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§

From the Banting and Best Department of Medical Research, University of Toronto, Charles H. Best Institute, Toronto, Ontario M5G 1L6, Canada

RNA polymerase II lacking the Rpb9 subunit 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 *RPB9* has a synthetic phenotype with the TFIIS gene. Disruption of *RPB9* 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 Δ*rpb9* cells. We set out to determine the relevant cellular role of yeast Rpb9 by assessing the ability of 20 different site-directed and deletion mutants of *RPB9* to complement the initiation and elongation defects of Δ*rpb9* cells *in vivo*. Rpb9 is composed of two zinc ribbons. The N-terminal zinc 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 transcription start sites suggests that this is not the primary cellular role for Rpb9. Genome-wide transcription profiling of Δ*rpb9* cells revealed only a few changes, predominantly in genes related to metabolism.

RNA polymerase II comprises 12 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 III, C11, also has been implicated in regulating RNA chain elongation (15).

Rpb9 comprises two zinc ribbon domains joined by a 30-amino acid linker. The C-terminal zinc ribbon is a sequence homologue of the zinc 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 zinc ribbon domain of Rpb9, like amino acid substitutions in the homologous part of TFIIS, completely eliminated elongation activity. Mutating the first zinc ribbon had no effect on elongation activity, although 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 (*MAT*a *ura3-52 his3-11,15 leu2-3,112 ade2-1 can1-100 ssd1-d2 trp1::hisG-URA3-hisG*) is the parent strain. YF2230 (*MAT*a *ura3-52 his3-11,15 leu2-3,112 ade2-1 can1-100 ssd1-d2 trp1::hisG-URA3-hisG rpb9::HIS3*) is a derivative of YF2221 deleted for *RPB9*. YF2222 (*MAT*a *ura3-52 his3-11,15 leu2-3,112 ade2-1 can1-100 ssd1-d2 trp1-1 ppr2::hisG-URA3-hisG*) is deleted for the TFIIS gene, and YF2234 (*MAT*a *ura3-52 his3-11,15 leu2-3,112 ade2-1 can1-100 ssd1-d2 trp1-1 ppr2::hisG-URA3-hisG rpb9::HIS3*) lacks both *RPB9* and the TFIIS gene.

Yeast Expression Plasmid

The yeast expression plasmid pRS314RPB9 containing the *RPB9* open reading frame plus ~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 Δ*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 wild-type *RPB9* corrects these defects. Haploid Δ*rpb9* cells were transformed with the *RPB9* yeast expression plasmids to test each mutant for the ability to complement the Δ*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 ~10,000, 2000, 400, and 80 cells were spotted onto solid medium and grown at 12, 30, or 37 °C for 2–6 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 ~10,000, 2000, 400, and 80 cells were spotted onto solid medium and grown at 30 °C for 3–8 days. Each mutant construct was compared with wild-type *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 yeast extract peptone liquid medium with 2% glucose. YF2230 cells transformed with each of the pRS314RPB9BE constructs was grown in liquid complete synthetic medium lacking tryptophan. All cultures were grown at 30 °C to $A_{600\ nm}$ = 0.2 to 1.0. Cells (5×10^7) were harvested, and total RNA was isolated using the RNeasy protocol (QIAGEN Inc.). The primer used for these experiments, 5′-AGAAGATAACACCTTTTTGAG-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 Inc.) and [γ-^{32}P]ATP. For each primer extension reaction, 15 μg of total RNA from the appropriate yeast strain was annealed with 0.4 pmol of the 5′-radiolabeled primer for 45 min at 52 °C. Reverse transcription from the annealed primer was done with Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) according to manufacturer's instructions. The reverse transcripts were collected by ethanol precipitation and resolved on a Tris borate/EDTA, 8.3 M urea, and 6% polyacrylamide gel and visualized by phosphorimaging.

RNA Isolation—Yeast cells were grown in yeast extract peptone 2% glucose medium at 30 °C with constant agitation and aeration to $A_{600\ nm}$ = 0.4–0.6. Cells were washed once with diethyl pyrocarbonate-treated water and resuspended in buffer containing 10 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 0.5% SDS. Total RNA was isolated 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 absorbance at 260 nm. Two independent RNA preparations were made for each mutant strain.

Preparation of Labeled cDNA Probes—For each DNA microarray, 50 μg of total RNA was reverse-transcribed using 400 units of Superscript II (Life Technologies, Inc.). The reverse transcription was primed with an AncT primer (T20VN, Sigma) and performed in the presence of dATP, dGTP, dTTP (final concentration of 168 μM each; Amersham Pharmacia Biotech), dCTP (final concentration of 50 μM; Amersham Pharmacia Biotech), and Cy3-labeled dCTP or Cy5-labeled dCTP (final concentration of 50 μM; Amersham Pharmacia Biotech). 20 units of RNasin (Promega) was also added to the reaction. The mixture (minus the enzyme) was heated at 65 °C for 5 min and then at 42 °C for 5 min; the reverse transcription enzyme was added; and the reaction was incubated at 42 °C for 2 h. The reverse transcription was stopped with EDTA (final concentration of 6.25 mM), and the RNA template was degraded by the addition of 10 N NaOH (final concentration of 0.5 N) and incubation at 65 °C for 20 min. The mixture was neutralized by the addition of 5 M acetic acid (final concentration of 0.5 M), and the labeled cDNA was precipitated by the addition of 1 volume of isopropyl alcohol and incubation on ice for 30 min. After rinsing with 70% EtOH, the labeled cDNA was resuspended in 5 μg of diethyl pyrocarbonate-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 (Roche Molecular Biochemicals). 2 μl of yeast tRNA (10 mg/ml; Sigma) and 2 μl of single-stranded salmon sperm DNA (10 mg/ml; Sigma) were also added to the hybridization
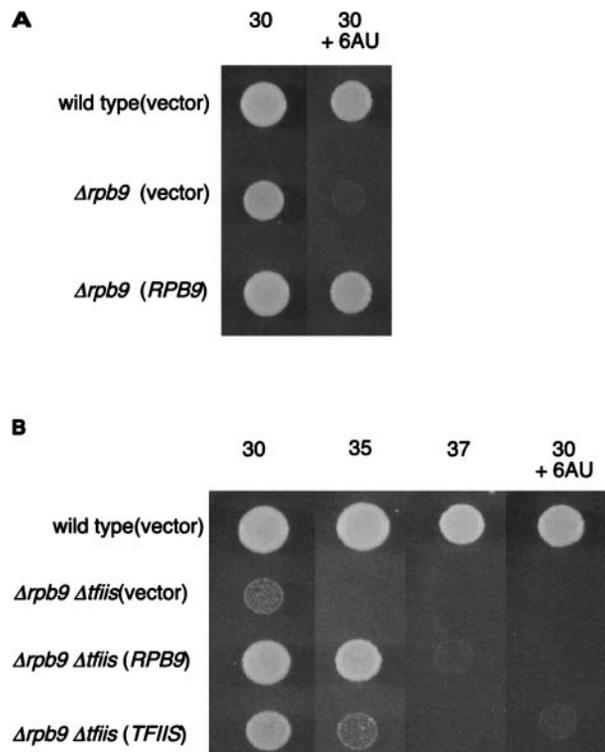
FIG. 1. **Phenotypes of Δ*rpb9* cells.** *A*, sensitivity of Δ*rpb9* cells to 6-azauracil. Approximately 10,000 cells of the YF2221 (wild-type) or YF2230 (Δ*rpb9*) strain containing the indicated plasmids were spotted onto agar containing synthetic complete medium lacking tryptophan and uracil and including, where indicated, 100 μg/ml 6-azauracil (*6AU*) and incubated at 30 °C. *B*, synthetic phenotype of Δ*rpb9* and Δ*tfiis* cells. Approximately 10,000 cells of the YF2221 (wild-type) or YF2234 (Δ*rpb9* Δ*tfiis*) strain containing the indicated plasmids were spotted onto 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.

buffer, and the solution was heated at 65 °C for 2 min. The solution was then applied under a coverslip to a custom-made yeast whole genome microarray (Microarray Center, Ontario Cancer Institute). The microarrays were incubated at 37 °C in a humid hybridization chamber for 8–12 h. Before scanning, the slides were washed with 0.1× SSC and 0.1% SDS (3 × 15 min at 50 °C), rinsed with 0.1× SSC (3 × 5 min at room temperature), and dried by centrifugation. A total of eight 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 QuantArray 2.0 software (GSI Lumonics).

RESULTS

The Phenotype of Δrpb9 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)[1] 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 zinc 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*

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[1] The abbreviation used is: pol IIΔ9, RNA polymerase II lacking the Rpb9 subunit.

FIG. 2. **Suppression of Δ*rpb9* sensitivity to 6-azauracil by the expression of the TFIIS gene on a high-copy plasmid.** Approximately 10,000, 2000, 400, or 80 cells of the YF2221 (wild-type), YF2230 (Δ*rpb9*), or YF2222 (Δ*tfiis*) strain containing the indicated plasmids were spotted onto 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).
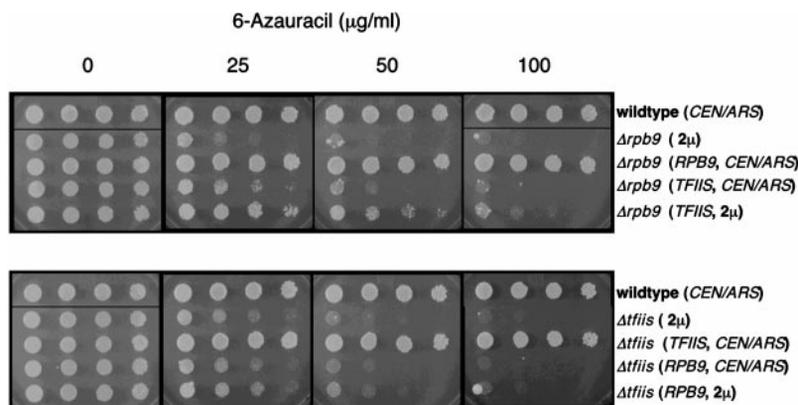
TABLE I

Summary of in vivo analyses of rpb9 mutant alleles

Rpb9 mutant	Phenotype	Start site	Elongation *in vitro*[b]
Rpb9	++[a]	++	++
Rpb9-(1–47)	−	++	−
Rpb9-(Δ12–27)	−	−	−
Rpb9-(Δ16–23)	−	++	−
Rpb9-(Δ36–70)	+	−	−
Rpb9-(Δ65–70)	++	++	−
Rpb9(D61A)	++	++	+
Rpb9(D65A)	++	++	+
Rpb9(R70A)	++	++	+
Rpb9-(Δ80–101)	++	++	−
Rpb9-(Δ89–95)	++	++	−
Rpb9(R91A)	++	++	−
Rpb9(R92A)	++	++	−
Rpb9(K93A)	++	++	−
Rpb9(D94A)	++	++	−

[a] ++, allele completely restores normal growth at 30 °C on synthetic complete solid medium lacking tryptophan; +, allele partially restores normal growth at 30 °C on synthetic complete solid medium lacking tryptophan; −, allele does not restore normal growth at 30 °C on synthetic complete solid medium lacking tryptophan.

[b] Reported previously (18).

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 zinc ribbon domain was important for elongation, as was the linker region connecting the two zinc ribbons composing 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 zinc ribbon, which is 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 whether there is also a genetic interaction between *RPB9* and TFIIS gene (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, 21). These phenotypes were also observed in our Δ*rpb9* strain, and normal growth was restored by expressing *RPB9* from a low-copy plasmid under the control of its own promoter. Yeast strains lacking the TFIIS gene 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 Δ*rpb9* strain for sensitivity to
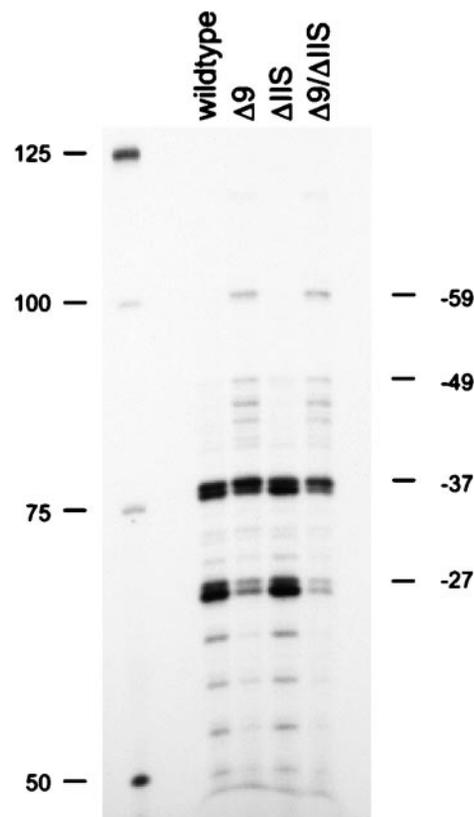

FIG. 3. **Primer extension analysis of the *ADH1* transcript from wild-type and deletion strains of *Saccharomyces cerevisiae*.** Total RNA was isolated from wild-type, Δ*rpb9* (Δ*9*), *tfiis* (Δ*IIS*), and Δ*rpb9/tfiis* (Δ*9*/Δ*IIS*) yeast strains. To map the transcription start site, primer extension assays were performed using a primer directed against the *ADH1* transcript. An autoradiogram of the reverse transcript is shown. The *first lane* contains DNA standards, with the sizes of the standards indicated (in bases) to the left. The size references indicated to the right refer to the number of bases upstream from the ATG codon in the transcript.

6-azauracil. The Δ*rpb9* strain grew more slowly on medium containing 6-azauracil than did the parent strain (Fig. 1). Transforming the Δ*rpb9* strain with the *RPB9* gene on a low-copy plasmid fully complemented the 6-azauracil sensitivity (Fig. 1).

The double deletion strain (Δ*rpb9*/Δ*tfiis*) was constructed, and its phenotype was tested to explore the genetic interaction between the TFIIS gene 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 (Fig. 1). These observations are consistent with a functional interaction between TFIIS and Rpb9 *in vivo*.

The Δ*tfiis* and Δ*rpb9* strains were each transformed with

high-copy plasmids bearing the wild-type TFIIS gene and *RPB9*, respectively (Fig. 2). These and various control strains were tested for growth on plates containing 0, 25, 50, and 100 μg/ml 6-azauracil. The TFIIS gene on a high-copy plasmid partially suppressed the 6-azauracil sensitivity of Δ*rpb9* cells. In contrast, *RPB9* on a high-copy plasmid did not suppress the 6-azauracil sensitivity of Δ*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 *RPB9* arise in part from defects in transcription elongation.

Complementing the Growth of Δrpb9 Cells with rpb9 Mutants—Rpb9 was shown originally to have a role in regulating the choice of the transcription start sites (9–11); 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 20 mutants were assayed for their ability to restore normal growth to haploid Δ*rpb9* and Δ*rpb9*/Δ*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 were monitored.

All of the 20 alanine-scanning Rpb9 mutants restored normal growth to Δ*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 I, first and second columns). Other mutants had no effect on growth rate, initiation *in vivo*, or elongation *in vitro* and are not included in Table I: Rpb9(R5A,F6A), Rpb9(R8A,D9A), Rpb9(R17A), Rpb9(E18A), Rpb9(D19A), Rpb9(K20A), Rpb9(E21A), Rpb9(R30A), Rpb9(E54A), and Rpb9(D72A), and Rpb9(K77A). These mutations are located in the N-terminal zinc ribbon and the linker domains.

Several *rpb9* alleles with internal or C- or N-terminal deletions in either of the two zinc domains had cell growth phenotypes (Table I, first and second columns). A C-terminal truncation mutant, Rpb9-(1–47), which contains the N-terminal zinc-binding domain and part of the linker region but lacks the second zinc domain, was unable to restore normal cell growth to Δ*rpb9* cells. Two deletion mutants in the first zinc (Zn1) region (Rpb9-(Δ12–27) and Rpb9-(Δ16–23)) also were unable to complement Δ*rpb9* cell growth. We conclude that Rpb9 requires both zinc-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 Transcription Start Site Selection in Vivo—*rpb9* cells exhibit altered preference for transcription start sites 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 the transcription start site between the wild-type parent and the Δ*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 initiation start site selection. 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 wild-type strain was compared with those in the Δ*rpb9*, Δ*tfiis*, and Δ*rpb9*/Δ*tfiis* strains. The RNA for this primer extension analysis was prepared from these strains after they were grown in a rich me-
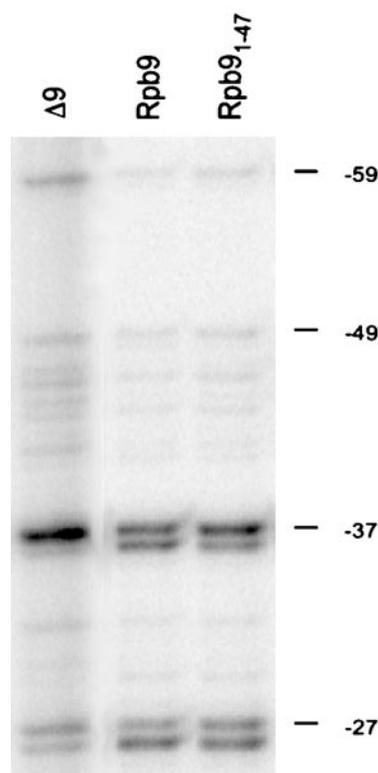

FIG. 4. **Primer extension analysis of the *ADH1* transcript from a yeast strain carrying the truncated Zn1 allele of *rpb9*.** Primer extension assays of the *ADH1* transcript were performed using total RNA prepared from the Δ*rpb9* yeast strain carrying either pRS314 or a derivative plasmid containing one of the various *rpb9* mutant alleles. Shown here is an autoradiogram displaying the reverse transcripts from the Δ*rpb9* strains transformed with control plasmid (Δ9), the plasmid encoding wild-type *RPB9* (Rpb9), or the truncated Zn1 *rpb9* allele (Rpb9$_{1-47}$). Primer extension results for each of the Δ*rpb9* mutants are presented in Table I.

dium. The patterns of initiation sites in the wild-type and Δ*rpb9* strains are easily distinguished; the reverse transcripts prepared from the Δ*rpb9* strain are longer and reflect an increase in the number of *ADH1* transcripts that start upstream of position −37 (Fig. 3). The deletion of the TFIIS gene appeared to have no influence on start site selection, even in conjunction with the deletion of *RPB9*. The Δ*tfiis* strain had a transcription initiation profile identical to that of the wild-type strain, and the profile for the *rpb9/tfiis* double knockout strain was identical to that of the Δ*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 I). One exception was an *rpb9* mutant containing only the Zn1 domain. This mutant, which expressed at wild-type levels,[2] was able to complement the start site defect (Fig. 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 zinc ribbon was

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[2] S. M. Orlicky, personal communication.

TABLE II

Functional clusters of transcription differences in Δrpb9 cells grown in rich medium compared with the isogenic parent

Shown are major functional gene clusters. Boldface groups share >85% sequence identity. ORF, open reading frame.

ORF	Gene name	Exp. 1	Exp. 2	Function	Description
Glycolysis					
YKL152C	*GPM1*	0.44	0.37	Glycolysis	Phosphoglycerate mutase
YGL253W	*HXK2*	0.39	0.37	Glycolysis	Hexokinase II (PII) (also called hexokinase B)
YFR053C	*HXK1*	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	Triose-phosphate isomerase
Amino acid and 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	Arginosuccinate synthetase
YEL046C	*GLY1*	0.44	0.58	Gly, Ser, Thr biosynthesis	Threonine aldolase
YDR046C	*BAP3*	0.38	0.52	Transport	Valine transporter
YBR249C	*ARO4*	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*-(5′-Phosphoribosyl)-anthranilate isomerase
YHR137W	*ARO9*	3.46	3.04	Aromatic amino acid metabolism	Aromatic amino acid aminotransferase II
YLR438W	*CAR2*	2.63	3.38	Arginine metabolism	Ornithine aminotransferase
YDR399W	*HPT1*	0.55	0.44	Purine biosynthesis	Hypoxanthine-guanine phosphoribosyltransferase
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	
YEL058W	*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
YLR156C	*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; homologue of Hsp90
YMR186W	*HSC82*	0.29	0.27	Protein folding	Constitutively expressed heat shock protein
YNL007C	*SIS1*	0.51	0.48	Translation	*sit4* suppressor, *dnaJ* homologue
YER001W	*MNN1*	0.47	0.55	Protein glycosylation	α-1,3-Mannosyltransferase
YFL022C	*FRS2*	0.45	0.53	Protein synthesis	Phenylalanyl-tRNA synthetase
YFL031W	*HAC1*	0.15	0.27	Unfolded protein response	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	rRNA processing	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

required for RNA cleavage in arrested transcription complexes and for reactivating arrested complexes in conjunction with TFIIS, 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 I). 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 TFIIS- and Rpb9-disrupted Cells—The similar phenotypes of the *tfiis*- and *rpb9*-deleted strains and their genetic interaction suggest that the proteins have a similar effect on elongation. To determine whether a common set of genes is regulated by the two transcription factors, we compared the patterns of gene expression in the Δ*rpb9*, Δ*tfiis*, and Δ*rpb9*/Δ*tfiis* strains using yeast DNA microarrays.

Disruption of either the TFIIS gene or *RPB9* in log-phase cells grown in rich medium had little effect on global gene expression (data not shown).[3] In Δ*rpb9* cells, where the effect was more dramatic, transcription of only 1–2% of yeast genes was altered more than 2-fold compared with the isogenic parent. Many of these genes belonged to metabolic clusters (Table II). For example, the expression of a set of glycolytic enzymes was decreased in the Δ*rpb9* cells. Although this observation

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[3] C. Seidel and C. M. Kane, unpublished data.

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. In general, the transcription profile correlated with the growth of the cells. There were more differences in the *rpb9/tfiis* double disruption than in the singly deleted *rpb9* and *tfiis* 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 and 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. The Zn1 domain would be predicted to be closer to the DNA template than would the Zn2 domain. The activity of the Zn1 domain in selecting transcription start sites is therefore consistent with its position in the RNA polymerase.

In addition to altered patterns of transcription 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 $\Delta rpb9$ cells, we conclude that the defect in start site selection does not appear to be the underlying basis for the defect in growth.

The Zn1 domain has a charged loop whose homologue in the Zn2 domain is essential for elongation activity. Point mutants or deletion mutants in the charged loop in the Zn1 domain do not appear to play an important role in selecting the transcription start sites. Each of the single amino acid substitution mutants within the loop as well as the $\Delta 16-23$ mutant, which has the entire predicted flexible loop removed, restored the wild-type pattern of initiation sites. The mechanism of action of the Zn1 domain likely differs than of the Zn2 domain.

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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