RNA polymerase II subunit Rpb9 regulates transcription elongation \textit{in vivo}

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Abstract

RNA polymerase II lacking the Rpb9 subunit, pol IIΔ9, uses alternate transcription initiation sites in vitro and in vivo and is unable to respond to the transcription elongation factor TFIIS in vitro. Here we show that the Rpb9 gene has a synthetic phenotype with the TFIIS gene. Disruption of the RPB9 gene in yeast also resulted in sensitivity to 6-azauracil, which is a phenotype linked to defects in transcription elongation. Expression of the TFIIS gene on a high-copy plasmid partially suppressed the 6-azauracil sensitivity of Δrpb9 cells. We set out to determine the relevant cellular role of yeast Rpb9 by assessing the ability of twenty different site-directed and deletion mutants of Rpb9 to complement the initiation and elongation defects of rpb9 cells in vivo. RPB9 is composed of two Zn-ribbons. The N-terminal Zn ribbon restored the wild-type pattern of initiation start sites but was unable to complement the growth defects associated with defects in elongation. Most of the site-directed mutants complemented the elongation-specific growth phenotypes and reconstituted the normal pattern of transcription initiation sites. The anti-correlation between the growth defects of cells disrupted for RPB9 and the selection of start sites of transcription suggests that this is not the primary cellular role for Rpb9. Genome-wide transcription profiling of rpb9 cells revealed only a few changes, predominantly in genes related to metabolism.
Introduction

RNA polymerase II comprises twelve subunits in yeast (1). Four of the subunits, Rpb1, Rpb2, Rpb3 and Rpb11, form a catalytic core that is homologous in structure and function to the prokaryotic core RNA polymerase (2,3). The other eight eukaryotic subunits are less well characterized. Five of these subunits, Rpb5, Rpb6, Rpb8, Rpb10 and Rpb12 are found in all three eukaryotic RNA polymerases (4-6). The other three, Rpb4, Rpb7, and Rpb9 are unique to RNA polymerase II, although both Rpb7 and Rpb9 have sequence homologues in RNA polymerases I and III (7). The gene for Rpb9 is not essential for yeast cell viability, but is essential in Drosophila (8).

Rpb9 has roles both in transcription initiation and in transcription elongation. In the initiation reaction, Rpb9 modulates the selection of the transcription start site. In cells lacking Rpb9 and in reconstituted transcription reactions lacking Rpb9, the population of start sites is shifted upstream at a variety of promoters (9-11). In the elongation reaction, Rpb9 is required, along with TFIIS, to effect transcription through blocks to elongation encoded by the DNA template (12). A role in the modulation of initiation and elongation is consistent with the localization of Rpb9 in the three-dimensional structure of yeast RNA polymerase II. Rpb9 is located at the tip of the so-called "jaws" of the enzyme, which is thought to function by clamping the DNA downstream of the active site (3,13,14). The Rpb9 homologue in RNA polymerase II, C11, also has been implicated in regulating RNA chain elongation (15).

Rpb9 comprises two Zn-ribbon domains joined by a 30 amino acid linker. The C-terminal Zn-ribbon is a sequence homologue of the Zn-ribbon in the transcription elongation factor TFIIS (16,17). The roles of each domain of Rpb9 in transcription elongation were determined by assaying a series of alanine-scanning mutants of Rpb9 in *in vitro* reactions (18). Alanine substitutions in the C-terminal Zn-ribbon domain of Rpb9, like amino acid substitutions in the homologous part of TFIIS, completely eliminate elongation activity. Mutating the first Zn-ribbon had no effect on elongation activity, though deleting this domain entirely abrogated activity. The linker region mediated the interaction of Rpb9 with the rest of the RNA polymerase. In this study, we used this series of mutations to probe the cellular role of Rpb9 in both initiation and elongation.
Materials and Methods

Yeast Strains

YF2221 (MATa ura3-52 his3-11,15 leu2-3, 2-112 ade2-1 can1-100 ssd1-d2 trp1::hisG-URA3-hisG) is the parent strain. YF2230 (MATa ura3-52 his3-11, 3-15 leu2-3, 2-112 ade2-1 can1-100 ssd1-d2 trp1::hisG-URA3-hisG rpb9::HIS3) is a derivative of YF2221 deleted for Rpb9. YF2222 (MATa ura3-52 his3-11,3-15 leu2-3, 2-112 ade2-1 can1-100 ssd1-d2 trp1-1 ppr2::hisG-URA3-hisG) is deleted for TFIIS, and YF2234 lacks both Rpb9 and TFIIS (MATa ura3-52 his3-11, 3-15 leu2-3, 2-112 ade2-1 can1-100 ssd1-d2 trp1-1 ppr2::hisG-URA3-hisG rpb9::HIS3).

Yeast Expression Plasmid

The yeast expression plasmid pRS314RPB9 containing the RPB9 open reading frame plus approximately 500 base pairs upstream and 2200 base pairs downstream was obtained from Dr. Rolf Furter (9). This plasmid was adapted by inserting a Bam HI restriction site immediately upstream of the start codon, and an Eco RI restriction site immediately downstream of the stop codon, creating the plasmid pRS314RPB9BE. These sites were inserted using the Quikchange protocol and Pfu I DNA polymerase (Stratagene). Incorporation of these restriction sites allowed for the insertion of each of the previously constructed rpb9 mutants into pRS314RPB9BE. The resulting plasmids, containing each of the rpb9 mutant alleles under control of the endogenous RPB9 promoter, were transformed into yeast to determine their effects on growth and the use of initiation start sites.

Growth Assays

The rpb9 yeast strain grows slowly at 30°C, is extremely sensitive to high and low temperature extremes, and is sensitive to the drug 6-azauracil. Expression of wildtype Rpb9 corrects these defects. Haploid rpb9 cells were transformed with the RPB9 yeast expression plasmids to test each mutant for the ability to complement the rpb9 growth phenotypes. To test for complementation of cold and temperature sensitivity, the cells were grown on solid synthetic complete yeast medium lacking tryptophan. Suspensions containing approximately 10000, 2000, 400 and 80 cells were spotted onto solid medium.
and grown at 12°C, 30°C, or 37°C for 2 to 6 days. Cells were grown on solid synthetic complete yeast medium lacking tryptophan and uracil, and containing 50 µg/ml 6-azauracil to measure ability to correct sensitivity to 6-azauracil. Suspensions containing approximately 10000, 2000, 400 and 80 cells were spotted onto solid medium and grown at 30°C for 3 to 8 days. Each mutant construct was compared to wildtype RPB9 with respect to ability to restore growth characteristics.

**Primer Extension**

Primer-extension assays were performed to identify the transcription start sites in the mutant yeast strains. Yeast strains YF2221, YF2222, YF2230 and YF2234 were grown in YP liquid medium with 2% glucose. YF2230 transformed with each of the pRS314RPB9BE constructs was grown in synthetic, complete liquid medium lacking tryptophan. All cultures were grown at 30°C to an optical density at 600 nm between 0.2 and 1.0. 5 X 10^7 cells were harvested and total RNA was isolated using the RNeasy protocol (Qiagen). The primer used for these experiments, 5’-AGAAGATAACACCTTTTGAG-3’ (Dalton Chemicals), is complementary to nucleotides +37 to +17 in the ADH1 gene. The primer was radiolabeled at the 5’-end by phosphorylating with polynucleotide kinase (New England Biolabs) and γ-32P-ATP. For each primer extension reaction, 15 µg of total RNA from the appropriate yeast strain was annealed with 0.4 picomoles of the 5’-radiolabeled primer for 45 minutes at 52°C. Reverse transcription from the annealed primer was done with MMLV reverse transcriptase (Gibco BRL) according to manufacturer’s instructions. The reverse transcripts were collected by ethanol precipitation and resolved on a 6% polyacrylamide, 8.3 M urea, TBE gel and visualized by phosphorimaging.

**RNA isolation.** Yeast cells were grown in YPD medium at 30°C with constant agitation and aeration, to an OD (600nm) of 0.4-0.6. Cells were washed once with DEPC-treated water and resuspended in TES buffer (10mM Tris-HCl, pH 7.5, 10mM EDTA, 0.5% SDS). Total RNA was isolated by using a hot phenol method (19), and further purified using a Qiagen RNeasy midi kit, essentially as described by the supplier. RNA concentrations were determined by measuring the optical density at 260nm. Two independent RNA preparations were made for each mutant strain.

**Preparation of labeled cDNA probes.** For each DNA microarray, 50µg of total RNA were reverse transcribed using 400U of SuperScript II (Gibco, Life Technologies). The
reverse transcription was primed with an AncT primer (T20VN, Sigma Genosys), and performed in the presence of dATP, dGTP, dTTP (Pharmacia, final concentration 168µM each), dCTP (Pharmacia, final concentration 50µM), and Cy3-dCTP or Cy5-dCTP (Amersham, final concentration 50µM). 20U of RNasin (Promega) were also added to the reaction. The mixture (minus the enzyme) was heated at 65°C for 5 min, then at 42°C for 5 min, the RT enzyme was added and the reaction was incubated at 42°C for 2 hours. The reverse transcription was stopped with EDTA (final concentration 6.25 mM), and the RNA template degraded by the addition of 10N NaOH (final concentration 0.5N), and incubation at 65°C for 20 min. The mixture was neutralized by the addition of 5M acetic acid (final concentration 0.5M), and the labeled cDNA was precipitated by the addition of one volume of isopropanol and incubating on ice for 30 min. After rinsing with 70% EtOH, the labeled cDNA was resuspended in 5 µg of DEPC-treated water.

**Hybridization.** For each DNA microarray 5 µl of purified Cy3-labeled cDNA, and 5 µl of purified Cy5-labeled cDNA were added to 75 µl of DIG Easy hybridization buffer (Boehringer Manheim). 2 µl of yeast tRNA (Sigma, 10mg/ml), and 2 µl of single-stranded salmon sperm DNA (Sigma, 10mg/ml) were also added to the hybridization, and the solution heated at 65°C for 2 min. The solution was then applied under a coverslip to a custom-made yeast whole genome microarray (Microarray Centre, Ontario Cancer Institute). The microarrays were incubated at 37°C in a humid hybridization chamber for 8-12 hours. Before scanning, the slides were washed in 0.1X SSC, 0.1% SDS (3 times 15 min at 50°C), rinsed in 0.1X SSC (3 times 5 min at room temperature), and dried by centrifugation. A total of 8 slides were hybridized for each mutant strain. The arrays were read on a laser confocal scanner (ScanArray 4000, GSI Lumonics), and the images obtained were quantified using the QuantArray 2.0 software (GSI Lumonics).
Results

The phenotype of rpb9 cells is consistent with a role in elongation

Our previous studies implicated Rpb9 in transcription elongation in vitro (12,18). In cells lacking Rpb9, a proportion of the Rpb9-deficient RNA polymerase II molecules (pol IIΔ9) initiated transcription at many promoters at upstream DNA sequences. This defect could be rescued by the addition of wild-type Rpb9 but not a mutant altered in the N-terminal Zn-ribbon domain. Subsequently, biochemical studies revealed a role for Rpb9 in transcription elongation in vitro (12,18). In our original study (12), the mutant enzyme, pol IIΔ9, was shown to have the same maximal elongation rate as did the wild-type RNA polymerase II, but stopped less frequently at DNA sequences (e.g. a sequence from the histone H3.3 intron) that promote pausing of the transcription complex. The addition of Rpb9 to pol IIΔ9 restored its in vitro elongation properties. Occasionally, the pol IIΔ9 enzyme did form arrested elongation complexes at the histone H3.3 arrest site. Unlike wild-type arrested complexes, these arrested pol IIΔ9 complexes were unable to be rescued by the addition of the elongation factor TFIIS. In general, these studies revealed a role for Rpb9 in transcription elongation. The parts of Rpb9 that contributed to the elongation activity were determined using a set of Rpb9 deletion and alanine-scanning mutants (18). These studies showed that the C-terminal Zn-ribbon domain was important for elongation as was the linker region connecting the two Zn-ribbons comprising Rpb9. The linker region was shown specifically to be important for the binding of Rpb9 to pol IIΔ9. We were unable to show that the N-terminal Zn-ribbon, which was important for start-site selection (10), played a role in elongation.

TFIIS and Rpb9 are linked biochemically and are related in structure: we were interested if there was also a genetic interaction between the RPB9 and TFIIS genes (the TFIIS gene is also known as PPR2). Yeast cells lacking the gene for Rpb9 are sensitive to both low and high temperature extremes and grow more slowly than do wild-type strains even at the optimal growth temperature (10). These phenotypes were also observed in our rpb9 strain, and normal growth was restored by expressing RPB9 from a low-copy plasmid under the control of its own promoter. Yeast strains deficient lacking TFIIS are sensitive to the drug 6-azauracil. This phenotype is thought to reflect a defect in transcription elongation (20). Since Rpb9 is required for the functional interaction between RNA polymerase II and TFIIS, we tested the rpb9 strain for sensitivity to 6-azauracil. The rpb9 strain grew more slowly on medium containing 6-azauracil than did the parent strain (Figure
1). Transforming the rpb9 strain with the RPB9 gene on a low-copy plasmid fully complemented the 6-azauracil sensitivity (Figure 1).

The double deletion strain (rpb9/tfiis) was constructed and its phenotype tested in order to explore the genetic interaction between TFIIS and RPB9. In agreement with the biochemical studies, the double mutant possessed a much more severe phenotype than did either of the individual gene disruptions (Figure 1). These observations are consistent with a functional interaction between TFIIS and Rpb9 in vivo.

The tfiis and rpb9 strains were each transformed with high-copy plasmids bearing the wild-type TFIIS or RPB9 genes respectively (Figure 2). These, and various control strains, were tested for growth on plates containing 0, 25, 50 and 100 µg/ml of 6-azauracil. The TFIIS gene on a high-copy plasmid partially suppresses the 6-azauracil sensitivity of rpb9 cells. In contrast, the RPB9 gene on a high-copy plasmid did not suppress the 6-azauracil sensitivity of tfiis cells. These data suggest that the elongation defect caused by the absence of Rpb9 can be restored partially by increasing the cellular concentration of TFIIS. We conclude that the effects on cell growth caused by disrupting the RPB9 gene arise in part from defects in transcription elongation.

Complementing the growth of rpb9 cells with Rpb9 mutants

Rpb9 was shown originally to have a role in regulating the choice of the transcription start sites (9-11) and subsequently Rpb9 was implicated in transcription elongation (12,18). Here, we analyzed the properties of the set of Rpb9 mutants in vivo to gain insight into the physiological role of Rpb9. The twenty mutants were assayed for their ability to restore normal growth to haploid rpb9 and rpb9 / tfiis yeast cells. To accomplish this, each strain was transformed with the low-copy plasmid pRS314 carrying each of the rpb9 mutant alleles and the phenotypes monitored.

All of the twenty alanine-scanning Rpb9 mutants restored normal growth to rpb9 cells. These mutants could, however, be divided into two classes: those that had no effect on any of the other Rpb9 properties (start-site selection and elongation; data not shown) and those that affected one or the other (Table 1, Columns 1 and 2). Other mutants had no effect on growth rate, initiation in vivo or elongation in vitro and were not included in the table: Rpb9 R5A, F6A, Rpb9 R8A, D9A, Rpb9 R17A, Rpb9 E18A, Rpb9 D19A, Rpb9 K20A, Rpb9 E21A, Rpb9 R30A, Rpb9 E54A, Rpb9 D72A, and Rpb9 K77A. These mutations are located in the N-terminal Zn-ribbon and the linker domains.

Several rpb9 alleles with internal or C-or N-terminal deletions in either of the two Zn domains had cell growth phenotypes (Table 1, columns 1 and 2). A C-terminal
truncation mutant Rpb9<sub>1-47</sub>, which contains the N-terminal zinc-binding domain and part of the linker region but lacks the second Zn domain, was unable to restore normal cell growth to rpb9 cells. Two deletion mutants in the Zn1 region (Rpb9<sub>Δ12-27</sub> and Rpb9<sub>Δ16-23</sub>) also were unable to complement rpb9 cell growth. We conclude that Rpb9 requires both Zn-binding regions for normal growth.

For all rpb9 mutants tested, the three phenotypes, temperature, cold and 6-azauracil sensitivity, were strongly correlated. This correlation suggests that the lack of Rpb9 is the primary defect that underlies all three phenotypes and that they are not secondary effects of the gene disruption.

Effect of rpb9 mutants on selection of transcription start site in vivo

rpb9 cells exhibit altered preference for the start sites of transcription initiation on a variety of promoters (9-11). In most cases, an upstream shift of the 5’ end of the transcript is observed. In this study, the ADH1 gene, which shows a distinctive difference in transcript start site between the wild-type parent and the rpb9 strains (10), was used to analyze the effect of the various rpb9 alleles on the use of initiation sites. A tfiis knockout strain and a rpb9/tfiis double-knockout strain were also analyzed to determine the effect of TFIIS on the selection of initiation start site. Primer-extension analysis was performed on RNA isolated from the different yeast strains using a primer directed against the 5’-end of the ADH1 gene.

The pattern of initiation sites in the wildtype strain was compared to the rpb9, tfiis, and rpb9/ tfiis strains. The RNA for this primer-extension analysis was prepared from these strains after they were grown in a rich medium. The patterns of initiation sites in the wild-type and the rpb9 strains are easily distinguished; the reverse transcripts prepared from the rpb9 strain are longer and reflect an increase in the number of ADH1 transcripts that start upstream of the -37 position (Figure 3). The deletion of the TFIIS gene appeared to have no influence on start-site selection, even in conjunction with the deletion of the RPB9 gene (data not shown). The tfiis strain had a transcript-initiation profile identical to the wild-type strain, and the profile for the rpb9/ tfiis double knockout was identical to that of the rpb9 strain. The synthetic phenotype that occurs in the rpb9/ tfiis double knockout strain does not appear to stem from a more severe defect in transcription initiation.

The majority of rpb9 alleles restored the normal pattern of initiation sites in rpb9-deleted yeast cells (see above and Table 1). One exception was an Rpb9 mutant containing only the Zn1 domain. This mutant, which expressed at wild-type levels (Stephen Orlicky, personal communication), was able to complement the start site defect (Figure 4) but not the
other activities. The properties of this mutant serve to uncouple the role of Rpb9 in transcription initiation from the roles in transcription elongation and cell growth. The phenotypes of the Rpb9 mutants are inconsistent with an important role for Rpb9 in transcription initiation.

**Correlation between *in vivo* and *in vitro* properties**

Our previous studies linking Rpb9 structure to function identified several parts of the protein important for transcription elongation activity (18). Specifically, the C-terminal Zn-ribbon was required for RNA cleavage in arrested transcription complexes and for re-activating arrested complexes in conjunction with TFIIKS and the linker region mediated the interaction of Rpb9 with RNA polymerase II. When tested for activity in yeast cells, every Rpb9 mutant that displayed a growth phenotype was inactive for transcription elongation. However, all of the alanine-scanning mutants that displayed reduced elongation activity had perfectly normal growth rates and were able to restore the normal pattern of initiation start sites (Table 1). We suggest that the *in vivo* complementation assays are less sensitive indicators of Rpb9 function than the *in vitro* assays.

**Comparison of genome-wide expression in TFIIKS and Rpb9 disrupted cells.**

The similar phenotypes of the *tfiis* and *rpb9*-deletion strains and their genetic interaction suggest that the proteins have a similar effect in elongation. To determine whether a common set of genes was regulated by the two transcription factors, we compared the pattern of gene expression in the *rpb9, tfiis* and *rpb9 / tfiis* strains using yeast DNA microarrays.

Disruption of either the TFIIKS gene of the RPB9 gene in log phase cells grown in rich medium had little effect on global gene expression (data not shown, Seidel and Kane, unpublished). In rpb9 cells, where the effect was more dramatic, transcription of only 1-2% of yeast genes was altered more or less than 2-fold compared with the isogenic parent. Many of these genes belonged to metabolic clusters (Table 2; for a complete list see http://january.med.utoronto.ca). For example, the expression of a set of glycolytic enzymes was decreased in the rpb9 cells. Although this observation suggests some form of metabolic response, the profile of gene expression did not resemble the global responses to glucose starvation (22). Further analysis will be required to determine whether the response is a direct effect on gene regulation or a more general indirect response.
In cells lacking both *TFIIS* and *RPB9*, the transcriptional changes were more pronounced, but the response resembled an amplified version of the rpb9 disruption (see http://january.med.utoronto.ca). In general, the transcription profile was correlated with the growth of the cells. There were more differences in the *rpb9/tfiis* double mutant than in the *rpb9*-deleted cells than in the *tfiis*-deleted cells. We were unable to relate any differences to specific elongation defects.
DISCUSSION

Rpb9 plays a role in selecting the sites of transcription initiation. Cells lacking Rpb9 initiate transcription at upstream sites on many promoters. We discovered that a derivative of Rpb9 containing only the Zn1 and linker domains of Rpb9 was sufficient to correct this defect. In addition, inactivating the Zn1 domain by deleting the majority of the region between the two pairs of cysteines, or mutating the first cysteine (10) destroyed its ability to select the wild-type initiation sites. Together, these two results suggest that the Zn1 domain regulates the selection of transcription-initiation sites.

In the crystal structure of yeast RNA polymerase II (3), Rpb9 is positioned near the largest subunit and is predicted to contact the DNA downstream of the active site. The Zn1 domain and the Zn2 domains are positioned on opposite sides of a protein wall that separates the DNA cleft from the back of the enzyme with respect to the DNA. Zn1 would be predicted to be closer to the template DNA than would Zn2. The activity of Zn1 in selecting start sites of transcription is therefore consistent with its position in the RNA polymerase.

In addition to altered patterns of transcript initiation sites, rpb9 strains also exhibit slow growth at optimal temperature, an increased sensitivity to high and low temperatures (20), and a lower tolerance for the drug 6-azauracil. One of the most significant observations of this work is that the temperature and drug phenotypes can be distinguished from the defect in start-site selection. Two mutations in the Zn1 domain were unable to correct the defect in start-site selection, yet corrected the growth defects. Therefore, in rpb9 cells, we conclude that the defect in start-site selection does not appear to be the underlying basis for the defect in growth.

Zn1 has a charged loop whose homologue in Zn2 is essential for elongation activity. Point mutants or deletion mutants in the charged loop in Zn1 do not appear to play an important role in selecting the start sites of transcription. Each of the single amino acid substitution mutants within the loop as well as the Δ16-23 mutant, which has the entire predicted flexible loop removed, restored the wildtype pattern of initiation sites. The mechanism of action of the Zn1 domain likely differs than for Zn2.

Rpb9 must assemble with RNA polymerase to restore elongation activity to RNA polymerase II. In vitro, we showed that the mutant comprising the Zn1 domain and part of the linker interacted poorly with pol IIΔ9 (18). However, this mutant was able to restore correct start site selection in vivo. The discrepancy between in vitro and in vivo observations is common; many of the mutants that bound poorly to pol IIΔ9 in vitro restored both start site preferences and growth characteristics in vivo. Recently we learned that the
polymerase-binding surface of Rpb9 consists of more than just the conserved D---
DPTLPR sequence; in the RNA polymerase crystal structure (3), there appear to be a range
of contacts between Rpb9 and RPB1. Many of these contacts involve the Zn1 domain,
suggesting that the strength of these interactions is sufficient for the assembly of the Zn1
domain into RNA polymerase II in the cell.
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Legends

**Fig. 1. Phenotypes of Δrpb9 cells.** *A.* Sensitivity of Δrpb9 cells to 6-azauracil. Approximately 10 000 cells of YF2221 (wild type), YF2230 (Δrpb9) containing the indicated plasmids were spotted on agar containing synthetic complete medium lacking tryptophan and uracil and including, where indicated, 100 µg/ml 6-azauracil, and incubated at 30 degrees. *B.* Synthetic phenotype of Δrpb9 and Δtfiis. Approximately 10 000 cells of YF2221 (wild type) or YF2234 (Δrpb9 Δtfiis) containing the indicated plasmids were spotted on agar containing synthetic complete medium lacking tryptophan and uracil and including, where indicated, 100 µg/ml 6-azauracil. The plates were incubated at 30 C for 2 days (without 6-azauracil), 30 C for 4 days (with 6-azauracil), 35 C for 2 days or 37 C for 2 days.

**Fig. 2. Suppression of Δrpb9 sensitivity to 6-azauracil by the expression of PPR2 on a high-copy plasmid.** Approximately 10 000, 2000, 400 or 80 cells of YF2221 (wild type), YF2230 (Δrpb9) or YF2222 (Δtfiis) containing the indicated plasmids were spotted on agar containing synthetic complete medium lacking tryptophan and uracil and including the indicated amounts of 6-azauracil. The plates were incubated at 30 C for 2 days (without 6-azauracil) or at 30 C for 6 days (with 6-azauracil).

**Figure 3:** Primer Extensions of the ADH1 Transcript from Wildtype and Deletion Strains of *S. cerevisiae*

Total RNA was isolated from wildtype, rpb9(Δ9), tfiis (ΔIIS) and rpb9 / tfiis (Δ9/ΔIIS) yeast strains. In order to map the transcript start site primer extension assays were performed using a primer directed against the *ADH1* transcript. An autoradiogram of the reverse transcript is shown. The leftmost lane contains DNA standards, with the sizes of the standards indicated (in bases) to the left of the figure. The size references indicated on the right of the figure refer to the number of bases upstream from the ATG codon in the transcript.

**Figure 4:** Primer Extension of the ADH1 Transcript from a Yeast Strain Carrying the Truncated Zn1 Allele of RPB9
Primer extensions assays of the ADH1 transcript were performed using total RNA prepared from the rpb9 yeast strain carrying either pRS314 or a derivative plasmid containing one of the various RPB9 mutant alleles. Shown here is an autoradiogram displaying the reverse transcripts from the rpb9 strains transformed with control plasmid (rpb9), the plasmid encoding wildtype RPB9 (Rpb9), or the Zn1 truncated RPB9 allele (Rpb9_1-47). Primer extension results for each of the RPB9 mutants are presented in Table 1.
Table 1. Summary of *in vivo* Analyses of RPB9 Mutant Alleles

| RPB9 Mutant | Phenotype | Start site | Elongation* |
|-------------|-----------|------------|-------------|
|             |           |            | In vitro    |
| RPB9        | ++        | ++         | + +         |
| RPB9<sub>1-47</sub> | –         | ++         | –           |
| RPB9<sub>Δ12-27</sub> | –         | –          | –           |
| RPB9<sub>Δ16-23</sub> | –         | ++         | –           |
| RPB9<sub>Δ36-70</sub> | +         | –          | –           |
| RPB9<sub>Δ65-70</sub> | ++        | ++         | –           |
| RPB9<sub>D61A</sub> | ++        | ++         | +           |
| RPB9<sub>D65A</sub> | ++        | ++         | +           |
| RPB9<sub>R70A</sub> | ++        | ++         | +           |
| RPB9<sub>Δ80-101</sub> | ++        | ++         | –           |
| RPB9<sub>Δ89-95</sub> | ++        | ++         | –           |
| RPB9<sub>R91A</sub> | ++        | ++         | –           |
| RPB9<sub>R92A</sub> | ++        | ++         | –           |
| RPB9<sub>K93A</sub> | ++        | ++         | –           |
| RPB9<sub>D94A</sub> | ++        | ++         | –           |

++ Allele completely restores normal growth at 30 degrees on synthetic complete solid medium lacking tryptophan.

+ Allele partially restores normal growth at 30 degrees on synthetic complete solid medium lacking tryptophan.

– Allele does not restore normal growth at 30 degrees on synthetic complete solid medium lacking tryptophan.

* Reported previously (Hemming et al, 1999)
Table 2

Functional clusters of transcription differences in rpb9 cells grown in rich medium compared to isogenic parent

| ORF       | Gene Name | Exp. 1 | Exp 2 | Function                          | Description                                                                                           |
|-----------|-----------|--------|-------|-----------------------------------|--------------------------------------------------------------------------------------------------------|
| **Glycolysis** |           |        |       |                                   |                                                                                                        |
| YKL152C   | GPM1      | 0.44   | 0.37  | glycolysis                        | Phosphoglycerate mutase                                                                                   |
| YGL253W   | HKX2      | 0.39   | 0.37  | glycolysis                        | Hexokinase II (PII) (also called Hexokinase B)                                                            |
| YFR053C   | HKX1      | 0.37   | 0.31  | glycolysis                        | Hexokinase I (PI) (also called Hexokinase A)                                                              |
| YOR344C   | TYE7      | 0.37   | 0.36  | glycolysis                        | basic region/helix-loop-helix/leucine-zipper protein                                                    |
| YCR012W   | PGK1      | 0.32   | 0.35  | glycolysis                        | 3-phosphoglycerate kinase                                                                                  |
| YAL038W   | CDC19     | 0.25   | 0.2   | glycolysis                        | Pyruvate kinase                                                                                          |
| YDR050C   | TPI1      | 0.15   | 0.2   | glycolysis                        | triosephosphate isomerase                                                                                |
| **Amino Acid & Nucleotide Biosynthesis/Metabolism** |           |        |       |                                   |                                                                                                        |
| YEL009C   | GCN4      | 0.57   | 0.36  | amino acid, purine biosynthesis   | transcriptional activator of amino acid biosynthetic genes                                               |
| YOL058W   | ARG1      | 0.52   | 0.47  | arginine biosynthesis             | argininosuccinate synthetase                                                                               |
| YEL046C   | GLY1      | 0.44   | 0.58  | Gly, Ser, Thr biosynthesis        | Threonine Aldolase                                                                                       |
| YDR046C   | BAP3      | 0.38   | 0.52  | transport                         | Valine transporter                                                                                        |
| YBR249C   | AR04      | 0.36   | 0.57  | aromatic amino acid biosynthesis  | DAHP synthase isoenzyme                                                                                  |
| YOR202W   | HIS3      | 9.78   | 4.78  | histidine biosynthesis            | imidazoleglycerol-phosphate dehydratase                                                                |
| YER081W   | SER3      | 7.5    | 3.91  | serine biosynthesis               | 3-phosphoglycerate dehydrogenase                                                                         |
| YDR007W   | TRP1      | 2.25   | 2.5   | tryptophan biosynthesis           | n-(3'-phosphoribosyl)-anthranilate isomerase                                                            |
| YHR137W   | AR09      | 3.46   | 3.04  | aromatic amino acid metabolism    | aromatic amino acid aminotransferase II                                                                   |
| YLR438W   | CAR2      | 2.63   | 3.38  | arginine metabolism              | ornithine aminotransfer                                                                                  |
| YDR399W   | HPT1      | 0.55   | 0.44  | purine biosynthesis               | hypoxanthine guanine phosphoribosyltransfer                                                               |
| YAR075W   | YAR075W   | 0.41   | 0.41  | unknown                           |                                                                                                        |
| YAR073W   | YAR073W   | 0.33   | 0.44  | unknown                           |                                                                                                        |
| YHR216W   | YHR216W   | 0.32   | 0.42  | purine biosynthesis               | IMP Dehydrogenase                                                                                       |
| YLR432W   | YLR432W   | 0.25   | 0.43  | unknown                           |                                                                                                        |
| YFL058W   | THI5      | 3.42   | 1.95  | pyrimidine biosynthesis           | thiamine regulated pyrimidine precursor biosynthesis                                                     |
| YNL332W   | THI12     | 3.01   | 2.47  | pyrimidine biosynthesis           | Involved in pyrimidine biosynthesis                                                                       |
| YEL021W   | URA3      | 2.77   | 2.63  | pyrimidine biosynthesis           | orotidine-5'-phosphate decarboxylase                                                                     |
| YJR156C   | THI11     | 2.54   | 1.86  | pyrimidine biosynthesis           | Thiamine biosynthetic enzyme                                                                              |
| YHR128W   | FUR1      | 0.2    | 0.27  | pyrimidine salvage pathway        | UPRTase                                                                                                 |
| **Protein Synthesis** |           |        |       |                                   |                                                                                                        |
| YPL240C   | HSP82     | 0.56   | 0.44  | protein folding                   | 82 kDa heat shock protein; homolog of Hsp90                                                             |
| YMR186W   | HSC82     | 0.29   | 0.27  | protein folding                   | constitutively expressed heat shock protein                                                               |
| YNL007C   | SIS1      | 0.51   | 0.48  | translation                       | sst4 suppressor, dnaJ homolog                                                                           |
| YER001W   | MNN1      | 0.47   | 0.55  | protein glycosylation             | Alpha-1,3-mannosyltransferase                                                                            |
| YFL022C   | FRS2      | 0.45   | 0.53  | protein synthesis                 | Phenylalanyl-tRNA synthetase                                                                             |
| YFL031W   | HAC1      | 0.15   | 0.27  | unfolded protein response         | bZIP (basic-leucine zipper) protein                                                                      |
| **RNA processing** |           |        |       |                                   |                                                                                                        |
| YHR163W   | SOL3      | 2.45   | 1.75  | tRNA splicing, putative           | homologous to Sol2p and Sol1p                                                                            |
| YDR021W   | FAL1      | 2.1    | 1.76  | rRNA processing                  | DEAD-box protein, putative RNA helicase                                                                  |
| YKL149C   | DBR1      | 0.55   | 0.31  | mRNA splicing                    | debranching enzyme                                                                                        |
| YCR035C   | RRP43     | 0.55   | 0.48  | mRNA splicing                    | Component of the exosome 3->5 exoribonuclease complex                                                    |
| YNL112W   | DBP2      | 0.47   | 2.02  | mRNA decay                       | ATP-dependent RNA helicase of DEAD box family                                                             |
| YDL048C   | STP4      | 0.36   | 0.44  | tRNA splicing                    | involved in tRNA splicing                                                                                |

Major functional gene clusters. Highlighted groups share >85% sequence identity.
**A**

Wild type (vector)

Δrpb9 (vector)

Δrpb9 (RPB9)

**B**

Wild type (vector)

Δrpb9 Δtfiis (vector)

Δrpb9 Δtfiis (RPB9)

Δrpb9 Δtfiis (TFIIS)

30 30 + 6AU

30 35 37

30 + 6AU
RNA polymerase II subunit Rpb9 regulates transcription elongation in vivo
Sally A. Hemming, David B. Jansma, Pascale F. Macgregor, Andrew Goryachev, James D. Friesen and Aled M. Edwards

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