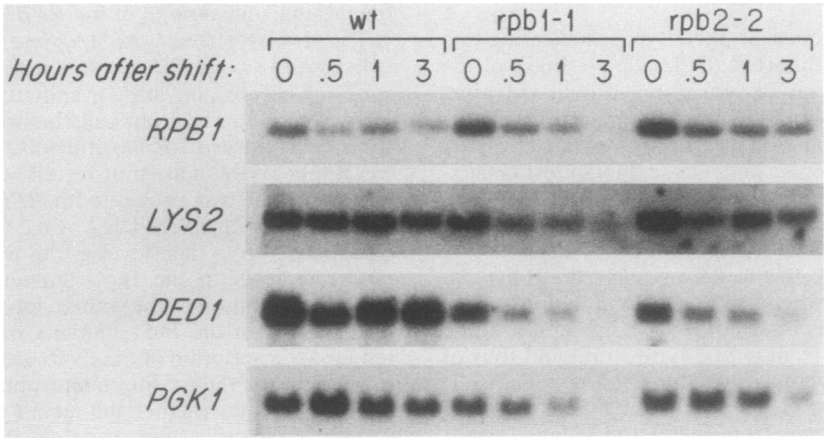
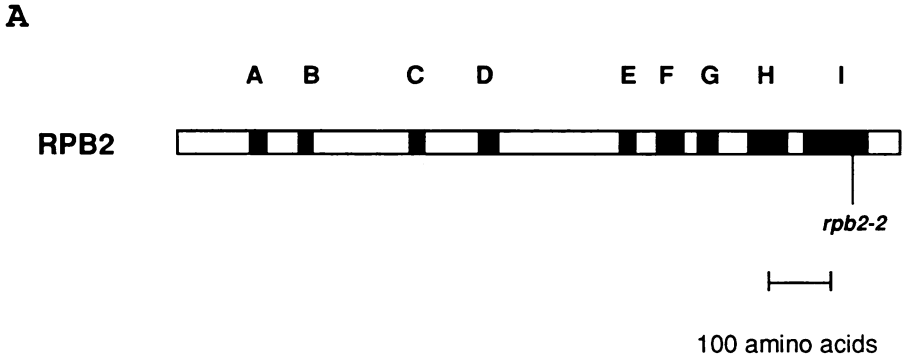


FIG. 6. Comparison of mRNA depletion rates in *rpb1-1* and *rpb2-2* mutants. The levels of specific mRNAs were measured by Northern analysis of total cellular RNA isolated from wild-type (Z96), *rpb2-2* (Z109), and *rpb1-1* (Z111) strains at various times after a shift to 36°C.



B

rpb2-2
D

S. cerevisiae	GEMERDCMIAHGAASFLKERL 1151
D. melanogaster	.....Q.S....Q..R...
E. coli	....VWALE.Y...YT.Q.M.

Region I

FIG. 7. The *rpb2-2* mutation. (A) Position of the *rpb2-2* mutation, shown relative to segments of sequence similarity observed between procaryotic and eucaryotic RNA polymerase subunits (■) (35). (B) Amino acid alteration produced by the *rpb2-2* mutation, shown relative to amino acid sequences from the homologous RNA polymerase subunits of *D. melanogaster* (7) and *E. coli* (28).

bly interacts with β; the σ and β subunits can be cross-linked to DNA sequences only five nucleotides apart in the *lac* UV5 promoter (32), and mutations in β can affect σ binding to the core enzyme (8). In addition, β mutants have been observed to alter promoter selectivity in vitro (9, 26). It is tempting to speculate that eucaryotic transcriptional activators may interact directly or indirectly with *RPB2* protein, in a manner analogous to the interaction between procaryotic β and σ subunits. If so, this interaction is only part of the story, because gene-specific defects (inositol auxotrophy) have been observed with mutations in other RNA polymerase II subunits (2, 30a, 36).

RNA polymerase II mutations can produce developmental defects in *Drosophila melanogaster* and in *Caenorhabditis elegans* (6, 10, 11, 23–25, 30). The *Drosophila* Ultrabithorax-like (*Ubl*) mutation, which mimics Ultrabithorax (*Ubx*), occurs in the gene for the largest RNA polymerase II subunit (*RpII215*). Other alleles of *Ubl* have been identified, one of which displays a Hyperabdominal-like (*Hab*) phenotype. *Caenorhabditis* RNA polymerase II mutations can produce defects in embryogenesis and in postembryonic development (30). How these RNA polymerase II mutations cause developmental defects is not known. However, RNA polymerase II mutations that produce gene-specific transcription defects like those exhibited by *S. cerevisiae rpb2-2* could account for some of the developmental defects observed with *Drosophila* and *Caenorhabditis* RNA polymerase II mutations.

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