Rpb4, a Non-essential Subunit of Core RNA Polymerase II of Saccharomyces cerevisiae Is Important for Activated Transcription of a Subset of Genes

Received for publication, December 5, 2000, and in revised form, May 26, 2001
Published, JBC Papers in Press, May 29, 2001, DOI 10.1074/jbc.M010952200

Beena Pillai, Vinaya Sampath, Nimisha Sharma, and Parag Sadhale‡

From the Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India 560 012

A major role in the regulation of eukaryotic protein-coding genes is played by the gene-specific transcriptional regulators, which recruit the RNA polymerase II holoenzyme to the specific promoter. Several components of the mediator complex within the holoenzyme also have been shown to affect activation of different subsets of genes. Only recently has it been suggested that besides the largest subunit of RNA polymerase II, smaller subunits like Rpb3 and Rpb5 may have regulatory roles in expression of specific sets of genes. We report here, the role of Rpb4, a non-essential subunit of core RNA polymerase II, in activation of a subset of genes in Saccharomyces cerevisiae. We have shown below that whereas constitutive transcription is largely unaffected, activation from various promoters tested is severely compromised in the absence of RPB4. This activation defect can be rescued by the overexpression of cognate activators. We have localized the region of Rpb4 involved in activation to the C-terminal 24 amino acids. We have also shown here that transcriptional activation by artificial recruitment of the TATA-binding protein (TBP) to the promoter is also defective in the absence of RPB4. Surprisingly, the overexpression of RPB7 (the interacting partner of Rpb4) does not rescue the activation defect of all the promoters tested, although it rescues the activation defect of the heat shock element-containing promoter and the temperature sensitivity associated with RPB4 deletion. Overall, our results indicate that Rpb4 and Rpb7 play independent roles in transcriptional regulation of genes.

Transcriptional regulation at the initiation step is one of the most important steps in the regulation of gene expression. Inducible (or repressible) genes, whose expression is varied in response to extra- or intracellular stimuli, often have complex promoters with upstream regulatory sites that help them respond to various stimuli (1). In contrast, the constitutively expressed genes have relatively simple promoters with invariant levels of gene expression.

It has been proposed relatively recently that the transcriptional activator responsive form of RNA polymerase II, the holoenzyme, includes the core polymerase II subunits, the general transcription factors, and a complex variously called the

mediator, upstream stimulatory activity, or coactivators (1, 2). The mediator-coactivator complex is an important although not essential component of the holoenzyme (1, 3). In fact, a number of different holoenzymes have been proposed to coexist; each one of which is able to respond only to a subset of activators/ regulators (4). It is increasingly becoming apparent that the subunits of the core polymerase may also play an active role in gene regulation by determining the interactions with either the mediators/coactivators or transcriptional activators (5–8).

The core RNA polymerase II of Saccharomyces cerevisiae is composed of twelve subunits, ten of which are essential for survival and five are shared between the three RNA polymerases (1). Rpb4, one of the non-essential subunits, is unique to eukaryotic RNA polymerase II in that it does not have a counterpart in the other two eukaryotic RNA polymerases (1, 9). It forms a subcomplex with one of the essential subunits, Rpb7, within the core RNA polymerase II. Biochemical analyses have revealed that the lack of the Rpb4 subunit also leads to the lack of the Rpb7 subunit in the immunoprecipitated polymerase (10, 11). The polymerase lacking Rpb4 and Rpb7 is inefficient in carrying out GAL1 promoter-directed transcription in vitro (10). Many groups including our own have shown recently that overexpression of RPB7 allows rescue of some of the phenotypes associated with the deletion of RPB4 (12–14).

Rpb4 has been proposed to have a role in survival under stress conditions, in yeast (15).

We show here that RPB4 affects transcriptional activation of a subset of genes. This effect on activated transcription is much more pronounced than its effect on constitutive transcription. We also show here that this defect in activated transcription can be overcome by overexpression of transcriptional activators but not by increased recruitment of the TATA-binding protein (TBP)† to the promoter. Rpb7, the interacting partner of Rpb4, when overexpressed, partially rescues temperature sensitivity and some heat shock element-driven transcription but not all activated promoters.

EXPERIMENTAL PROCEDURES

Strains and Media—The yeast strains used in this study were SY10-1: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, rpb4Δ::HIS3/pPS2; SY10-2: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, rpb4Δ::HIS3/pNS114; SY10-3: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, rpb4Δ::HIS3/pPS4; SY10-4: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, rpb4Δ::HIS3/pNS118; SY21-1: Mat a, ade2-1, his3Δ200, ura3-52, leu2-3, 112, trp1Δ901, rpb4Δ::HIS3/pPS2; SY21-2: Mat a, ade2-1, his3Δ200, ura3-52, leu2-3, 112, trp1Δ901, rpb4Δ::HIS3/pPS2; SY23-1: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, trp1Δ901, cyh2Δ, rpb4Δ::HIS3/pPS2; SY23-2: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, trp1Δ901, cyh2Δ, rpb4Δ::HIS3/pNS114. All the plasmids were transformed and amplified in Escherichia coli strain DH5α [supE44

† The abbreviations used are: TBP, TATA-binding protein; HSE, heat shock element; kb, kilobase; ORF, open-reading frame.
Accl168, (Δ80 lacZΔM15) hosR17 recA1 gyrA96 thi-1 relA1. The plasmids used in this study are described in Table I. Common media used for routine growth of yeast cultures and the manipulations of DNA were made as described (16). The yeast transformations were carried out by a modified lithium acetate method, which does not involve heat shock of the yeast cells (17).

Construction of Plasmids—The construction of β-galactosidase fusions to pPS232 heat shock element (HSE), pPS31, and LexAop (LexA operator) have been previously described (18–21). The 3.9-kb EcoRI fragment from pPS76 containing the actin-β-galactosidase fusion was cloned downstream of the P<sub>ACT1</sub>-LacZ reporter, URA3, 2μ in pPS31, this study. The yeast transformation was carried out by a modified lithium acetate method, which does not involve heat shock of the yeast cells (17).

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Alias</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pPS2</td>
<td>YCplac11</td>
<td>p&lt;sub&gt;TEF2&lt;/sub&gt;-TEF2 GAL1, TRP1, CEN, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS4</td>
<td>YCplac33</td>
<td>pSCH135 HSE element-LacZ fusion, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS5</td>
<td>YEplac181</td>
<td>pHS11 HSE element-LacZ fusion, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS24</td>
<td>pJS359</td>
<td>P&lt;sub&gt;TEF2&lt;/sub&gt;-LacZ reporter, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS30</td>
<td>pGB9T</td>
<td>GAL4 DBD vector, TRP1, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS31</td>
<td>pGAD424</td>
<td>GAL4 AD vector, LEU2, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS59</td>
<td>pCL-1</td>
<td>GAL4 ORF under P&lt;sub&gt;GAL1&lt;/sub&gt; in Ycp50</td>
<td>This study</td>
</tr>
<tr>
<td>pNS95</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>pNS76</td>
<td>pSB20</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt;-ACT1-LacZ, TRP1, CEN</td>
<td>This study</td>
</tr>
<tr>
<td>pNS79</td>
<td>pCU53</td>
<td>CUP1 ORF under P&lt;sub&gt;GAL1&lt;/sub&gt; in pPS189</td>
<td>This study</td>
</tr>
<tr>
<td>pNS80</td>
<td>pSB53</td>
<td>CUP1 ORF under P&lt;sub&gt;GAL1&lt;/sub&gt; in pPS190</td>
<td>This study</td>
</tr>
<tr>
<td>pNS98</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>pNS99</td>
<td>—</td>
<td>—</td>
<td>This study</td>
</tr>
<tr>
<td>pNS111</td>
<td>pZJHSE137</td>
<td>HSE element-LacZ fusion, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pNS114</td>
<td>—</td>
<td>RPB4 gene in pPS2, LEU2, CEN</td>
<td>This study</td>
</tr>
<tr>
<td>pNS118</td>
<td>—</td>
<td>RPB4 gene in pPS4, URA3, CEN</td>
<td>This study</td>
</tr>
<tr>
<td>pPS121</td>
<td>pGAB</td>
<td>Bidirectional P&lt;sub&gt;GAL1&lt;/sub&gt;-GAL1 pr, ACT1-LacZ under P&lt;sub&gt;GAL1&lt;/sub&gt;, URA3, CEN</td>
<td>This study</td>
</tr>
<tr>
<td>pPS122</td>
<td>pSCH135</td>
<td>P&lt;sub&gt;HIS3&lt;/sub&gt;-LacZ, URA3, 2μ</td>
<td>U. Vijayaraghavan</td>
</tr>
<tr>
<td>pBP130</td>
<td>—</td>
<td>SPT15(TBP) ORF cloned as fusion to the LexA DBD in pPS139</td>
<td>This study</td>
</tr>
<tr>
<td>pNS132</td>
<td>—</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt;-ACT1-LacZ in pPS190</td>
<td>This study</td>
</tr>
<tr>
<td>pPS139</td>
<td>pEC202</td>
<td>LexA-DBD vector, HIS3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS141</td>
<td>pSH18–34</td>
<td>LexAop-LacZ reporter, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pNS166</td>
<td>—</td>
<td>P&lt;sub&gt;TEF2&lt;/sub&gt;-ACT1-LacZ in pPS189</td>
<td>This study</td>
</tr>
<tr>
<td>pNS185</td>
<td>—</td>
<td>P&lt;sub&gt;TEF2&lt;/sub&gt;-RPB7 in pPS55</td>
<td>This study</td>
</tr>
<tr>
<td>pPS189</td>
<td>pABE448</td>
<td>P&lt;sub&gt;TEF2&lt;/sub&gt;-RPB7 in pPS826 URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pPS190</td>
<td>pABE446</td>
<td>P&lt;sub&gt;TEF2&lt;/sub&gt;-RPB7 in pPS826 URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pBP192</td>
<td>—</td>
<td>RPB4 gene in pBP211</td>
<td>This study</td>
</tr>
<tr>
<td>pBP211</td>
<td>—</td>
<td>P&lt;sub&gt;ACT1&lt;/sub&gt;-LacZ reporter, URA3, 2μ</td>
<td>This study</td>
</tr>
<tr>
<td>pBP212</td>
<td>—</td>
<td>Rpb4–2 allele, Rpb4 without the C-terminal 24 amino acids</td>
<td>This study</td>
</tr>
<tr>
<td>pBP215</td>
<td>—</td>
<td>P&lt;sub&gt;ADH1&lt;/sub&gt;-LexA-TBP-T&lt;sub&gt;ADH1&lt;/sub&gt; in pPS30</td>
<td>This study</td>
</tr>
<tr>
<td>pPS231</td>
<td>p146 GAL1-LacZ</td>
<td>P&lt;sub&gt;GAL1&lt;/sub&gt;-LacZ, URA3, CEN</td>
<td>PJ Bhattacharyya</td>
</tr>
<tr>
<td>pPS232</td>
<td>p4-TEF2 GAL1</td>
<td>P&lt;sub&gt;TEF2&lt;/sub&gt;-GAL1 ORF, TRP1, CEN</td>
<td>This study</td>
</tr>
<tr>
<td>pNS245</td>
<td>—</td>
<td>A region of 18S rRNA gene in pPS55</td>
<td>This study</td>
</tr>
</tbody>
</table>

β-Galactosidase Assays—the promoter activities of different constitutive and activator promoters in p RBP4 and RPB4 strains were analyzed by assaying the appropriate strains under the following growth conditions. GAL1 and GAL10: strains were grown in SC medium + 2% glucose + 2% agar. Cells were restreaked at equal densities onto plates containing SC medium + 2% glucose + 2% agar. The plates were incubated at 25, 34, or 37°C for 3 days before they were photographed.

β-Galactosidase Assays—the promoter activities of different constitutive and activator promoters in p RBP4 and RPB4 strains were analyzed after growing the appropriate strains under the following growth conditions. GAL1 and GAL10: strains were grown in SC medium + 2% glucose until mid-log phase and then subcultured into SC medium + 2% galactose for 3–5 h (inducing condition). INO1: strains were grown in SC medium + 2% glucose until mid-log phase in culture before they were assayed (derepressing condition). PHO5: strains were grown in SC medium + 2% glucose until mid-log phase and subcultured into SC medium without phosphate (derepressing condition) or with 0.5 mM ammonium phosphate. HSE: strains were grown in SC medium + 2% glucose until mid-log phase and one-half volume of culture was given a heat shock at 37°C for 1 h (inducing condition). CUP1: strains were grown in SC medium + 2% glucose until mid-log phase and subcultured into SC medium with (inducing condition) or without 100 μM CuSO<sub>4</sub>.

The assay for the activity of constitutive promoters tested, P<sub>ADH1</sub>, P<sub>GAL1</sub>, and P<sub>TEF2</sub>, was performed by growing the strains in SC medium + 2% glucose until mid-log phase. Cells from both p RBP4 and RPB4 strains were pelleted and β-galactosidase assays were performed by using the glass bead method as described previously (22).
FIG. 1. The presence of RPB4 affects activated transcription from several activated promoters very severely but constitutive promoters are relatively unaffected. All the promoters except $P_{ADH1}$ and $P_{CUP1}$-$\beta$-galactosidase fusions were tested in the strains SY10-1 (rpb4Δ) and SY10-2 (RPB4) transformed with the appropriate plasmids. The $P_{ADH1}$ activity was tested in the strains SY10-3 (rpb4Δ) and SY10-4 (RPB4) and $P_{CUP1}$ activity was tested in the syngenic strain SY21-2 because of lack of appropriate selection markers in the strain SY10. The rpb4Δ and RPB4 strains with the appropriate promoter-reporter fusions are represented as rpb4Δ and RPB4. The $\beta$-galactosidase activities reflecting the promoter strengths are expressed as $\beta$-gal units in bar graphs. The results represented are averages of three independent experiments performed with three transformants of each strain. Standard deviations are indicated by error bars. The inducing and non-inducing conditions and probes used are described under "Experimental Procedures." a, the constitutive promoters of $ADH1$, $TEF2$, and $GPD1$ genes were tested from the respective promoter-$\beta$-galactosidase reporter plasmids, pNS99, pNS166, and pNS132 in rpb4Δ and RPB4 strains. $b$, the five activated promoters from the genes $GAL1$, $GAL10$, $INO1$, $PHO5$, and the promoter-containing Heat shock element (HSE) were tested in rpb4Δ and RPB4 strains under non-inducing (−) and inducing (+) conditions. The ratio of the $\beta$-galactosidase activity in RPB4 to that in rpb4Δ strains under non-inducing (U) and inducing conditions (I) is represented on top of each bar graph. For $INO1$ promoter fusions, no detectable level of $\beta$-galactosidase activity was found in non-inducing conditions. Hence, only the inducing condition results are represented. $c$, the activated promoter of the $CUP1$ gene was tested in rpb4Δ and RPB4 strains under non-inducing (−) and inducing (+) conditions. $d$, the endogenous transcript level of the $GAL1$ and $CUP1$ genes were determined in rpb4Δ and RPB4 strains under non-inducing (−) and inducing (+) conditions using Northern analysis. The 18 S rRNA levels were used as loading controls.

RESULTS

Activated Transcription Is Defective in a Strain Deleted for RPB4—Activation of transcription from yeast promoters involves complex interactions between the upstream activating sequence (UAS)-bound transcriptional activator, the mediator/coactivator complexes, and the core RNA polymerase II, which leads to increased recruitment of the transcription-competent complex to the nearby promoter (1). Recent reports have suggested that the smaller subunits of core RNA polymerase II, Rpb3 and Rpb5, may act as contact points for transcriptional activators (5, 6). We have analyzed the role of another small subunit of RNA polymerase II, Rpb4, in activated transcription. Earlier reports suggest a role for Rpb4 in transcription initiation based on the inability of the RNA polymerase II purified from rpb4Δ cells to initiate transcription from a UAS $GAL1$-CYC1 promoter in vitro (10). We have used several well studied yeast promoters fused to the $\beta$-galactosidase reporter gene and tested their activities in the absence and presence of RPB4. The $\beta$-galactosidase activities were measured from cells grown in synthetic medium for the three constitutive promoters. For the six regulated promoters, the $\beta$-galactosidase activities were measured from cells grown under inducing and non-inducing conditions. We observed that the activities of the promoters of the constitutive genes $ADH1$, $TEF2$, and $GPD1$ (from plasmids pNS99, pNS166, and pNS132) in rpb4Δ and RPB4 strains were reduced by 1.3-3-fold in rpb4Δ as compared with wild type (Fig. 1a). This suggests that the constitutive promoters tested are not significantly affected by the absence of RPB4.

Of the six regulated promoters tested, five promoters of the genes $GAL1$, $GAL10$, $INO1$, and $PHO5$, and a promoter containing HSE (from plasmids pPS231, pPS121, pPS24, pPS122, and pPS111) showed highly inefficient regulated expression in the absence of RPB4 (Fig. 1b). The ratio of the $\beta$-galactosidase activities in the wild-type strain to that in rpb4Δ has been calculated under inducing (I) and non-inducing (U) conditions and is represented on the top of each bar graph. It is obvious that the lack of RPB4 results in a drastic reduction in promoter activity under inducing conditions (from 14–320-fold depending on the promoter) but the activity under non-inducing conditions is affected to a much lesser extent in each case. This indicates that the reduced induction level is not just a reflection of reduced basal/uninduced levels of the promoter activity. On the other hand, the sixth inducible promoter tested, $P_{CUP1}$ (from the plasmid, pNS98) showed a reduction in activity of

diolabeled probes. [32P]dATP (PerkinElmer Life Science Products, BLU512H)-labeled DNA probes were generated using a random-primed DNA labeling kit (Amersham Pharmacia Biotech, RPN1604). The protocol suggested by the manufacturer was strictly followed. Routine hybridization and posthybridization procedures were followed. The blots were subjected to phosphor imaging analysis using a Fuji Phosphor Imager.
only about 3-fold in a rpb4Δ strain as compared with a wild-type strain even under inducing conditions (Fig. 1c).

Using another approach to study the transcriptional activity from the induced promoters, we tested the endogenous expression levels of some of the genes in rpb4Δ and RPB4 strains. The steady-state mRNA levels of CUP1 are similar in rpb4Δ and RPB4 strains even under inducing conditions (Fig. 1d). The RPB4 strain shows a drastic increase in the mRNA levels of the GAL1 transcript under inducing conditions as compared with the mRNA levels in non-inducing conditions. However, the GAL1 transcript is hardly visible in the rpb4Δ strain even under inducing conditions. This pattern of defective expression in rpb4Δ strain as compared with the RPB4 strain was also seen for the PHO5 and INO1-activated promoters (data not shown).

In summary, the results from the β-galactosidase assays and the Northern analysis suggest that Rpb4 is involved in activated transcription. In the absence of Rpb4, transcription from constitutive promoters is less efficient, but the activated transcription from several promoters is severely compromised. These results also suggest that regulation of CUP1 transcription does not involve Rpb4 and is mechanistically different from other activated promoters (see “Discussion”).

Overexpression of Rpb7 Specifically Rescues Defects in Heat Shock Promoter Activity in the Absence of Rpb4.—Previously we have shown that some of the stress phenotypes associated with the absence of RPB4 are partially rescued by heat shock element-containing promoter activities. The inducing and non-inducing conditions tested are represented by PTEF2 under inducing conditions and PTEF2 under non-inducing conditions, respectively. The inducing and non-inducing conditions used are described under “Experimental Procedures.”

The observation that activated transcription is inefficient in the absence of Rpb4, and overexpression of activators partially rescues the activation defect of rpb4Δ (Fig. 2a). On the other hand, as shown in Fig. 2b, β-galactosidase activities from the P GAL10 and P INO1 promoter-reporter fusions in rpb4Δ strain are very similar to their activities in the rpb4Δ strain. This suggests that overexpression of RPB7 in rpb4Δ strain can rescue the defect in activated transcription from the HSE-containing promoter but not from the GAL10 and INO1 promoters.

Defects in Activated Transcription Are Partially Compensated by Overexpression of the Cognate Transcriptional Activators.—It was earlier observed that the purified polymerase lacking both subunits, Rpb4 and Rpb7, was defective in promoter-specific transcription initiation in vitro, which could be rescued partially by the addition of a potent chimeric transcriptional activator (10). Because we found that in vivo (unlike the in vitro result) activated transcription is significantly affected in the absence of RPB4, we decided to test if overexpressed cognate activators will have a rescuing effect on the specific-activated promoters. We overexpressed two specific transcriptional activators, GAL4 and INO2, from the ADH1 promoter (30) and tested the effect on the activity of the GAL10 and INO1 promoters, respectively, in the absence of RPB4. Both the activators when expressed at higher than normal levels partially rescued the activation defect of rpb4Δ (Fig. 3). In the case of GAL4 overexpression, the uninduced levels in rpb4Δ are also significantly higher (see “Discussion,” Ref. 31). Under inducing conditions, the GAL10 promoter activity is even higher than under non-inducing conditions, indicating that the rescue of activated transcription in rpb4Δ is specific to overexpression of the activator. To test whether the effect of overexpression of transcriptional activator is specific to the cognate regulated promoter and not a nonspecific general effect on transcription, we also tested the promoter activities in the presence of an excess of the non-cognate transcriptional activators. As shown in Fig. 3, the activated promoters are affected by overexpression of only the cognate activator. This confirmed that the effect of the activators is specific and not an effect on transcription in general.

The observation that activated transcription is inefficient in the absence of Rpb4, and overexpression of activators partially rescues the activation defect of rpb4Δ strains could be explained by direct or indirect contact between activators and Rpb4. Previously, the mammalian transcriptional activator, EWS-Fli1, has been shown to interact directly with the human homolog of Rpb7 (8). We used the directed two-hybrid assay to
test whether there is a physical interaction between either Rpb4 or Rpb7 and the GAL4 and INO2 activators. Neither of the activators interacted with either of the subunits (data not shown).

Removal of a C-terminal Region of RPB4 Leads to Defective Activated Transcription—The comparison of the Rpb4 protein sequence (using the PRODOM data base) across species shows that the protein has a conserved C-terminal region whereas the N-terminal sequence appears unique to the S. cerevisiae protein (32). The 150 amino acid long domain, 16118 (as numbered in the PRODOM data base) is shared by all the homologs in this alignment and has several invariant residues (Fig. 4a) (32). Using a convenient HindIII restriction site we decided to alter the invariant sequence PSL to PS (STOP). This manipulation deletes the remaining 24 amino acids from the C terminus of Rpb4, estimated to be highly conserved (identical in four or more of the six homologs compared). We tested if this change in the sequence affects the role of RPB4. We observed that the mutant rpb4–2 is defective in rescue of temperature sensitivity of the rpb4Δ strain even at 34 °C (Fig. 4b). The deletion mutant was as defective as the rpb4Δ strain (data not shown).

DISCUSSION

Over the last decade, there has been a tremendous increase in our understanding about mechanisms of activation of transcription in eukaryotes, with major contributions coming from the yeast system. It has been reported that the transcription machinery exists in the form of a holoenzyme comprising RNA polymerase II, the general transcription factors, and the mediator/coactivator complex. An important concept that has emerged is that the holoenzyme is a flexible complex that can respond to activators (1, 2). The composition of the holoenzyme differs substantially depending on the purification method employed and today it is accepted that these differences may actually reflect in vitro differences (4). In most cases, the differences have been found to be in the ancillary factors and components of the mediator/coactivator complexes.

Fig. 4. The C-terminal conserved region of Rpb4 is essential for its function. a, the alignment of all known homologs of Rpb4 using the program PRODOM Version 2000.1 identified a conserved C-terminal domain, 16118. The invariant residues in the 150 amino acid long domain are indicated in white with black background and those identical in at least four sequences are in black with gray background. The residues deleted from the S. cerevisiae RPB4 allele, rpb4–2, are underlined. b, the rpb4–2 allele does not rescue the temperature-sensitive phenotype of rpb4Δ. rpb4Δ strain (SY10) transformed with either Vector, RPB4, or rpb4–2 were assayed for temperature sensitivity. rpb4Δ strain with vector, or rpb4–2 allele, or RPB4 are represented as rpb4Δ, rpb4–2, and RPB4, respectively. c, the C-terminal 24 amino acids are essential for the activation function of Rpb4. The strains as described in b were transformed with the P_{GAL10}–LacZ plasmid and were assayed for the activity of the P_{GAL10} under non-inducing (−) and inducing (+) conditions. The results presented are averages of three independent experiments with three transformants, and the standard deviations are represented by error bars.
Until recently, the core RNA polymerase II was considered an integral part of all holoenzymes. Since then it has been reported that subunits Rpb3 and Rpb5 of the yeast core polymerase actually affect the activation of subsets of genes showing that core subunits of the RNA polymerase II can have regulatory roles. Core RNA polymerase II subunits may determine the composition of the holoenzyme, probably through the interactions with other components, in turn affecting the activation of a subset of genes.

As reported here, Rpb4 falls into the category of those core subunits, which affect activation of a subset of genes. Choder et al. (15) have shown that rpb4Δ is defective in stress response based on its inability to survive under extreme temperatures and in stationary phase. Brendel and Karlin (37) have proposed based on its inability to survive under extreme temperatures somehow allows RNA polymerase II to transcribe at extreme temperatures in vitro. While this manuscript was in preparation, Tan et al. (39) reported that Rpb4 is required for basal transcription in in vitro assays whereas in vivo studies show that basal and activated transcription are both defective. Our in vivo results show that the constitutive promoters are not significantly affected in the absence of RPB4 (Fig. 1a). We have clearly shown using both promoter β-galactosidase fusions and Northern analyses of steady-state levels of endogenous transcripts that the absence of RPB4 affects many if not all of the activated transcription units tested (Fig. 1b). The only activated promoter tested that is not defective in the absence of RPB4 is P\text{CUP1} (Fig. 1, c and d). This promoter is also known to be one of the few yeast promoters that does not require the holoenzyme for activation suggesting that the CUP1 gene is activated by alternative mechanisms (40, 41). Therefore, it is not entirely surprising that Rpb4 is dispensable for activation of CUP1. The activation defect is also shown by a nonsense mutant lacking a small, highly conserved 24-amino acid stretch at the C terminus of the protein.

Several investigators have reported that Rpb4 and Rpb7 subunits form a subcomplex. The close proximity of these two proteins within the core polymerase has been demonstrated using electron microscopic analysis of crystals of RNA polymerase II (42). Our own studies earlier showed that overexpression of its interacting partner, RPB7, compensates for the lack of RPB4 in the temperature sensitivity assay suggesting that one of the functions of Rpb4 is to stabilize the interaction of Rpb7 with the rest of the polymerase (12). Rpb7 has also been identified by Tan et al. (39) as a multicopy suppressor of temperature sensitivity of rpb4Δ. It also partially restored the steady-state levels of transcripts from various non-heat shock genes whose expression was affected in rpb4Δ. However, in our studies using promoter-reporter fusions, we do not find a similar rescue. This difference implies that Rpb7 overexpression increases the steady-state levels of transcripts, probably through a post-transcriptional effect. Sro9p another suppressor reported by Tan et al. (39) has also been shown to rescue rpb4Δ by increasing mRNA stability. That the overexpression of RPB7 rescues the HSE activity is in accordance with the above result, and similar observations reported by others (Fig. 2) (12–14). This observation suggests that the two subunits may have different roles in expression of stress and non-stress-related genes.

To investigate further the role of RPB4 in activation of transcription, we decided to test if the defect in activation is because of defective interaction, either direct or indirect, with some of the activators. In either case, then excess of transcriptional activator may be able to rescue the defect. It was indeed seen that overexpression of the cognate activators partially rescued activation from the corresponding promoters. Overexpression of Gal4 results in relatively high levels of transcription under non-induced conditions. This is most likely because of the fact that the constitutively expressed Gal4 is present in excess over the negative regulator Gal80 thereby releasing active Gal4 even under non-inducing conditions (31). The fact that under inducing conditions the level of transcription is significantly higher than under non-inducing conditions suggests that the overexpression is indeed rescuing activated transcription even in the absence of Rpb4. The human homolog of Rpb7 has been shown to interact directly with transcriptional activators (8). We have seen that activators do not interact directly with Rpb4 even though their overexpression rescues the transcriptional activation defect of rpb4Δ. The effect of Rpb4 on activation is probably mediated through other proteins.

Previous reports show that transcriptional activation in the absence of true activators is possible if one tethers TBP to a strong DNA binding domain and recruits it to the promoter (33, 34). In vivo, TBP occupancy at promoters increases on activation of the gene (35, 36). These reports have suggested that at least one of the ways in which activators function is by increasing the recruitment of the holoenzyme to the promoter. Because we find that the tethered TBP was ineffective in activation in the absence of RPB4, we conclude that the holoenzyme recruitment through tethering of TBP is not sufficient for activation in the absence of this subunit. In summary, our results show that RPB4 plays an important role in activation of a subset of genes in the model eukaryote, S. cerevisiae. This function is not shared directly by its interacting partner, Rpb7.

Acknowledgments—We thank Drs. E. Golemis, U. Vijayaraghavan, L. Nover, J. Bhat, S. Chavez, and T. Platt for gifts of various plasmids used in this study. We thank Drs. V. Sarangdhar, C-M. Chiang, and the members of our laboratory for encouragement and useful discussions.

REFERENCES

Acad. Sci. U. S. A. 97, 3148–3153


Rpb4, a Non-essential Subunit of Core RNA Polymerase II of Saccharomyces cerevisiae Is Important for Activated Transcription of a Subset of Genes
Beena Pillai, Vinaya Sampath, Nimisha Sharma and Parag Sadhale

doi: 10.1074/jbc.M010952200 originally published online May 29, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010952200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 21 of which can be accessed free at
http://www.jbc.org/content/276/33/30641.full.html#ref-list-1