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RNA polymerase subunit RPB5 plays a role in transcriptional activation

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ABSTRACT **A mutation in RPB5 *(rpb5–9)*, an essential RNA polymerase subunit assembled into RNA polymerases I, II, and III, revealed a role for this subunit in transcriptional activation. Activation by GAL4-VP16 was impaired upon *in vitro* transcription with mutant whole-cell extracts. *In vivo* experiments using inducible reporter plasmids and Northern analysis support the *in vitro* data and demonstrate that RPB5 influences activation at some, but not all, promoters. Remarkably, this mutation maps to a conserved region of human RPB5 implicated by others to play a role in activation. Chimeric human-yeast RPB5 containing this conserved region now can function in place of its yeast counterpart. The defects noted with *rpb5–9* are similar to those seen in truncation mutants of the RPB1-carboxyl terminal domain (CTD). We demonstrate that RPB5 and the RPB1-CTD have overlapping roles in activation because the double mutant is synthetically lethal and has exacerbated activation defects at the GAL1/10 promoter. These studies demonstrate that there are multiple activation targets in RNA polymerase II and that RPB5 and the CTD have similar roles in activation.**

Regulated gene expression in eukaryotes enlists the action of a multitude of individual proteins and protein complexes to activate or repress mRNA transcription. The signals initiated and transmitted by these proteins ultimately influence the multisubunit enzyme RNA polymerase II. Purified *Saccharomyces cerevisiae* RNA polymerase II is composed of 12 polypeptides, RPB1–RPB12 (1, 2). The amino acid composition of RPB1 and RPB2 revealed that they are orthologs of the bacterial β' and β subunits, respectively. RPB3 (and to a lesser extent, RPB11) appear to have some functions similar to the bacterial α subunit, but the amino acid sequences of the remaining eight subunits do not reveal any clear clues to their functions.

The RPB5, RPB6, RPB8, RPB10, and RPB12 subunits are shared components of RNA polymerases I, II, and III. These five subunits are assembled along with a unique set of additional subunits to form either of the three classes of enzyme. The common subunits play an essential role in transcription because all are required for yeast cell viability (1). However, their specific contribution to regulated gene expression is still an enigma.

RNA polymerase II associates with about 30 other proteins to form the RNA polymerase II holoenzyme, the molecular machine that supports activation and repression of transcription *in vitro* and *in vivo* (3–5). Although the proteins that mediate signals to the RNA polymerase II holoenzyme are the subject of intensive study, the final targets of this signaling relay are not well defined. Therefore, functional studies on the series of steps and signals that regulate RNA polymerase are still in their infancy.

In contrast, more detailed mechanistic studies of gene activation have been performed in bacteria. These led to the identification of multiple activation target sites on bacterial RNA polymerase, and in some cases, definition of the molecular outcomes (e.g., open complex formation, promoter clearance) upon binding of activators to their targets on polymerase (6). All four RNA polymerase subunits have been implicated as activation targets, with α and σ appearing to be the favored targets with natural activators (6–13). Therefore, eukaryotes likely engage at least as many target sites in RNA polymerase II because their genes are subject to many more layers of control by a panoply of regulatory proteins.

Surprisingly, only one site on RNA polymerase II, the carboxyl-terminal domain (CTD) of RPB1, has been shown to be required for normal activated transcription *in vivo* and *in vitro* (14, 15). The CTD in *S. cerevisiae* is composed of 26 contiguous repeats at the carboxyl-terminal end of RPB1 that are either identical to, or closely resemble, the consensus sequence YSPTSPS (16, 17). Its crucial role in both negative and positive regulation was later understood at the molecular level when it was demonstrated that this domain interacts with protein complexes called SRBs or mediators (4, 5, 18). The SRB/mediator proteins along with RNA polymerase II, GAL11, and the general factors TFIIB, TFIIF, and TFIIH comprise the yeast RNA polymerase II holoenzyme. Therefore, studies of the CTD led to a link between RNA polymerase II and the rest of the players in regulated gene expression.

With the CTD playing such a pivotal role in transcription, as well as being a feature exclusive to RNA polymerase II, its inferred role as a predominant processor of activation signals to RNA polymerase was widely accepted. The following work demonstrates that a second subunit, RPB5, also plays a role in activation. Interestingly, its mode of action is not entirely independent of the CTD because the two appear to act as targets for similar activators. These observations uncovered a concrete function of the yeast RPB5 subunit and extend our understanding of activation by revealing that multiple subunits can participate in this process.

MATERIALS AND METHODS

Media and Strain Construction. Yeast extract/peptone/dextrose (YPD), synthetic complete (SC), synthetic dextrose, and minimal medium for inositol starvation were prepared according to standard recipes (19). 5-Fluoroorotic acid was added to 1 mg/ml.

The *rpb5–9* mutant allele was generated by PCR mutagenesis (20), and the yeast strain containing the mutant was identified by using plasmid shuffle followed by exposure to

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Abbreviations: CTD, carboxyl-terminal domain; HBx, hepatitis B virus X activator protein; UAS, upstream activating sequence; YPD, yeast extract/peptone/dextrose; SC, synthetic complete; wt, wild type.

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the high and low temperature to reveal its conditional phenotype (21) (see Table 1). The exact location of the mutation then was identified by DNA sequence analysis. The plasmid containing the *rpb5*Δ2::*URA3hisG* allele was constructed by replacing a 650-bp *Eco*RI fragment in 1.5-kb *RPB5*-containing a *Xba*I–*Spe*I fragment with *URA3 hisG* cassette (22) followed by ligation to pGEM-9Zf(−). To construct the *rpb5–9*/*rpb1*Δ104 double mutant, the WY187 diploid was first created by mating of Z22 and Z551 (a.k.a. C6; ref. 15). WY188 was created by transformation of WY187 with the DNA fragment of *rpb5*Δ2::*URA3hisG* from pRP534 followed by selection of Ura^+ transformants. WY188 was plated on 5-fluoroorotic acid (5-FOA) to remove *URA3*, then transformed with pRP514 to generate WY189. WY190 was isolated after tetrad analysis of WY189 followed by selection for $His^+Leu^+5\text{-}FOA^s$ spores.

***In Vitro* Transcription.** Whole-cell extracts from wild-type (wt) (N222) and mutant (WY186) strains were prepared with glass beads as described (23). Transcription reactions were based on published procedures (14), performed at 23°C for 30 min with 250 μg of extract and 500 ng of template plasmid $pGAL4CG^-$ (24), in a 25-μl reaction mixture, and initiated by addition of nucleotides. For activated transcription, $pGAL4CG^-$ was incubated with GAL4-VP16 for 5 min on ice before starting the transcription reaction.

β-Galactosidase Assays and Northern Analysis. The reporter plasmids, pJH359 (15), pLGSD5 (25), and pHYC3 (26), containing a *CYC1* TATA element and either the *INO1*, *GAL10,* or *HIS4* upstream activating sequence (UAS), respectively, were individually transformed into WY185 (wt) and WY186 (*rpb5–9*). Cells were harvested after exposure to nonactivating or activating growth conditions (described below). Cell extracts were prepared, enzyme levels were assayed, and unit calculations were performed according to standard methods (27).

For Northern analysis of *INO1*, cells were grown at 30°C to midlogarithmic phase in minimal medium containing 400 μM inositol. Cells were washed then incubated with agitation for 10 hr in the minimal medium containing 10 μM inositol. For analysis of *GAL1* expression, cells were grown to midlogarithmic phase at 30°C in SC containing 2% raffinose as the sole carbon source. Cells were washed then incubated with agitation for 3 hr at 30°C or 37°C in SC containing 5% galactose and 2% raffinose as carbon sources. For *HIS4*, cells were grown at 30°C in synthetic dextrose media supplemented with lysine and adenine to midlog phase. 3-Aminotriazole was added to a final concentration of 10 mM followed by incubation with agitation for 3 hr at 30°C or 37°C. For *PHO5*, cells were grown at 30°C in either low-phosphate YPD (28) or high-phosphate YPD, and cultures were started at a OD_{600} of 0.01 and harvested at approximately OD_{600} of 0.5. In low- and high-phosphate YPD, potassium phosphate was added to a final concentration of 0.1 mM and 7.5 mM, respectively.

Total RNA was prepared by standard methods (27), and 20 μg of total RNA was loaded into each lane. Northern blots were hybridized with radioactively labeled DNA probes and band intensities were quantified by using a PhosphorImager. The probes used were labeled with ^{32}P by using the Random Primer Labeling Kit (Boehringer Mannheim), and unincorporated counts were removed by gel filtration using Quick Spin Columns (Boehringer Mannheim). The plasmid names and fragment sizes used as gene-specific probes were as follows: *INO1* (pN333) 0.9-kb *Hin*dIII/*Cla*I; *GAL1* (pGAL1-GAL10)-2.1-kb *Eco*R I; *HIS4* (pFW45) 1.4-kb *Eco*RI/*Sal*I; *ACT1* (pN162) 1.4-kb *Hin*dIII/*Eco*RI; *U3* (pJD161) 0.5-kb *Bam*HI/*Hpa*I; and *PHO5* (pN973) 625-bp *Bam*HI/*Sal*I.

Complementation by Yeast-Human RPB5 Chimeras. Fused genes for yeast-human chimeras were prepared by PCR (29). All constructs contained ≈750 bp of yeast DNA corresponding to sequences upstream from the initiating ATG and had *Kpn*I sites added to both ends of the fragments. Final PCR products were cloned into the *Kpn*I site of the yeast multicopy vector YEplac181. WY184 was transformed with each plasmid, and transformants were tested for the growth in the presence of 5-fluoroorotic acid on SC-His^-Leu^- plates.

Table 1. Strains and plasmids

Strain/plasmid	Genotype/description
N222	*MATα ura3-52 his3Δ200 leu2-3, 112 lysΔ201 ade2*
Z22	*MATα ura3-52 his3Δ200 leu2-3,112*
Z551	*MAT***a** *ura3-52 his3Δ200 leu2-3, 112 rpb1Δ187*::*HIS3* [pC6 (*LEU2 rpb1Δ104*)]
WY184	*MATα ura3-52 his3Δ200 leu2-3, 112 lysΔ201 ade2 RPB5Δ1*::*HIS3* [pRP510 (*URA3 RPB5*)]
WY185	*MATα ura3-52 his3Δ200 leu2-3, 112 lysΔ201 ade2 RPB5Δ1*::*HIS3* [pRP58 (*LEU2 RPB5*)]
WY186	*MATα ura3-52 his3Δ200 leu2-3, 112 lysΔ201 ade2 RPB5Δ1*::*HIS3* [pRP514 (*LEU2 rpb5-9*)]
WY187	*MAT***a**/*MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3, 112/leu2-3, 112 RPB1/rpb1Δ187*::*HIS3* [pC6 (*LEU2 rpb1Δ104*)]
WY188	*MAT***a**/*MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3, 112/leu2-3, 112 RPB1/rpb1Δ187*::*HIS3, RPB5/rpb5Δ2*::*URA3hisG* [pC6 (*LEU2 rpb1Δ104*)]
WY189	*MAT***a**/*MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3, 112/leu2-3, 112 RPB1/rpb1Δ187*::*HIS3, RPB5/rpb5Δ2*::*hisG* [pC6 (*LEU2 rpb1Δ104*)] [pRP524 (*URA3 rpb5-9*)]
WY190	*MAT***a** *ura3-52 his3Δ200 leu2-3, 112 rpb1Δ187*::*HIS3 rpb5Δ2*::*hisG* [pC6 (*LEU2 rpb1Δ104*)] [pRP524 (*URA3 rpb5-9*)]
pRP58	1.5-kb *RPB5*-containing *Xba*I–*Spe*I fragment in pRS415 (*LEU2 CEN*)
pRP510	1.5-kb *RPB5*-containing *Xba*I–*Spe*I fragment in pRS416 (*URA3 CEN*)
pRP514	*rpb5-9* in pRS415 (*LEU2 CEN*)
pRP524	*rpb5-9* in pRS416 (*URA3 CEN*)
pRP525	1.5-kb *RPB5*-containnig *Xba*I–*Spe*I fragment in YEplac181 (*LEU2* 2 μm)
pRP526	Hybrid gene for yRPB5 (1-139)-hRPB5 (135-210) chimera* in YEplac181 (*LEU2* 2 μm)
pRP527	Hybrid gene for yRPB5 (1-120)-hRPB5 (115-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP528	Hybrid gene for yRPB5 (1-78)-hRPB5 (73-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP529	Hybrid gene for hRPB5 (1-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP530	Hybrid gene for yRPB5 (11-139)-hRPB5 (1-8, 135-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP531	Hybrid gene for yRPB5 (45-139)-hRPB5 (1-42, 135-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP532	Hybrid gene for yRPB5 (67-139)-hRPB5 (1-66, 135-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP533	Hybrid gene for yRPB5 (121-139)-hRPB5 (1-114, 135-210) chimera in YEplac181 (*LEU2* 2 μm)
pRP534	*rpb5Δ2*::*URA3hisG* allele in pGEM-9Zf(−)

*Amino acid positions of yeast RPB5 and human RPB5 regions encoded by the hybrid genes are shown in parenthesis.

RESULTS

A Mutation in the Common Subunit RPB5 Maps to a Region Implicated in Activation. To search for clues about the role of RPB5 in transcription, we created random mutations throughout the ORF for this 215-aa subunit and screened for cold- and temperature-sensitive mutants at 12°C or 37°C, respectively. One mutant, designated *rpb5–9*, carried a single mutation, resulting in a V111G substitution that rendered the yeast cells cold-sensitive and mildly temperature-sensitive (Fig. 1*A*). Interestingly, this mutant falls within a conserved 48-aa region analogous to that in human RPB5 shown by others to interact with hepatitis B virus X activator protein (HBx) *in vivo* and *in vitro* (Fig. 1*B*) (30, 31). This conserved region is represented in yeasts and higher eukaryotes but not archaebacterial orthologs of RPB5 (Fig. 1*B*) (32).

***rpb5–9* Is Activation Impaired *in Vitro*.** Based on the human RPB5 and HBx interaction, we tested whether the *rpb5–9* mutant had any effect on activation. We used an *in vitro* transcription assay to test the ability of the mutant extracts to support basal and activated transcription from a defined promoter (Fig. 2*B*). Although the levels of basal transcription for the mutant and wt extracts were equivalent, the mutant was significantly impaired in its ability to activate transcription with GAL4-VP16 (Fig. 2*B*). Quantification revealed that the transcript yield increased by approximately 10-fold when GAL4-VP16 was added to wt extracts before transcription, compared with only about 1.5-fold when using the mutant extract (Fig. 2*C*). These results suggest that this mutation specifically affects activation because the basal levels of transcription are not affected. These results are also consistent with the predicted connection between this region of RPB5 and activation initially revealed by the human RPB5-HBx interaction.

***rpb5–9* Mutant Cells Have Activation Defects that Mirror CTD Mutants.** To test for activation *in vivo*, we used two approaches. We measured expression of inducible genes by using *lac*Z reporter plasmids and Northern analysis. wt and *rpb5–9* cells containing *lac*Z reporter plasmids that allow quantification of activation at the *INO1*, *GAL10*, and *HIS4* promoters were grown under uninduced or induced conditions (described in *Materials and Methods*). Cells were harvested at the appropriate time, cell extracts were prepared, and β-galactosidase activity was measured for each sample. The results indicated that activation at the *INO1* UAS was strikingly affected, activation at the *GAL10* UAS was only moderately affected, and there was no effect on activation at the *HIS4* UAS in mutant *rpb5–9* cells (Fig. 3).

We then used Northern analysis to look at the effect of the *rpb5–9* mutation on the synthesis of new transcripts upon activation of the four genes, *INO1*, *GAL1, PHO5,* and *HIS4*. With this approach, we again saw a dramatic decrease in activation as assessed by the levels of *INO1* and *PHO5* transcript (Fig. 4), a more marginal effect for *GAL1* (Fig. 4), and no effect on *HIS4* (not shown). The results of both *in vivo* experiments corroborate our *in vitro* transcription data and also revealed that this mutation has variable affects on the levels of transcription of four genes upon activation.

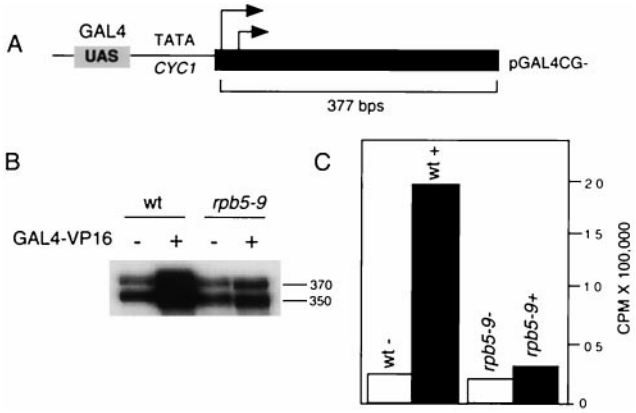

FIG. 2. *In vitro* transcription of *rpb5–9* reveals an activation defect. (*A*) DNA template used for transcription. Arrows represent approximate transcription start sites within the 377-bp G-less cassette. (*B*) Transcription products from wt and mutant (*rpb5–9*) whole-cell extracts with (+) or without (−) the addition of GAL4-VP16. mRNA sizes after gel electrophoresis and autoradiography are indicated. (*C*) PhosphorImager quantification of results shown in *B*.

rpb5–9 appears to specifically alter the overall transcript yield upon activated, but not basal, transcription by multiple supporting experiments. First, our *in vitro* transcription experiments with the mutant extracts revealed that wt levels of transcript were synthesized in the absence of added activator (Fig. 2). Second, we also looked at the transcript levels of several constitutive genes in mutant and wt cells and found no significant differences (data not shown). Finally, transcript levels of one inducible gene tested (*HIS4*) are unaffected. Taken together, these results suggest that *rpb5–9* causes a decrease in activation of a subset of genes. Thus, RPB5 appears to be a target for transcriptional activation by some activators.

Yeast-Human RPB5 Chimeras Support Yeast Cell Growth. Heterocomplementation experiments using the human counterparts of the yeast common subunits demonstrated that all, except RPB5, support normal yeast cell growth (33–35). Because we have demonstrated that yeast RPB5 contributed to transcriptional activation, the inability of the human ortholog to function in yeast was possibly the result of a species-specific interaction between RPB5 and activators. To test this possibility, we created six human-yeast chimeras and found two combinations that could support normal yeast cell growth (Fig. 5). Curiously, substitution of the putative yeast activator-

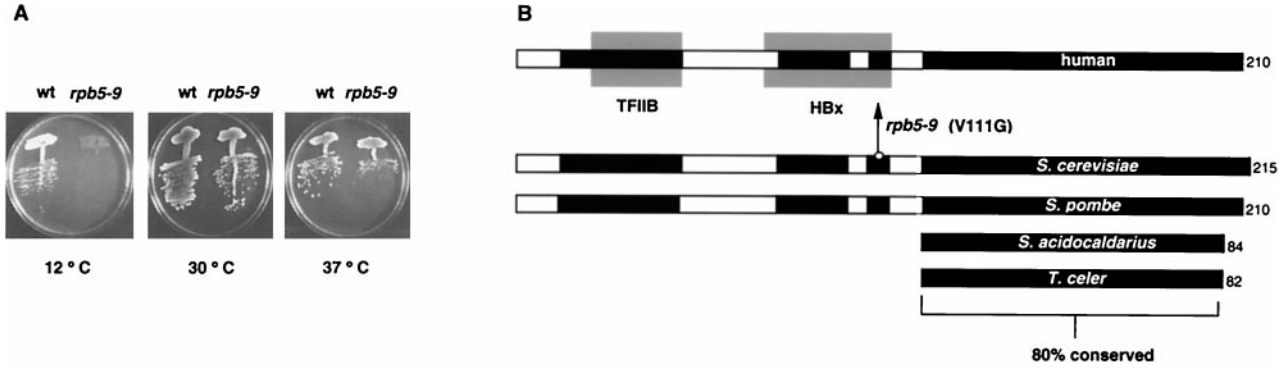

FIG. 1. *rpb5–9* maps to a region implicated in transcriptional activation. (*A*) YPD plates were inoculated with isogenic wt and mutant (*rpb5–9*) cells and incubated at the temperatures indicated. (*B*) Gray shaded boxes represent TFIIB- and HBx-binding regions delineated within human RPB5. Black shaded regions represent areas of high sequence conservation. The number of amino acids comprising each protein is shown to the right.

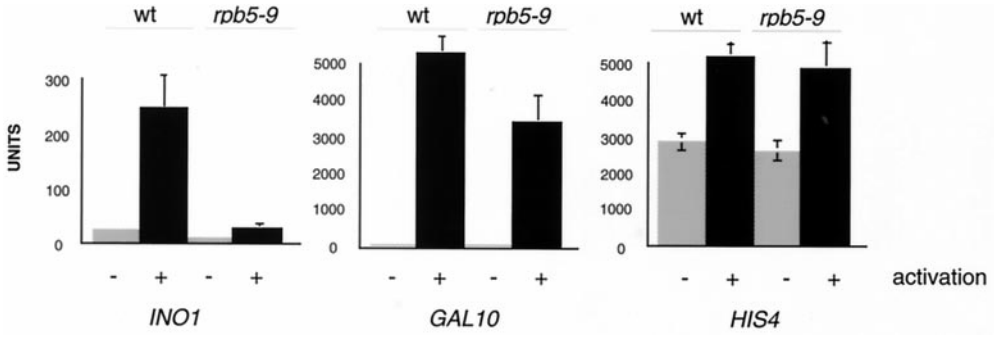

FIG. 3. Defective utilization of some UAS elements in *rpb5–9.* Assays were performed on extracts prepared from cells harvested after growth under activating (+) or nonactivating (−) conditions. Units represented as nmol *O*-nitrophenyl-β-D-galactosidase cleaved per min per mg of protein.

binding region alone was not sufficient for heterocomplementation. Functional chimeras required nearly the entire amino terminal two-thirds of the yeast subunit sequence, comprising regions corresponding to both the putative TFIIB- and activator-binding regions.

We have several conditional mutants (cold-sensitive and/or temperature-sensitive) spanning yeast RPB5 residues 140–215 that have only one substitution mutation resulting in a single amino acid change. One mutant, *rpb5–17*, is in residue 147, only eight amino acids after the last yeast residue in the two functional yeast-human chimeras. Because alteration of a single amino acid in this region causes distinct growth defects not seen in the chimeras that did complement, it is clear that complementation was not simply the result of the ability of a truncated yeast RPB5 to function normally in yeast.

Although we were unable to measure the levels of chimeric protein made in the cell for each construction, the overwhelming success with subunit complementation experiments also suggests that human genes are efficiently translated in yeast and the proteins made are stable. For 10 of the 12 subunits tested to date, all except RPB5, could be complemented with their full-length human counterpart. In fact, comparison of a codon usage map for the 210-aa human RPB5 subunit compared with the fully complementing ≈2,000-aa human RPB1 subunit revealed that all codons in human RPB5 are represented at least once in human RPB1.

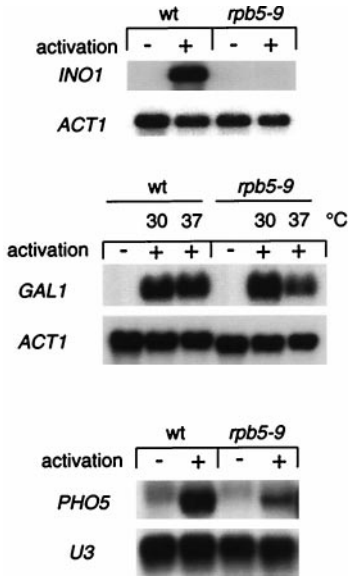

FIG. 4. Deficient activation of *INO1*, *GAL1,* and *PHO5* transcripts in *rpb5–9*. RNA was prepared from cells grown at 30°C under nonactivating (−) or activating (+) conditions. RNA also was prepared from cells grown at 37°C for *GAL1* activation. *ACT1* (actin mRNA) and *U3* (RNA polymerase II transcribed snRNA) were included as loading controls.

RPB5 and the CTD Have Overlapping Functions. Interestingly, the results obtained for *rpb5–9* from both *in vivo* methods closely parallel those seen with the only other mutation in RNA polymerase II causing activation deficiencies, a truncation mutation in the CTD designated *rpb1Δ104*. These similarities in mutant phenotypes suggested that RPB5 and the CTD may function jointly. We used two approaches to test this possibility, one genetic and one biochemical.

In the genetic approach, we created a yeast cell with both the *rpb5–9* and *rpb1Δ104* mutations and tested whether the cells are viable. The double mutants were synthetically lethal at 37°C (Fig. 6 *A* and *B*). In its most conservative interpretation, synthetic lethality (a.k.a. synthetic enhancement) between two conditional alleles (that are not null alleles) indicates that the two genes perform similar functions. Therefore, our results suggest that RPB5 and the CTD have overlapping roles in activation.

To strengthen the genetic conclusion, we specifically tested for additive effects in activation by analyzing activation of *GAL1*, a gene that was only partially affected in either CTD or RPB5 mutant. By using Northern analysis, we compared the level of message upon activation of the double mutant relative to wt and either single mutant at the permissive (30°C) or nonpermissive (37°C) temperature (Fig. 6 *C* and *D*). These results revealed a more marked activation defect in the double mutant at both temperatures; however, the most dramatic effect is noted when RNA was prepared from cells grown at the nonpermissive temperature. These experiments represent another form of synthetic enhancement, consistent with the synthetic lethal phenotype of the double mutant. Taken together, they both indicate that the CTD and RPB5 play similar roles in activation.

DISCUSSION

We isolated a mutant in the common subunit RPB5 with an amino acid change in a region implicated in activation based on interaction studies of its human counterpart. We demonstrated that this mutant does not respond normally to an activator *in vitro* or to some activators *in vivo*. Because this phenotype mirrored that seen with a CTD truncation mutant, we demonstrated that RPB5 and the CTD have overlapping functions in activation by studying the phenotypes of a RPB5/CTD double mutant.

This conclusion is supported by subunit interaction studies documenting a strong interaction between RPB5 and RPB1 *in vitro* (36, 37). RPB5 also is known to bind three proteins with roles in activation, TFIIB, HBx, and $TAF_{II}68$ (30, 31, 38). The role of RPB1 and RPB5 in activation is also consistent with studies demonstrating that they both photocrosslink to DNA (39). Finally, the region of human RPB5

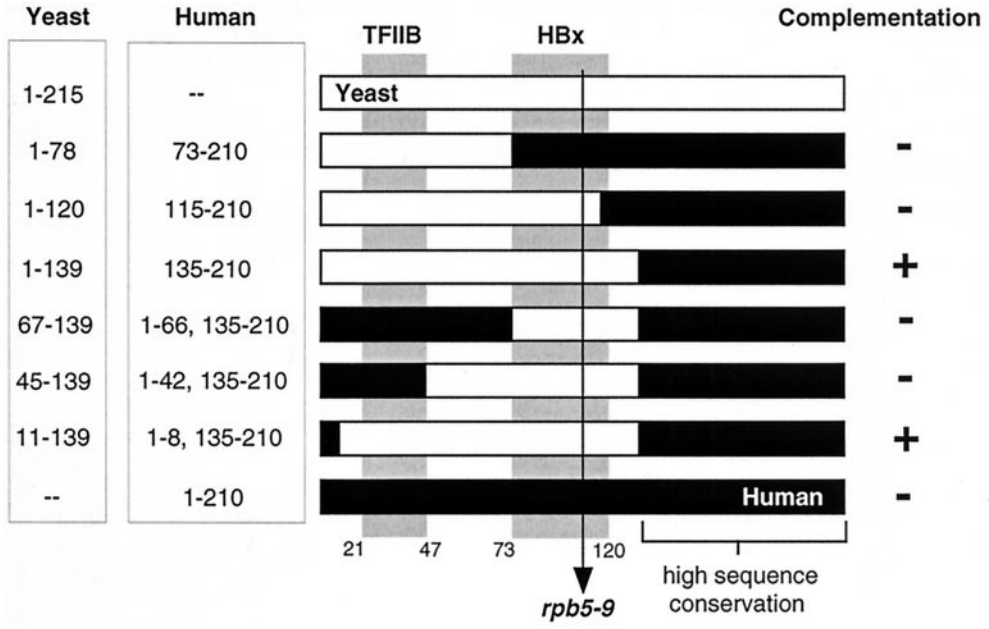

FIG. 5. Yeast-human RPB5 chimeras function in place of their yeast counterpart. Open bars represent *S. cerevisiae* sequences, black bars represent human sequences (sizes are approximate). Specific amino acid boundaries are indicated on the left. The gray shaded regions and corresponding numbers delineate the TFIIB and HBx binding regions in human RPB5 relative to the *rpb5–9* point mutant.

that binds HBx and TFIIB (and the analogous regions in yeast) is predicted to be mostly surface exposed according to secondary structure estimates (40). The two latter observations are consistent with RPB5 being positioned in the complex such that it is exposed and accessible for interaction with other proteins.

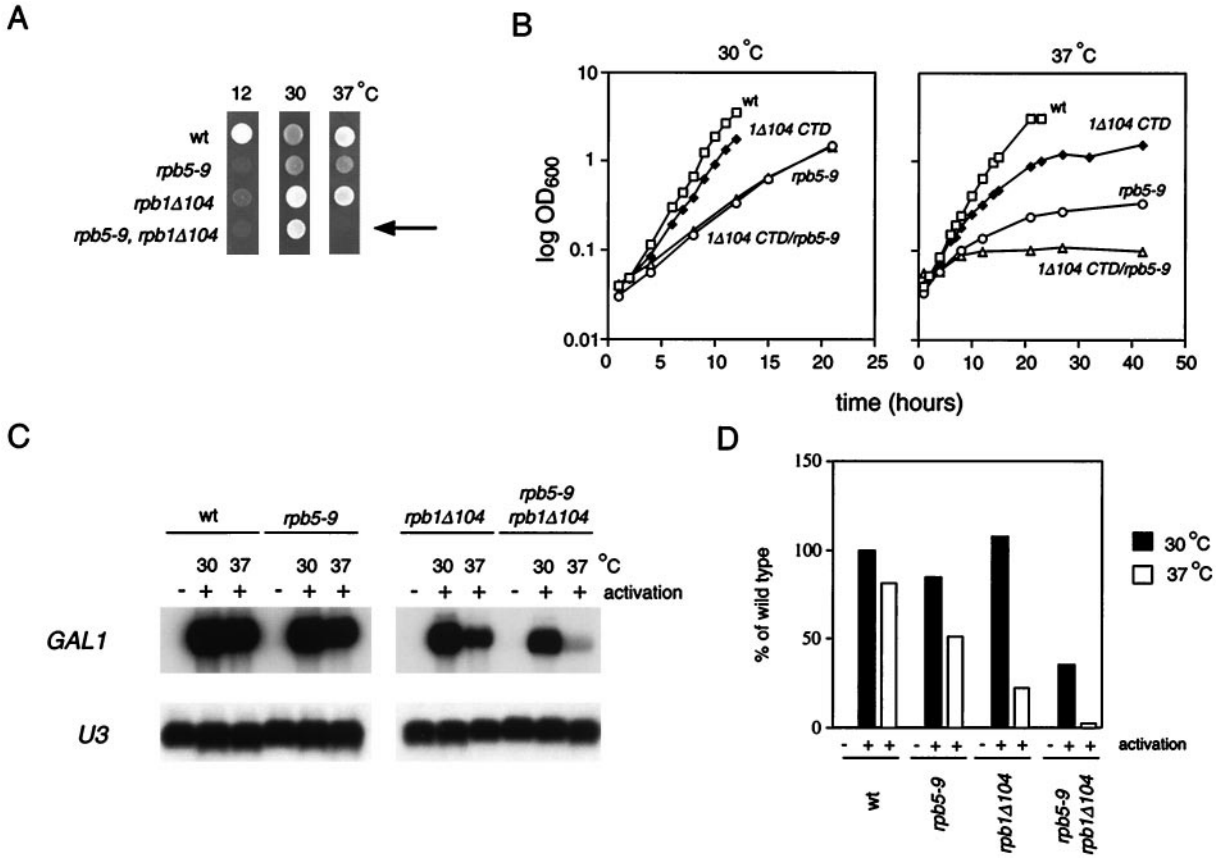

FIG. 6. RPB5 and the CTD have overlapping functions. (*A*) Equal amounts of yeast cells were spotted onto YPD plates and tested for growth at the temperatures indicated. The RPB5/CTD double mutant failed to grow at 37°C (indicated by the arrow). (*B*) Growth curves of isogenic wt and mutant strains grown at the permissive (30°C) and nonpermissive temperatures (37°C). (*C*) RNA prepared from cells grown at, or shifted to, the indicated temperatures. *U3*, snRNA transcript of RNA polymerase II used as loading control. (*D*) Quantification and normalization of the results in *C*.

Details associated with the mechanism of activation remain elusive because of the complexity of proteins exerting influence at any given promoter (41). Sorting out how the cacophony of signals from mediators, activators, and general transcription factors at promoters are orchestrated to consistently reach their target and engage the desired effect on the RNA polymerase II holoenzyme is a daunting undertaking. Toward this broad goal, individual components of the transcriptional machinery that do influence activation have been identified. The list now includes two RNA polymerase subunits (RPB1 and RPB5), TFIIB (42, 43), TATA-binding protein (44–48), and SRB/mediator proteins (49–51). While select mutations in these components diminish or abolish activation, it is unclear how they function coordinately in activation.

Several potential mechanistic clues have been unearthed by these studies. First, the fact that a complementing RPB5 chimera appears to display species specificity, not only in the putative activator-binding region but also in the TFIIB-binding region, suggests a functional interaction between the RPB5, the CTD, and TFIIB. This specificity seems logical because the spectrum of activators is markedly different between the two organisms and is consistent with work by others demonstrating species specificity for TFIIB as well (42). However, the species-specific region of TFIIB is near the middle of the protein and does not correspond to that required for interaction with RPB5 (the amino terminal 60 amino acids).

Second, the observation that both CTD and RPB5 mutants have variable effects on activation may help pinpoint what types of activators target these subunits. Also, because activation refers to any effect resulting in an overall increase in transcript yield, it is important to clarify that the activation assays we implemented do not distinguish between stimulation of initiation versus elongation. It has been documented that some activators stimulate only initiation whereas others stimulate both initiation and elongation (4). These differential outcomes from activators may result from specific, independent direct, or indirect contacts with distinct target sites.

Finally, RPB5 is a common subunit. Consequently, it is of interest to see how this mutant affects activation of RNA polymerase I and RNA polymerase III. At this time, however, it is difficult to envision why a subunit shared by all three RNA polymerases is a target for the same types of activators as another subunit unique to RNA polymerase II.

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1. Young, R. A. (1991) *Annu. Rev. Biochem.* **60,** 689–715.
2. Sentenac, A., Riva, M., Thuriaux, P., Buhler, J.-M., Treich, I., Carles, C., Werner, M., Ruet, A., Huet, J., Mann, C., *et al*. (1992) in *Transcriptional Regulation*, eds. McKnight, S. L. & Yamamoto, K. R. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 1, pp. 27–54.
3. Carlson, M. (1997) *Annu. Rev. Cell. Dev. Biol.* **13,** 1–23.
4. Greenblatt, J. (1997) *Curr. Opin. Cell. Biol.* **9,** 310–319.
5. Koleske, A. J. & Young, R. A. (1995) *Trends Biochem. Sci.* **20,** 113–116.
6. Hochschild, A. & Dove, S. L. (1998) *Cell* **92,** 597–600.
7. Dove, S. L., Joung, J. K. & Hochschild, A. (1997) *Nature (London)* **386,** 627–630.
8. Ebright, R. H. & Busby, S. (1995) *Curr. Opin. Genet. Dev.* **5,** 197–203.
9. Ishihama, A. (1992) *Mol. Microbiol.* **6,** 3283–3288.
10. Lee, J. H. & Hoover, T. R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 9702–9706.
11. Niu, W., Kim, Y., Tau, G., Heyduk, T. & Ebright, R. H. (1996) *Cell* **87,** 1123–1134.
12. Miller, A., Wood, D., Ebright, R. H. & Rothman-Denes, L. B. (1997) *Science* **275,** 1655–1657.
13. Savery, N. J., Lloyd, G. S., Kainz, M., Gaal, T., Ross, W., Ebright, R. H., Gourse, R. L. & Busby, S. J. (1998) *EMBO J.* **17,** 3439–3447.
14. Liao, S. M., Taylor, I. C., Kingston, R. E. & Young, R. A. (1991) *Genes Dev.* **5,** 2431–2440.
15. Scafe, C., Chao, D., Lopes, J., Hirsch, J. P., Henry, S. & Young, R. A. (1990) *Nature (London)* **347,** 491–494.
16. Chao, D. M. & Young, R. A. (1991) *Gene Expression* **1,** 1–4.
17. Corden, J. L. (1990) *Trends Biochem. Sci.* **15,** 383–387.
18. Bjorklund, S. & Kim, Y. J. (1996) *Trends Biochem. Sci.* **21,** 335–337.
19. Treco, D. A. & Lundblad, V. (1993) in *Current Protocols in Molecular Biology*, ed. Janssen, K. (Wiley, New York), Vol. 2, pp. 13.1.1–13.1.7.
20. Muhlrad, D., Hunter, R. & Parker, R. (1992) *Yeast* **8,** 79–82.
21. Boeke, J. D., Trueheart, J., Natsoulis, G. & Fink, G. R. (1987) *Methods Enzymol.* **154,** 164–175.
22. Alani, E., Cao, L. & Kleckner, N. (1987) *Genetics* **116,** 541–545.
23. Wootner, M., Wade, P. A., Bonner, J. & Jaehning, J. A. (1991) *Mol. Cell. Biol.* **11,** 4555–4560.
24. Lue, N. F., Flanagan, P. M., Sugimoto, K. & Kornberg, R. D. (1989) *Science* **246,** 661–664.
25. Guarente, L., Yocum, R. R. & Gifford, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7410–7414.
26. Hinnebusch, A. G., Lucchini, G. & Fink, G. R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 498–502.
27. Kaiser, C., Michaelis, S. & Mitchell, A. (1994) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
28. Rubin, G. M. (1974) *Eur. J. Biochem.* **41,** 197–202.
29. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. & Pease, L. R. (1989) *Gene* **77,** 51–59.
30. Cheong, J. H., Yi, M., Lin, Y. & Murakami, S. (1995) *EMBO J.* **14,** 143–150.
31. Lin, Y., Nomura, T., Cheong, J., Dorjsuren, D., Iida, K. & Murakami, S. (1997) *J. Biol. Chem.* **272,** 7132–7139.
32. Klenk, H. P., Palm, P., Lottspeich, F. & Zillig, W. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 407–410.
33. McKune, K. & Woychik, N. A. (1994) *Mol. Cell. Biol.* **14,** 4155–4159.
34. McKune, K., Moore, P., Hull, M. W. & Woychik, N. A. (1995) *Mol. Cell. Biol.* **15,** 6895–6900.
35. Shpakovski, G. V., Acker, J., Wintzerith, M., Lacroix, J. F., Thuriaux, P. & Vigneron, M. (1995) *Mol. Cell. Biol.* **15,** 4702–4710.
36. Acker, J., de Graaff, M., Cheynel, I., Khazak, V., Kedinger, C. & Vigneron, M. (1997) *J. Biol. Chem.* **272,** 16815–16821.
37. Miyao, T., Honda, A., Qu, Z. & Ishihama, A. (1998) *Mol. Gen. Genet.* **259,** 123–129.
38. Bertolotti, A., Melot, T., Acker, J., Vigneron, M., Delattre, O. & Tora, L. (1998) *Mol. Cell. Biol.* **18,** 1489–1497.
39. Kim, T. K., Lagrange, T., Wang, Y. H., Griffith, J. D., Reinberg, D. & Ebright, R. H. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 12268–12273.
40. King, R. D. & Sternberg, M. J. (1996) *Protein Sci.* **5,** 2298–2310.
41. Stargell, L. A. & Struhl, K. (1996) *Trends Genet.* **12,** 311–315.
42. Shaw, S. P., Wingfield, J., Dorsey, M. J. & Ma, J. (1996) *Mol. Cell. Biol.* **16,** 3651–3657.
43. Tansey, W. P. & Herr, W. (1997) *Science* **275,** 829–831.
44. Kim, T. K., Hashimoto, S., Kelleher, R. J., 3rd, Flanagan, P. M., Kornberg, R. D., Horikoshi, M. & Roeder, R. G. (1994) *Nature (London)* **369,** 252–255.
45. Lee, M. & Struhl, K. (1995) *Mol. Cell. Biol.* **15,** 5461–5469.
46. Arndt, K. M., Ricupero-Hovasse, S. & Winston, F. (1995) *EMBO J.* **14,** 1490–1497.
47. Stargell, L. A. & Struhl, K. (1995) *Science* **269,** 75–78.
48. Stargell, L. A. & Struhl, K. (1996) *Mol. Cell. Biol.* **16,** 4456–4464.
49. Lee, Y. C., Min, S., Gim, B. S. & Kim, Y. J. (1997) *Mol. Cell. Biol.* **17,** 4622–4632.
50. Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G. & Ptashne, M. (1995) *Cell* **81,** 359–368.
51. Hengartner, C. J., Thompson, C. M., Zhang, J., Chao, D. M., Liao, S. M., Koleske, A. J., Okamura, S. & Young, R. A. (1995) *Genes Dev.* **9,** 897–910.