THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 51, Issue of December 19, pp. 51566–51576, 2003
Printed in U.S.A.

The Conserved and Non-conserved Regions of Rpb4 Are Involved in Multiple Phenotypes in *Saccharomyces cerevisiae**

Received for publication, June 4, 2003, and in revised form, August 11, 2003
Published, JBC Papers in Press, October 6, 2003, DOI 10.1074/jbc.M305863200

Vinaya Sampath‡, Nambudiry Rekha§¶, N. Srinivasan§‖, and Parag Sadhale‡**

From the ‡Department of Microbiology & Cell Biology and §Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India

Rpb4, the fourth largest subunit of RNA polymerase II in *Saccharomyces cerevisiae*, is required for many phenotypes, including growth at high and low temperatures, sporulation, pseudohyphal growth, activated transcription of a subset of genes, and efficient carbon and energy metabolism. We have used deletion analysis to delineate the domains of the protein involved in these multiple phenotypes. The scRpb4 protein is conserved at the N and C termini but possesses certain non-conserved regions in the central portion. Our deletion analysis and molecular modeling results show that the N- and C-terminal conserved regions of Rpb4 are involved in interaction with Rpb7, the Rpb4 interacting partner in the RNA polymerase II. We further show that the conserved N terminus is required for efficient activated transcription from the *INO1* promoter but not the *GAL10*- or the *HSE*-containing promoters. The N terminus is not required for any of the stress responses tested: growth at high temperatures, sporulation, and pseudohyphal growth. The conserved C-terminal 23 amino acids are not required for the role of Rpb4 in the pseudohyphal growth phenotype but might play a role in other stress responses and activated transcription. From the deletion analysis of the non-conserved regions, we report that they influence phenotypes involving both the N and C termini (interaction with Rpb7 and transcription from the *INO1* promoter) but not any of the stress-responsive phenotypes tested suggesting that they might be involved in maintaining the two conserved domains in an appropriate conformation for interaction with Rpb7 and other proteins. Taken together, our results allow us to assign phenotype-specific roles for the different conserved and non-conserved regions of Rpb4.

Transcriptional regulation by RNA polymerase II and its associated proteins lies at the core of eukaryotic gene expression. *Saccharomyces cerevisiae* RNA polymerase II is a complex of 12 subunits, Rpb1–12, named in the order of decreasing size. Rpb1, Rpb2, and Rpb3·Rpb11 are homologs of *Escherichia coli* RNA polymerase β′, β, and α subunits, respectively. Rpb5, 6, 8, 10, and 12 are shared by all three RNA polymerases I, II, and III. Rpb9 and Rpb4 are the two non-essential subunits in this complex (1). Rpb4 forms a sub-complex with Rpb7 that is easily dissociated from the polymerase under mild denaturing conditions and is involved in promoter dependent initiation of transcription (2). This sub-complex is associated sub-stoichiometrically with the rest of the polymerase under logarithmic growth phase, but under stationary phase conditions the stoichiometry increases to one (3, 4). These observations have led to the hypothesis that this sub-complex forms the eukaryotic counterpart of the bacterial σ subunit (2).

The Rpb4·Rpb7 sub-complex has been reported from many species (5–8). Recently Rpb4·Rpb7 like sub-complex has also been demonstrated in RNA polymerases I and III (9, 10). Rpb7 is very highly conserved across species (11, 12), and the lethality of sc*rpb7*Δ can be rescued by overexpression of homologs from other species.[1] Rpb4 is conserved from archaea to humans with 12 Rpb4-like sequences reported in the GenBank™ data base (13). Till date, only the *Homo sapiens* (Hs), *Arabidopsis thaliana* (At), *Drosophila melanogaster* (Dm), *Schizosaccharomyces pombe* (Sp), and *Methanococcus jannaschii* (Mj) proteins have been shown to be *bona fide* Rpb4 homologs (5–8).

*rpb4*Δ yeast strain grows at moderate temperatures, albeit slowly, and exhibits a variety of stress response defects (14). It cannot survive at temperatures above 32 °C or below 15 °C. It also shows poor recovery from stationary phase. Previous work from our group has shown that *rpb4*Δ strains are also defective for sporulation and show enhanced pseudohyphal morphology, two hallmark responses to nutritional starvation (15). Transcriptional activity and global mRNA synthesis in *rpb4*Δ strains is significantly reduced on prolonged exposure to high temperatures (*in vitro* and *in vivo*) or stationary phase conditions (3, 16, 17). In addition, in certain genetic backgrounds *rpb4*Δ strains exhibit Na^+/Li^+ ion sensitivity (18). Some but not all of these phenotypes can be rescued by overexpression of *RPB7* suggesting that one of the roles of Rpb4 is to stabilize the interaction of Rpb7 with the rest of the polymerase (19–22).

Apart from these stress response defects, *rpb4*Δ also affects transcription of many genes even under moderate growth conditions. Using promoter-reporter studies, we have shown previously that *rpb4*Δ strains exhibit defects in activated transcription of a subset of genes. This defect is partially rescued by overexpression of cognate transcriptional activators (23). Whole genome transcriptional analysis of *rpb4*Δ strains reveals defects in carbon and energy metabolism at moderate temperatures and, additionally, the transcription of ribosomal protein genes in response to mild heat shock (15).

Because Rpb4 affects many different phenotypes, we hypoth-

* This work has been supported in part by grants from the Council of Scientific and Industrial Research, the University Grants Commission, the Department of Science and Technology, and the Indian Council of Medical Research (to P. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Supported by a fellowship from Council of Scientific and Industrial Research, Government of India.

‖ Supported by the award of Senior Fellowship in Biomedical Sciences by the Wellcome Trust, UK.

** To whom correspondence should be addressed. Tel.: 91-80-293-2292; Fax: 91-80-360-2697; E-mail: pps@mcbl.iisc.ernet.in.

[1] S. Singh, N. Rekha, B. Pillai, V. Singh, A. Naorem, V. Sampath, N. Srinivasan, and P. Sadhale, unpublished data.

TABLE I
List of plasmids used in this study

Plasmid name	Alias	Description	Reference
pPS2	Ycplac111	Yeast cloning vector, LEU2, CEN	(44)
pPS12	pYES2	Yeast cloning vector, P_{GAL1}, URA3, 2μ	Invitrogen
pPS24	pJH359	P_{INO1}-LacZ, URA3, 2μ	(45)
pPS31	pGAD424	GAL4AD fusion vector, LEU2, 2μ	Invitrogen
pPS40		RPB4 ORF in pPS12, URA3, 2μ	(12)
pPS111	pZJHSE137	HSE element-P_{CYC1}-LacZ fusion, URA3, 2μ	(46)
pPS121	pGAB	P_{GAL10}-LacZ, URA3, CEN	U. Vijayaraghavan
pPS141	LexADBD-Rpb7	RPB7 ORF in frame with LexADBD, HIS3, 2μ	(12)
pPS143	pJK103	LexAop2-LacZ, URA3, 2μ	(12)
pVS151	pGAD424Δ RI	pPS31-EcoRI site, LEU2, 2μ	This study
Deletions of Rpb4 in the gene context in pPS2 vector, LEU2, CEN			
pNS114	1–221	Full-length RPB4 gene in pPS2	(19)
pBP212	1–198	C-terminal 23 aa truncated Rpb4	(23)
pVS378	33–221	N-terminal 32 aa truncated Rpb4	This study
pVS379	33–198	N- and C-terminal truncated Rpb4	This study
pVS366	Δ95–105	Deletion of acidic stretch in Rpb4	This study
pVS368	Δ66–80	Deletion of basic stretch in Rpb4	This study
pVS370	Δ66–105	Deletion of acidic and basic stretch in Rpb4	This study
pVS384	Δ106–140	Deletion of 106–140aa in Rpb4	This study
pVS385	Δ66–140	Deletion of entire non-conserved region in Rpb4	This study
Deletions of Rpb4 as fusions to GAL4AD in pPS31 vector, LEU2, 2μ			
pVS153	1–221	Full-length RPB4 ORF in pPS31	This study
pVS288	1–198	C-terminal 23 aa truncation of Rpb4	This study
pRS217	33–221	N-terminal 32 aa truncation of Rpb4	This study
pVS356	33–198	N- and C-terminal truncation of Rpb4	This study
pVS372	Δ95–105	Deletion of acidic stretch in Rpb4	This study
pVS374	Δ66–80	Deletion of basic stretch in Rpb4	This study
pVS376	Δ66–105	Deletion of acidic and basic stretch in Rpb4	This study
pVS387	Δ106–140	Deletion of 106–140 aa in Rpb4	This study
pVS386	Δ66–140	Deletion of entire non-conserved region in Rpb4	This study

esized that different domains of Rpb4 could be involved in different phenotypes. Correlating a region of the protein to a given phenotype will allow us to isolate phenotype-specific protein partners. Earlier work from our group had localized the stress-responsive domain to the C-terminal 23 aa[2] of Rpb4 (23). In this study, we have generated systematic deletions of the conserved and non-conserved regions of Rpb4. Our analysis shows that both the conserved N and C terminus of Rpb4 are involved in interacting with Rpb7. We further report that the conserved N terminus is not involved in the stress responsive functions of Rpb4 but plays a role in activated transcription from certain promoters. We also show that the non-conserved regions of Rpb4 also affect a similar set of phenotypes as the N-terminal region indicating a role for these non-conserved regions in maintaining conformation of scRpb4.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions

The yeast strains used were (i) SY10 (*Mat a, his3Δ200, ura3–52, leu2–3,112, lys2–1, rpb4Δ::HIS3*); (ii) SYD1011 (*Mata/α, his3Δ200/his3Δ200, ura3–52/ura3–52, leu2–3,112/leu2–3,112, lys2–1/lys2–1, rpb4Δ::HIS3/rpb4Δ::HIS3*); (iii) SY19 (EGY191, *Matα, his3-Δ200, trp1–901, ura3–52, leu2:pLEU2-LexAop2*) (12). These strains were transformed with the appropriate plasmids listed in Table I and assayed for various phenotypes. Yeast strains were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) or in Synthetic Drop-out (SD) medium and manipulated as per standard protocols (24). Yeast transformations were performed using the modified lithium acetate protocol, which does not involve heat treatment of cells (25). All manipulations of plasmids were performed in *Escherichia coli* strain DH5α (supE44, *delta lacU169 (phi 80 lacZ delta M15), hsdR17, recA1, endA1, gyrA96, thi-1, relA1*) as per standard protocols (24).

[2] The abbreviations used are: aa, amino acid(s); Sc, *S. cerevisiae*; ORF, open reading frame; GAL4AD, Gal4 activation domain; lexADBD, LexA DNA binding domain; SD, synthetic drop-out medium; SLAD, synthetic low ammonia dextrose medium; HSE, heat shock element.

Construction of Plasmids

Mutants of *RPB4* were expressed in their gene context for assays of temperature sensitivity, sporulation, pseudohyphal growth, and activated transcription in *rpb4Δ* strains. For two-hybrid interaction analysis, the various deletions of the *RPB4* open reading frame (ORF) were expressed as fusions to Gal4 activation domain (GAL4AD) in the plasmid pPS31 (pGAD424, Invitrogen).

RPB4 Mutants in Gene Context—The full-length Rpb4 protein is 221 aa long. Construction of the C-terminal 23 aa deletion (Rpb4-(1–198), pBP212) has been detailed elsewhere (23). The *RPB4* upstream sequence encompassing the promoter was amplified using the primer Rpb4 PS2f (5′-GCGGATCCATGTTCCCTTGCACAATGG-3′) and Rpb4 RIdelr (5′-CCGGAATTCCATTTTTCTATATTC-3′) such that an EcoRI site immediately followed the Rpb4 ATG and cloned in pGEM-Teasy vector (Promega). The EcoRI fragment from this construct was used to replace the EcoRI fragment in pNS114 (Rpb4, 1–221 aa) and in pBP212 to generate the N-terminal 32-aa deletion (Rpb4 (33–221), pVS378) and the N- and C-terminal deletion (Rpb4 (33–198), pVS379), respectively. The internal deletions of *RPB4* were generated by overlap extension PCR method using deletion-specific primers and the *RPB4* upstream and downstream primers, Rpb4PS2f and Rpb4PS1r (5′-GCGTCGACTCACTCACCGTTTGGCACC-3′). The deletion-specific primers used were: Δ66–80 (f: 5′-GAAGCTCTTGTAGAACATTTGAAGCACGAA-3′ and r: 5′-TTCGTGCTTCAAATGTTCTACAAGAGCTTC-3′); Δ95–105 (f: 5′-GAAACTACGGCAGTAGTCAACGCTGATGAT-3′ and r: 5′-ATCATCAGCGTTGACTACTGCCGTAGTTTC-3′); Δ66–105 (f: 5′-GAAGCTCTTGTAGAAGTCAACGCTGATGAT-3′ and r: 5′-ATCATCAGCGTTGACTTCTACAAGAGCTTC-3′); Δ106–140 (f: 5′-CTGGATGAAGATGACTTGAAAAATACCATG-3′ and r: 5′-CATGGTATTTTTCAAGTCATCTTCATCCAG-3′); and Δ66–140 (f: 5′-GAAGCTCTTGTAGAATTGAAAAATACCATG-3′ and r: 5′-CATGGTATTTTTCAATTCTACAAGAGCTTC-3′). These PCR products were cloned in pGEM-Teasy vector and then sub-cloned into pPS2 using BamHI and SalI sites in the Rpb4PS2f and Rpb4PS1r primers, respectively.

RPB4 Deletions as Fusions to GAL4AD—ORFs of *RPB4* and its deletions were cloned in-frame in pGAD424 (pPS31) or the pGAD424ΔRI (pVS151, EcoRI site destroyed by Klenow fill-in reaction to alter the reading frame for the multiple cloning sites). The *RPB4* ORF was cloned as a BamHI-XhoI fragment from pPS40 in pVS151 to generate pVS153. The HindIII fragment of pPS40 (Rpb4-(1–198)) was

cloned into pPS12 and further sub-cloned as a BamHI fragment in pVS151 to generate the C-terminal 23-aa deletion of Rpb4 (pVS288). The EcoRI-XhoI fragment of pPS40 was cloned in pPS31 to generate the N-terminal deletion (Rpb4-(33–221), pRS217). The EcoRI-SalI fragment of pBP212 was cloned in-frame in pPS31 to generate the N- and C-terminal deletion (Rpb4-(33–198), pVS356). Overlap Extension PCR using the set of deletion-specific primers listed above, primers that bind to pPS31 vector sequences (f: 5′-CACAACCAATTGCCTCCTCTAAC-3′ and r: 5′-CCAAAGCTTCTGAATAAGCCCTC-3′) and pVS153 as a template was used to generate the *RPB4* internal deletions. These PCR products were cloned into pGEM-Teasy. The *RPB4* deletion ORFs were sub-cloned using the sites flanking the ORF into pVS151. Due to differences in the cloning strategies, the C-terminal truncation in the gene context is shorter than the truncation in the ORF context by 1 residue at the 198th position. However, to avoid confusion, both these constructs have been labeled Rpb4-(1–198).

Temperature Sensitivity, Sporulation, and Pseudohyphal Growth

The assays for temperature sensitivity, sporulation, and pseudohyphal growth using *rpb4*Δ strains containing plasmids expressing either *RPB4* deletion mutants or appropriate controls were done essentially as described earlier (15, 23). Assays for temperature sensitivity were done on SD medium plus 2% dextrose plates at 25 °C and 37 °C. Assays for sporulation and pseudohyphal growth were performed at 25 °C on 1% potassium acetate plates and synthetic low ammonia dextrose (SLAD) plates, respectively.

Activated Transcription

The ability of haploid *rpb4*Δ strains containing *RPB4* deletion plasmids to activate transcription from the *GAL10*, *INO1*, and *HSE* promoters were assayed using promoter-LacZ reporters as earlier described (23). β-Galactosidase assays were performed by glass bead disruption method essentially as described previously (26).

Two-hybrid Analysis

Two-hybrid analysis of interaction between LexA DNA binding domain (DBD)-Rpb7 fusion and the GAL4AD fusions of Rpb4 and deletion mutants was performed in strain SY19 (EGY191) transformed with LexAop2-LacZ reporter plasmid (JK103) (12). This particular combination of plasmids was preferred as the extended multiple cloning sites in the pPS31 plasmid allowed the construction of various GAL4AD-Rpb4 fusions. However, it precludes the use of the Leu2 reporter integrated in EGY191. Strains to be assayed for interaction were grown in SD plus 2% galactose plus 1% xylose plus tryptophan until mid log phase and harvested for β-galactosidase assays. β-Galactosidase assays were performed by chloroform permeabilization method as described previously (27).

Comparative Modeling of S. cerevisiae Rpb4p Using an Archaeal Homolog

Although, during the course of this work, structures of the multisubunit complex of RNA polymerase from *S. cerevisiae* has been published (28, 29),[3] the structures are of low resolution (4.1 and 4.2 Å) and for Rpb7 and Rpb4 the positions of Cα atoms alone are available. Hence detailed analysis of the side-chain interactions between Rpb4 and Rpb7 is not possible. So we generated models of Rpb4 and Rpb7 using archaeal complex structure, and we ensured that the features of the model built, such as similarity of the positioning of secondary structures in the fold are consistent with the features observed in the low resolution structures of yeast complex.

The structure of Rpo_E1 and Rpo_F1 proteins from *M. jannaschhi* is available in the Protein Data Bank (30). We have used this structure as a template to model Rpb7 and Rpb4 from *S. cerevisiae*, respectively. The sequence identity between archaeal Rpb4 and *S. cerevisiae* Rpb4 is about 21%. The Rpb4 proteins are significantly different, with iterative position-specific iterative blast failing to pick up the other as homologs at any relaxed *E* values. For Rpb4, the ClustalW (31) package was used to correctly align the N and C termini of the Rpb4 protein with Rpb4 sequence from an archaeal homolog. The alignment accuracy was assessed after looking at the residue conservation at the solvent buried positions and consulting the alignment arrived at by PFAM data base (32), which is a manually curated data base that considers the experimental observations in obtaining alignments. A three-dimensional model of both Rpb7 and Rpb4 has been generated using a suite of programs encoded in COMPOSER (33, 34) and incorporated in SYBYL (Tripos Inc., St. Louis, MO). The methods used for modeling is as discussed previously (35).

[3] PDB codes 1nik and 1nt9.

RESULTS

S. cerevisiae Rpb4 Has Distinct Conserved and Non-conserved Regions—The *S. cerevisiae* protein is considerably larger than the other eukaryotic homologs being 221 aa in size as against ~140 aa for the other homologs. Alignment of these homologs (Fig. 1*A*) shows that the N-terminal 45 aa (60 aa for the scRpb4 protein) and C-terminal 80 aa have high sequence similarity. The additional residues in *S. cerevisiae* protein are mainly localized to three stretches in the central region: residues 39–49, 69–95, and 107–140 (numbering as per Sc protein). A 15-aa long stretch of mainly basic residues ("Basic stretch") is part of 69–95 residue unique stretch. Amino acids 95–105 are composed of entirely acidic residues ("Acidic stretch") that show weak conservation among the homologs. In this study, we have ascertained the functions of the conserved (N- and C-terminal aa), the weakly conserved ("Acidic stretch"), and non-conserved ("Basic stretch" and aa 107–140) residues in the scRpb4 protein using deletion analysis. The deletions made in the protein are depicted in Fig. 1*B*. These deletions were analyzed for their ability to complement the defects of *rpb4*Δ when expressed in the context of the *RPB4* gene. For analysis of interaction with Rpb7, the different regions were expressed as fusion proteins with the GAL4AD and tested by the two-hybrid assay (see "Experimental Procedures" for further details)

The Conserved N Terminus of Rpb4 Is Involved in Interaction with Rpb7—Because the Rpb4·Rpb7 sub-complex is highly conserved, we sought to understand the role of the conserved residues of Rpb4 in this interaction. We used a directed two-hybrid assay to test the interaction between the LexADBD-Rpb7 fusion and the GAL4AD-Rpb4 fusions (full-length, 1–221; C-terminal truncation, 1–198; N-terminal truncation, 33–221; or the N- and C-terminal truncations, 33–198). As can be seen in Fig. 2*A*, deletions of either the N- or the C-terminal conserved regions affect the interaction with Rpb7 suggesting that these residues are important for the interaction. Western blotting of protein extracts from these strains with anti-Gal4 antibodies showed that, although the N-terminal truncation does not affect the stability of the protein, the C-terminal truncation reduces protein levels below detectable limits under conditions of our assay (data not shown). However, as shown below (Fig. 3*C*) this destabilization does not completely eliminate the functioning of the protein. The two-hybrid result suggests a role for the conserved N-terminal region in interaction with Rpb7. A role for the C-terminal Rpb4 residues in interaction with Rpb7 cannot be predicted based on these results alone.

Molecular Modeling of Rpb4·Rpb7 Complex Predicts a Role for the Rpb4 N and C Termini in the Interaction with Rpb7—To understand the structural basis of this interaction, we generated a molecular model of the scRpb4·Rpb7 complex based on the crystal structure of archaeal homologs (30). The model for scRpb7 and its homologs from other lower eukaryotic homologs shows very high structural conservation with the archaeal homolog and has been detailed elsewhere.[1] The C-terminal region of scRpb4 shows high sequence similarity with the homologous mjF protein. The N-terminal region of Rpb4 is poorly conserved between these homologs (as against the homology among eukaryotic Rpb4 proteins). The alignment arrived at by consulting the best match of the residues in the region among all the known homologs of Rpb4 was used for model building. The model generated for the heterodimeric interaction between Rpb4 and Rpb7 for *S. cerevisiae* proteins is shown in Fig. 2*B*. The model for scRpb4 is shown in *blue*, and the regions interacting with Rpb7 are colored *orange*. The non-conserved re-

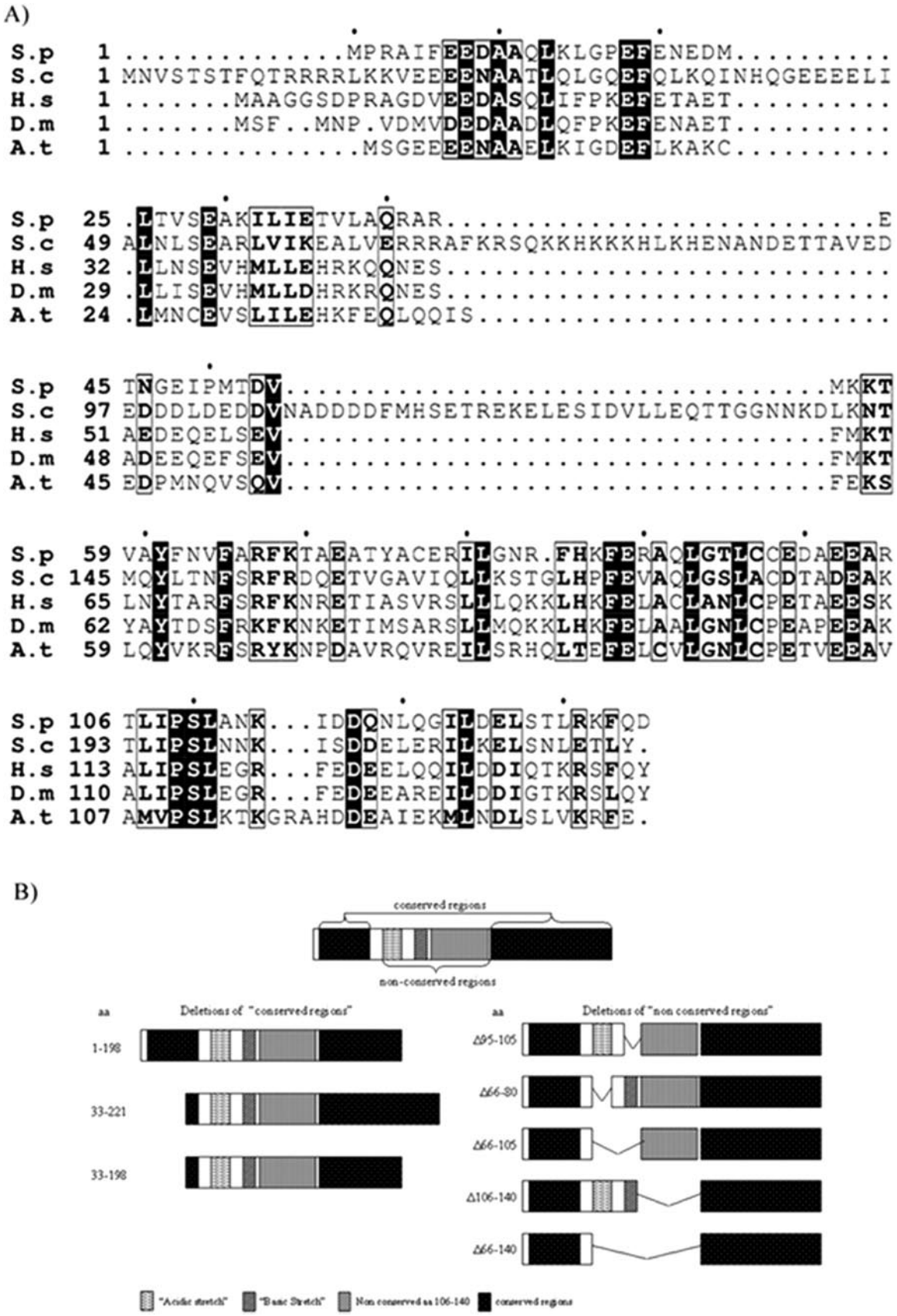


FIG. 1. **Sequence features of Rpb4.** *A*, alignment of Rpb4 sequences from eukaryotes. The protein sequences of Rpb4 homologs from *S. cerevisiae* (*S.c*), *Schizosaccharomyces pombe* (*S.p*), *Homo sapiens* (*H.s*), *Arabidopsis thaliana* (*A.t*), and *Drosophila melanogaster* (*D.m*) were aligned using ClustalW and modified for print representation using ESPript 2.0 (43). The residues *outlined in black boxes* are identical across all homologs, whereas the residues *outlined in white boxes* are similar across all homologs. *B*, deletions generated in this study. In the first set of deletions, the conserved regions (denoted by *black-filled regions*) were truncated either at the N or the C terminus or both simultaneously keeping the rest of the protein intact. In another set of deletions, the non-conserved regions were deleted singly (the Acidic stretch is denoted by *diagonal lines*, the Basic stretch is denoted by *waves*, and the aa 106–140 is denoted by *crossed hatches*) or in combination.

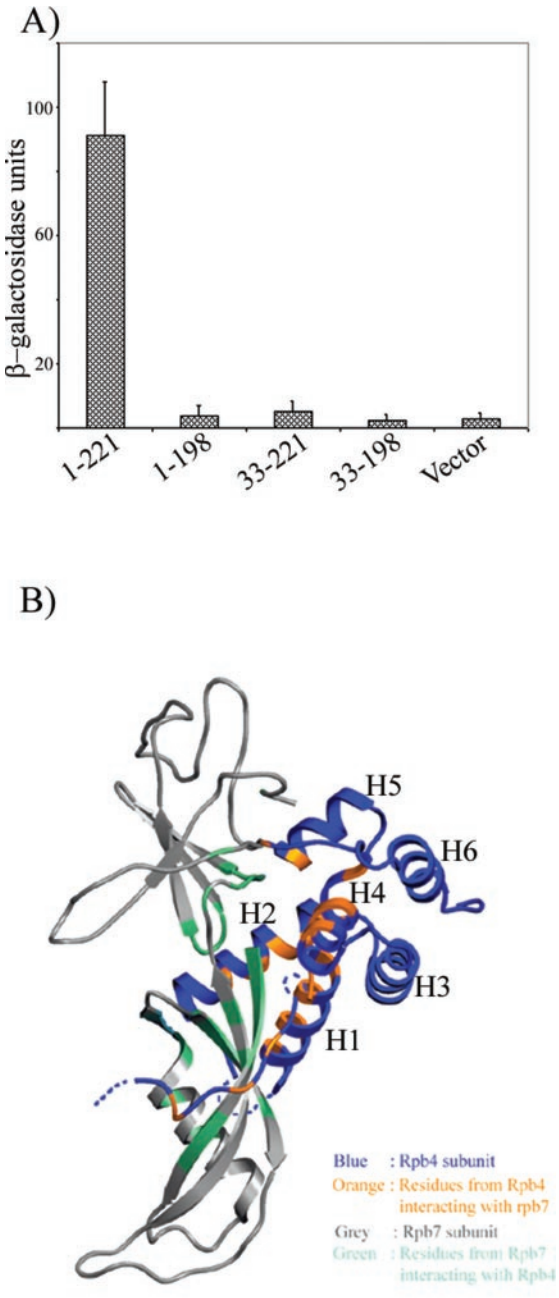


FIG. 2. **The conserved N- and C-terminal aa of Rpb4 are involved in interaction with Rpb7.** *A*, two-hybrid analysis of LexADBD-Rpb7 interaction with GAL4AD Vector, Rpb4-(1–221), and the indicated conserved region truncations. *Plotted here* are the average β-galactosidase activities and standard deviations thereof from two independent experiments with three different transformants each. *B*, Molecular Model of scRpb4·Rpb7 generated based on the crystal structure of mjF·mjE homologs. The Rpb4 model is represented in *blue* with the Rpb7 interaction regions colored *orange*. The six helices in the Rpb4 structure have been numbered *H1–H6*. The residues not modeled due to lack of similarity are represented as *dotted lines*. The Rpb7 model is represented in *gray* with the regions involved in interaction with Rpb4 in *green*. The Rpb7 interaction regions on Rpb4 map to the N and C termini.

gions of scRpb4 could not be modeled and are shown here as *dotted lines*. The overall fold of the interaction model is suggested to be similar to that of mjF·mjE sub-complex. The sequence of scRpb4 is consistent with the fold of mjF protein in which six C-terminal α-helices fold into a "belt" around the middle of scRpb7. The N-terminal conserved region adds a strand onto the conserved β-sheet of Rpb7. The region implicated in binding with Rpb7 from Rpb4 in the N-terminal region is solvent-exposed in the tertiary structure, and all the inter subunit interactions are mediated by interactions across the main-chain atoms of the participating residues from both the subunits. It is possible that some residues from this region contribute to the main-chain interactions that are responsible for inter-subunit interactions. The regions in the conserved C terminus of Rpb4 involved in interaction with Rpb7 map mainly to the helices H1, H2, and H4 (helix numbering scheme of archaeal homolog followed here). Some residues of the helix H5 are also involved in interacting with Rpb7 in the heterodimer. These interactions are shown in Fig. 2*B*.

The recent publication of the crystal structures of the 12-subunit RNA polymerase II from two different groups (28, 29) allowed us to compare our model with the Rpb4·Rpb7 substructures. Both the crystal structures do not have the sequence of both the Rpb7 and Rpb4 traced, and only the Cα positions are available in the data deposited at PDB (36). Furthermore, the residue numbering of Rpb4 in one of the crystal structures (PDB id: 1nik) is arbitrary as annotated at PDB. Comparison of the subunit structures extracted from the crystal structure (PDB id: 1nt9) and that of Rpb4 model shows that, although the N-terminal region is not well defined in crystal structure, there is excellent correspondence (root mean square deviation < 3 Å) at the helical region at the C terminus between residue numbers 160 and 221. Therefore, we believe that the model for scRpb4·Rpb7 generated based on the crystal structures of archaeal homologs can be used for understanding interactions of Rpb4·Rpb7 with each other and the rest of the polymerase. Taken together, our two-hybrid analysis and the molecular modeling studies indicate that the Rpb7 interaction domain localizes to the conserved N- and C-terminal regions of Rpb4.

Differential Requirement for the Conserved Regions of Rpb4 in Stress Response—Rpb4 has been implicated to have stress-specific roles, because *rpb4*Δ strains are temperature-sensitive, defective in sporulation, and predisposed to pseudohyphae formation (15). Earlier work from our group has shown that the C-terminal 23 aa are required for growth at high temperatures and sporulation (15, 23). To analyze the role of the N-terminal conserved residues, we transformed haploid and diploid *rpb4*Δ strains with plasmids expressing the full-length Rpb4 or the truncations of the conserved regions in the context of the Rpb4 promoter and terminator sequences (Fig. 1*B*). Assays of temperature sensitivity in haploid *rpb4*Δ strains (Fig. 3*A*) showed that deletion of the N-terminal 32 aa (33–221) does not affect the ability of Rpb4 to rescue growth of *rpb4*Δ strains at 37 °C. Neither the C-terminal truncation (1–198) nor the N- and C-terminal truncation (33–198) was able to rescue the defect. The results in the homozygous diploid *rpb4*Δ strain were similar (data not shown).

S. cerevisiae undergoes sporulation to form tetrads (four haploid spores enclosed in an ascus) in response to severe starvation usually involving lack of nitrogen and fermentable carbon source. We analyzed the ability of the Rpb4 truncations to rescue the sporulation defect of *rpb4*Δ strain as compared with the wild-type protein. This was measured as percentage of tetrads in each population after 3 days in sporulation medium. As can be seen from Fig. 3*B*, the N-terminal truncation (33–221) supports sporulation as well as full-length Rpb4, whereas the 1–198, and the 33–198 mutant proteins are defective for sporulation as is the *rpb4*Δ strain.

In the presence of a fermentable carbon source and limiting amounts of a nitrogen source, *rpb4*Δ strains show pronounced cell elongation, unipolar budding patterns, and irregular colony morphology associated with pseudohyphal growth phenotype,

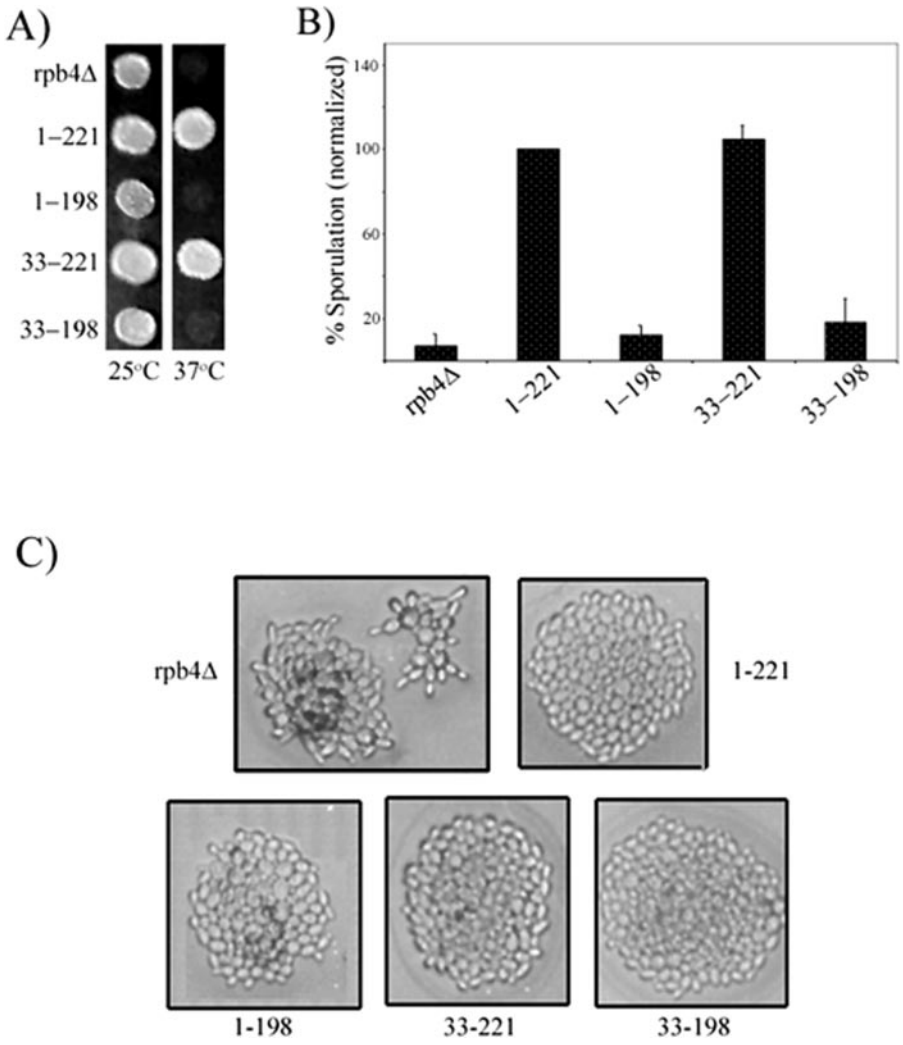


FIG. 3. **The conserved C-terminal aa but not the N-terminal aa of Rpb4 are required for stress response.** *A*, haploid *rpb4*Δ strains with vector (rpb4Δ), full-length Rpb4-(1–221), or the deletions of conserved regions were assayed for temperature sensitivity. Photographs were taken after 3-day incubation at permissive (25 °C) and non-permissive (37 °C) temperatures. *B*, diploid *rpb4*Δ strains with vector (rpb4Δ), full-length Rpb4-(1–221), or the deletions of conserved regions were assayed for sporulation. The number of tetrads in 500 cells of a population were counted, and percent sporulation values for each strain (normalized to full-length Rpb4 sporulation levels) were calculated. Plotted here are averages and standard deviations thereof of three independent experiments with three transformants each. *C*, diploid *rpb4*Δ strains with vector (rpb4Δ), full-length Rpb4-(1–221), or the deletions of conserved regions were assayed for pseudohyphal growth on SLAD (low nitrogen-containing SD media) plates. Shown here are representative colony morphologies for the strains 18 h after spotting.

whereas *rpb4*Δ strains expressing full-length Rpb4 show bipolar budding patterns resulting in rounded colonies (Fig. 3*C* and Ref. 15). To analyze the role for the conserved regions of Rpb4 in this phenotype, we spotted diploid *rpb4*Δ strains containing the truncations on SLAD plates and monitored growth patterns after 18 h. As can be seen in Fig. 3*C*, deletion of the N- and the C-terminal conserved regions does not affect the ability of Rpb4 to restore normal growth patterns to *rpb4*Δ strains. These results suggest that the N-terminal conserved stretch is not involved in stress-specific functions of Rpb4, whereas the C-terminal conserved residues are required for growth at high temperatures and sporulation but not for pseudohyphal growth.

Some Activated Promoters Require Both the Conserved Regions of Rpb4 for Effective Transcription—Apart from its stress-specific roles, Rpb4 is required for transcription from many activated promoters like the P_{GAL10}-, P_{INO1}-, and HSE-containing promoters even at moderate temperatures (23). To test the requirement of the conserved regions in activated transcription from the P_{GAL10}-, P_{INO1}-, and HSE-containing promoters, we transformed *rpb4*Δ strains containing these independent promoter-LacZ fusion reporters with the truncations listed above and assayed for β-galactosidase activity. The β-galactosidase units are represented as normalized to activity from *rpb4*Δ strains containing vector plasmids. As can be seen from Fig. 4 (*A–C*) loss of the conserved C-terminal residues affects the ability of Rpb4 to rescue activated transcription from the *GAL10* (23) and also the *HSE* and *INO1* promoters. On the other hand, deletion of the N-terminal conserved residues does not significantly affect activated transcription from the P_{GAL10}- and HSE-containing promoters (Fig. 4, *A* and *B*) but reduces transcription significantly from the P_{INO1} promoter (Fig. 4*C*). The activity of the N-terminal deletion is nearly 70–80% of the full-length protein activity for the *GAL10*- and *HSE*-containing promoters but less than 20% for the *INO1* promoter. These results suggest that the *INO1* promoter requires both the N- and C-terminal regions of Rpb4 for effective transcription, whereas transcription from P_{GAL10}- and *HSE*-containing promoters depends on only the C-terminal conserved regions of Rpb4.

Deletion of the Non-conserved Residues in Rpb4 Affects Interaction with Rpb7—scRpb4 has certain unique stretches in its primary sequence that are not conserved among the different homologs. After analyzing the requirement of the conserved regions of Rpb4 in various phenotypes, we wanted to test if the non-conserved (aa 66–80 and 107–140), and weakly conserved aa 95–105 residues play any role in these phenotypes. Toward this aim we generated a series of deletions in Rpb4 that lack these stretches singly or in combination (Fig. 1*B*) and expressed them as described for the conserved region deletions above.

We used two-hybrid analysis to analyze the effect of deletion of non-conserved regions on interaction of Rpb4 with Rpb7. Analysis of the interaction between Rpb4 mutant-GAL4AD and the LexA DBD-Rpb7 shows that loss of any one of the non-conserved regions of Rpb4 affects interaction with Rpb7 (Fig.

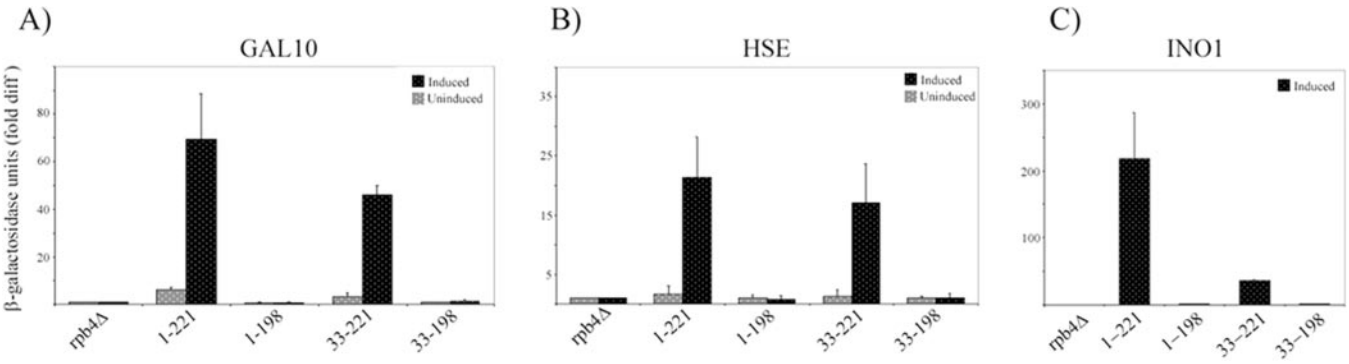


FIG. 4. **Activated transcription from *INO1* promoter but not the *GAL10* and *HSE* requires both the N- and the C-terminal aa of Rpb4.** Haploid *rpb4*Δ strains with P_{GAL10}-LacZ fusions (*A*), HSE-containing promoter-LacZ fusion (*B*) or P_{INO1}-LacZ fusions (*C*) transformed with vector (rpb4Δ), full-length Rpb4-(1–221) or the deletions of conserved regions were assayed for β-galactosidase activity under uninduced (*gray bars*) and induced (*black bars*) conditions. β-Galactosidase units for each strain were normalized to the vector control (*-fold diff*). Plotted here are average β-galactosidase units (*-fold diff*) and standard deviations thereof from two independent experiments with three transformants each.

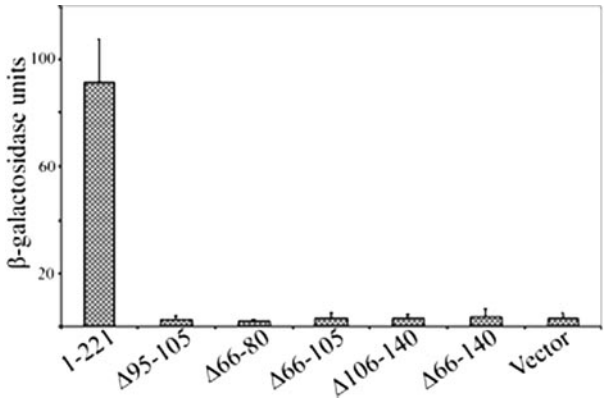


FIG. 5. **Deletion of the non-conserved regions of Rpb4 abrogates interaction with Rpb7.** Two-hybrid analysis of LexADBD-Rpb7 interaction with Vector, full-length Rpb4-(1–221) or the internal deletions as listed. Plotted here are average β-galactosidase units and standard deviations thereof from two independent experiments with three transformants each.

5). To rule out that the differences observed did not arise from differential stability of these mutant fusion proteins, we ascertained their steady-state levels using anti-Gal4 antibodies. We find that all the mutant fusion proteins are expressed in this strain background as expected (data not shown). This suggests that these non-conserved regions are also involved in interaction with Rpb7 either directly or indirectly (also see "Discussion").

The Non-conserved Regions Are Not Involved in Stress-specific Roles of Rpb4—We next determined the requirement of the non-conserved regions of Rpb4 in rescue of temperature sensitivity, sporulation defect, and predisposition to pseudohyphal growth of *rpb4*Δ strains. These deletions were made in the context of the Rpb4 gene and transformed into *rpb4*Δ haploid and diploid strains along with the appropriate controls. Deletion of most of the non-conserved regions does not affect the ability of Rpb4 to support growth of haploid (Fig. 6*A*) or diploid *rpb4*Δ strains (data not shown) at higher temperatures of 37 °C. Only the deletion of the entire Sc-specific region (from aa 66–140) moderately compromises growth at higher temperatures. This effect is seen only in haploid and not in diploid *rpb4*Δ strains (data not shown). We analyzed the ability of these deletions to support sporulation in diploid *rpb4*Δ strains in comparison to full-length Rpb4. As can be seen from Fig. 6*B*, deletion of none of the non-conserved regions affects the ability of Rpb4 to rescue the sporulation defect of *rpb4*Δ strains. To assay for their ability to reverse the pseudohyphal growth predisposition of *rpb4*Δ strains, these mutants were spotted on pseudohyphae-inducing SLAD plates. The difference in morphology assayed after 18 h shows that (Fig. 6*C*) none of the internal deletions of *RPB4* can be distinguished from wild-type *RPB4* suggesting that these regions are not involved in this phenotype. Taken together, the results in Fig. 6 suggest that the non-conserved regions of Rpb4 do not play a significant role in stress response.

Transcription from Some Activated Promoters Requires the Non-conserved Regions of Rpb4—We also analyzed the requirement for these non-conserved regions of Rpb4 in activated transcription from the P_{GAL10}-, P_{INO1}-, and HSE-containing promoters as explained above. As can be seen from Fig. 7 (*A* and *B*), deletion of the non-conserved regions either singly or in combination does not affect activated transcription from the P_{GAL10}- and HSE-containing promoters (activity for all deletions nearly 80–100% of the full-length activity). However, activated transcription from P_{INO1} is affected by deletion of the non-conserved regions (Fig. 7*C*). The deletion of both the basic and acidic regions (aa 66–105) and the deletion of the entire Sc-specific region (from 66 to 140) have more pronounced effects (activity is reduced to ~10% of full-length protein) on activated transcription than deletion of individual stretches (activity is between 20 and 40% of full-length protein). This suggests that the non-conserved regions of Rpb4 are involved in activated transcription from the P_{INO1} promoter but not the P_{GAL10}- and HSE-containing promoters.

DISCUSSION

Regulation of gene expression has been thought to involve contact between the DNA-bound activators and the Holoenzyme components (co-activators and general transcription factors) leading to increased recruitment of the polymerase (1). Modulation of RNA polymerase II composition could be an additional level of regulation in gene expression (37). The Rpb4·Rpb7 sub-complex is an ideal candidate for such a regulation, because the association of the complex with the rest of the polymerase is sub-stoichiometric under logarithmic growth conditions and becomes stoichiometric under stationary phase conditions (2). This sub-complex is also easily dissociated from the rest of the polymerase under mild denaturing conditions and is absent from polymerase purified from a mutant of Rpb1, Rpb6, and from *rpb4*Δ strains (3, 38, 39). Rpb4 plays a role in many stress-responsive phenotypes like growth at high and low temperature, sporulation, pseudohyphal growth, etc. (14, 15). It is also involved in activated transcription from a subset of promoters, in carbon and energy metabolism at moderate temperatures (15, 21, 23). Delineation of the roles played by this subunit in multiple pathways requires an understanding of its domainal organization.

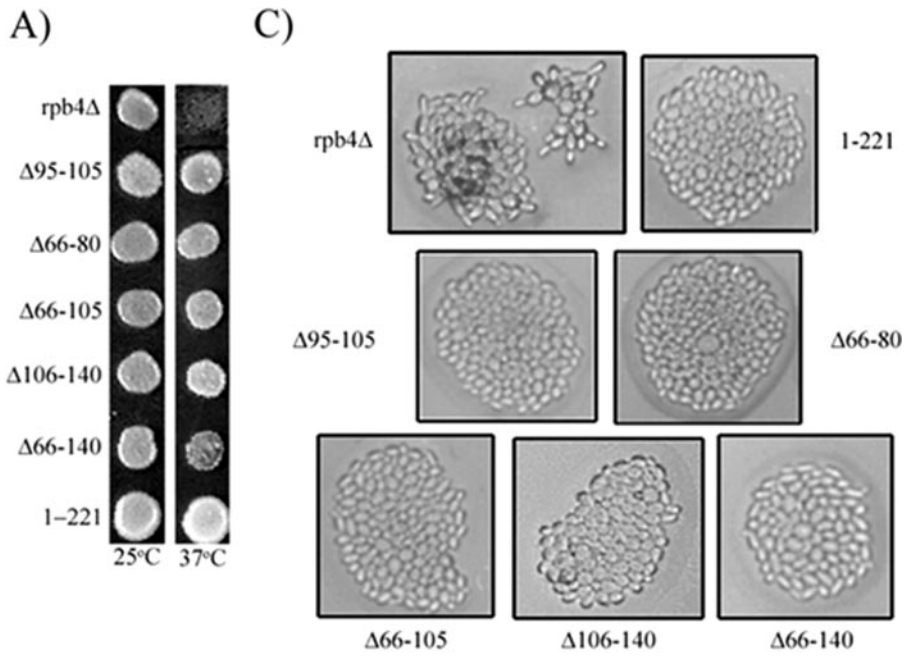
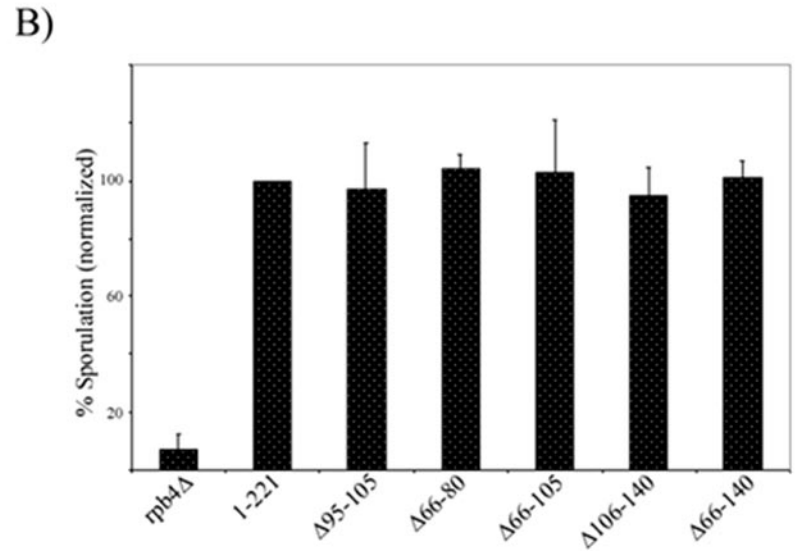


FIG. 6. **The non-conserved regions of Rpb4 are not involved in stress response.** Assays for temperature sensitivity (*A*), sporulation (*B*), and pseudohyphal growth (*C*) were carried out as described in the legend to Fig. 3 and under "Experimental Procedures." The results for the vector and full-length controls are the same as the ones shown in Fig. 3 and are shown here only for reference.



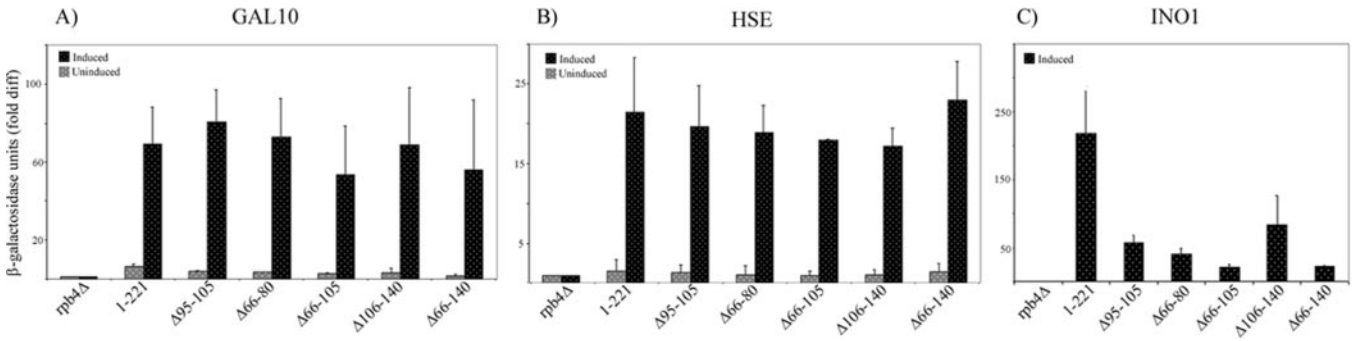


FIG. 7. **The non-conserved regions of Rpb4 are involved in activated transcription from the *INO1* promoter but not the *GAL10* or *HSE* containing promoters.** P_{GAL10}- (A), HSE containing promoter- (B) and P_{INO1}- (C) driven LacZ fusions were assayed for the non-conserved region deletions of Rpb4 as described in Legend to Fig. 4 and "Experimental Procedures" section.

A comparison of the Rpb4 primary sequence of the eukaryotic homologs reveals that the N-terminal 45 aa and the C-terminal 80 aa of the proteins can be denoted as "conserved regions" (Fig. 1*A*). However, the scRpb4 has unique stretches in the central region (aa 66–140) that has interesting features like a high density of basic (aa 66–80) or acidic (aa 95–105) residues. Our analysis of various deletion mutants in these regions (Fig. 1*B*) was aimed at understanding their roles in controlling the multiple phenotypes observed in *rpb4*Δ strain.

We have shown that the Rpb7 interaction domain in Rpb4 involves the conserved N-terminal 32 aa, because deletions of this domain abolishes two-hybrid interaction with Rpb7 (Fig.

2*A*). This observation is validated by molecular modeling of the scRpb4·Rpb7 complex (Fig. 2*B*) based on the crystal structure of mjF·mjE subunit complex, which reveals that the scRpb4 N-terminal and C-terminal regions are involved in the interaction. The N-terminal region forms a β-addition loop with the conserved three-stranded β-sheet of Rpb7. The C-terminal regions fold into α-helices that are involved in contact with Rpb7. The C-terminal 23 aa maps onto a helix after a region that has conserved surface-exposed residues and residues involved in binding Rpb7 in the heterodimer. The truncation of the C-terminal 23 residues could affect the folding of the regions preceding it (156–185), thereby affecting the binding to Rpb7. This region of the Rpb4 structure has structural similarity to the HRDC domain of the RecQ helicase (30). Hence, this region could be a structural domain such that any truncations in this region might result in misfolding and thereby compromise binding to Rpb7.

The role for the conserved N- and C-terminal regions of Rpb4 in interaction with Rpb7 is also validated by other observations made previously (i) Rpb4 and Rpb7 from many different systems interact with each other *in vivo* suggesting that a conserved region is used for interaction. (ii) The spRpb4·Rpb7 and AtB15.9/19.5 homologs form hybrid dimers with scRpb4·Rpb7 *in vitro* (6, 7) suggesting that Rpb4·Rpb7 interaction region is conserved among these species. (iii) Using two-hybrid analysis, the hsRpb7 interaction region in hsRpb4 has been mapped to the N-terminal 1–92 aa (5). Although this region encompasses some of the conserved regions, the C-terminal 23-aa conserved region is not involved in this interaction. This suggests that the hsRpb4 and scRpb4 have evolved differently to interact with their respective Rpb7 proteins. In keeping with this, the hsRpb7 protein interacts very weakly with scRpb4 (5).

The observation that deletion of any of the non-conserved regions in Rpb4 also abrogates interaction with Rpb7 (Fig. 5) throws up interesting possibilities. The simplest explanation could be that these regions are also involved in the interaction. However, this possibility will have to be discounted given that Rpb4·Rpb7 interaction is conserved from archaea to humans and none of the homologs have these regions. The other possibility is that these regions are involved in maintaining the conserved N- and C-terminal region helices, Helix 1 and Helix 2 (Fig. 2*B*), in an appropriate conformation for interaction with Rpb7. The exact structural fold adopted by these non-conserved regions is not yet known. Based on the 12-subunit crystal structures (28, 29) and the model building of scRpb4·Rpb7 interaction, it can be predicted that the basic stretch immediately following Helix 1 extends the Helix 1 further away from Helix 2. The other non-conserved regions probably adopt a fold that will bridge Helix 1 to Helix 2. Therefore, any deletion in the non-conserved region of the protein will affect the juxtaposition of the helices 1 and 2 of the conserved regions and hence the interaction between Rpb4 and Rpb7. To further understand the roles played by the non-conserved regions of Rpb4 in interaction with Rpb7, point mutants of Rpb4 defective for interaction with Rpb7 need to be isolated and characterized. A genetic screen to isolate these mutants is underway.

We have earlier reported that the conserved C-terminal 23 aa of Rpb4 are required for growth at high temperatures and efficient sporulation (15). Our analysis of deletions of the conserved and non-conserved regions of Rpb4 allows us to predict that the deletion of only the C-terminal conserved region affects these phenotypes. Lack of the N-terminal region, or any of the non-conserved regions, does not affect the ability of Rpb4 to function as well as the full-length protein in these phenotypes (Figs. 3*A*, 3*B*, 6*A*, and 6*B*). It is to be noted here that the deletion of the entire Sc-specific region (Δ66–140) has a marginal effect on the growth of *rpb4*Δ strains at 37 °C but not on sporulation. These observations are in keeping with the fact that spRpb4 and hsRpb4 complement the temperature sensitivity of the *rpb4*Δ strain (5, 6). The C-terminal 23 aa of scRpb4 is more conserved in spRpb4 than the hsRpb4, possibly explaining the robust growth of *rpb4*Δ with spRpb4 as compared with hsRpb4 (11, 12). Our analysis for the region of Rpb4 involved in inhibiting pseudohyphal growth of *rpb4*Δ strains did not provide any conclusive answers. Deletions of any of the conserved and non-conserved regions do not affect the ability of Rpb4 to rescue pseudohyphal growth of *rpb4*Δ strains suggesting that these regions are not involved in this phenotype (Figs. 3*C* and 6*C*). As these deletions encompass most of the protein, we surmise that the regions not deleted in this process (aa 141–198) are the regions involved in this phenotype. This could possibly be involved in interactions with novel proteins involved in inhibiting pseudohyphal growth.

Many groups, including ours, have shown that overexpression of Rpb7 can rescue the temperature sensitivity associated with *rpb4*Δ suggesting that one of the roles of Rpb4 is to stabilize interaction of Rpb7 with RNA polymerase II (19, 20, 22). It has also been hypothesized that the temperature sensitivity of *rpb4*Δ is due to the weakened interaction of Rpb7 with the rest of the polymerase. The fact that the N-terminal deletion of Rpb4 is compromised for interaction with Rpb7 but is still able to rescue temperature sensitivity of *rpb4*Δ (Figs. 2*A* and 3*A*) suggests that Rpb4 and Rpb7 have independent abilities to rescue temperature sensitivity of *rpb4*Δ.

We find a similar pattern of results for domains of Rpb4 involved in activated transcription from the P_{GAL10}- and HSE-containing promoters as for the temperature sensitivity and sporulation. The C-terminal 23 aa might play a role in all these phenotypes. The N-terminal 32 aa deletion retains ~70–80% of full-length activity from the P_{GAL10}- and HSE-containing promoters, whereas the internal deletions are indistinguishable from the full-length protein (Figs. 4 (*A* and *B*) and 7 (*A* and *B*)). On the other hand, transcriptional activation from the *INO1* promoter requires both the N- and the C-terminal regions. Deletion of the N-terminal region of Rpb4 or the internal deletions of the non-conserved regions lowers activity from the P_{INO1} promoter. Deletion of the entire Sc-specific region (aa 66–140) or deletion of the aa 66–105 has more pronounced effects on the activity than deletions of individual stretches (Figs. 4*C* and 7*C*). As the case with the domains involved in interaction with Rpb7, it is not possible to differentiate at this point of time, whether these non-conserved regions are actually required for the activity from the *INO1* promoter or their deletion affects the conformation of the N- and C-terminal regions that are essential for this phenotype. The difference in the requirements for activation from the *GAL10*- and *HSE*-containing promoters and the *INO1* promoter could reflect differential interaction of Rpb4 with other proteins involved in transcription from these promoters. It is also worthwhile to note that the *INO1* promoter is known to be extremely sensitive to perturbations in RNA polymerase II activity. Reduction of the levels of Rpb1 or certain mutants of Rpb1 affects activity of the *INO1* promoter (40).

We have not assessed the protein levels of the mutants when expressed from their own promoters in CEN plasmids, because they are below the detection limits of the currently available anti-Rpb4 antibodies. The C-terminal truncation expressed from the Rpb4 promoter or as the fusion to GAL4AD rescues the pseudohyphal predisposition of *rpb4*Δ strains (Fig. 3*C* and data not shown). This suggests that, although the truncation affects protein stability (as assessed by the anti-Gal4AD Western blots) the reduced protein levels are sufficient to rescue this

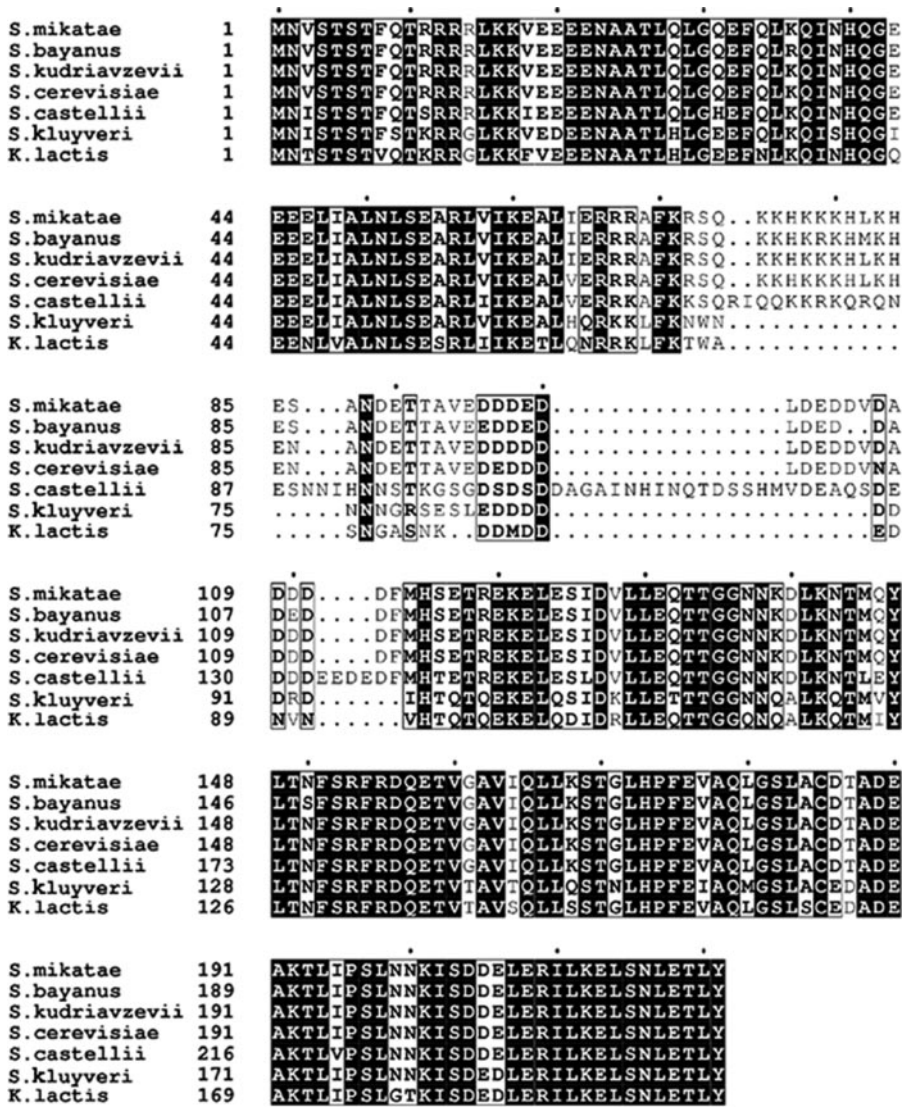


FIG. 8. **Alignment of Rpb4 sequences from closely related hemiascomycetes yeast species.** Rpb4 protein sequences from the species indicated were aligned using ClustalW and modified for print representation using ESPript 2.0 (43). The residues *outlined in black boxes* are identical across all homologs, whereas the *residues outlined in white boxes* are similar across all homologs.

particular phenotype. The finding that the deletion of the C-terminal region affects all the other phenotypes can be explained either by an absolute role for this region or the requirement for a more stable protein in the other phenotypes. Because the deletions of the conserved N terminus or all of the non-conserved regions do not affect any of the stress-responsive functions of Rpb4, it is possible to surmise that the C-terminal conserved regions of the protein (aa 140–221) are involved in these phenotypes.

Contrary to our expectations, we do not find any specific requirement for the non-conserved regions in *S. cerevisiae*-specific phenotypes. One explanation for their presence specifically in scRpb4 could be that they help stabilize a weaker conservation of scRpb4 in the N and the C termini. Analysis of the conserved regions between scRpb4 and the other homologs did not suggest any significant difference in the conserved region in the scRpb4 that could be stabilized by these non-conserved regions (data not shown). The other explanation could be that this is evolutionary "baggage" maintained by scRpb4, because it does not affect its function. However, the more complex systems have not maintained these extra sequences. Analysis of the pattern of conservation among closely related species would allow us to predict the evolutionary history of these sequences. Availability of the homologous sequence from *Kluveromyces lactis* (41) and 5 sequences from closely related *Saccharomyces* species (42) allowed us to compare the evolution of these regions. Rpb4 sequences from the evolutionarily distant *K. lactis* (diverged ~150 million years ago) and the *Saccharomyces* sensu lacto group (*S. castellii*) and petite negative group (*S. kluyveri*) show very high sequence similarity with *S. cerevisiae* in aa 112–140 region not conserved in higher eukaryotes (Fig. 8, compare with Fig. 1*A*). They also show a significant sequence similarity in the acidic stretch (aa 95–105). These sequences however lack nearly two-thirds of the "Basic Stretch." Interestingly, *S. castellii* sequence has a 24-aa unique insertion in between the acidic stretch. Rpb4 sequences from the *Saccharomyces* sensu stricto group (*S. bayanus*, *S. mikatae*, and *S. kudriazverii*) are almost identical to *S. cerevisiae* sequence throughout as expected from sequences that have diverged only a few million years ago. This analysis shows that there has been a progressive loss of the non-conserved regions, because the organisms have diverged with higher eukaryotes retaining only the functionally important N- and C-terminal regions.

It is interesting to note that, unlike in *S. cerevisiae*, spRpb4 is an essential protein and in *S. pombe* and higher eukaryotes, the sub-complex does not dissociate easily from the rest of the polymerase (6, 7). It is not yet clear whether the non-conserved regions of scRpb4 promote easier dissociation from the rest of the polymerase and, therefore, regulate activity under certain conditions. It is possible that, in the absence of the non-conserved regions, the higher eukaryotic Rpb4s have evolved a

stronger interaction with the rest of the polymerase and associated proteins and hence are important for growth and survival under all conditions.

In conclusion, our deletion analysis has allowed us to demarcate the roles for the conserved N- and C-terminal regions. Both these regions are important for interaction with Rpb7 and for transcription from the *INO1* promoter. The C-terminal conserved region may be involved in the stress-responsive role of Rpb4 and in activated transcription from a subset of genes. The non-conserved regions do not seem to be involved directly in any of these phenotypes. In fact, these regions seem to be required to maintain the conserved N- and C-terminal regions in an appropriate conformation, and their deletion affects only those phenotypes (interaction with Rpb7 and activated transcription from the *INO1* promoter) that require both the N- and the C-terminal conserved regions.

Acknowledgments—We thank Drs. E. Golemis, L. Nover, and U. Vijayaraghavan for the gift of various strains and plasmids and J. Bhat for the gift of anti-Gal4 antibodies used in this study. We are grateful to Dr. P. Cliften and Dr. M. Johnston and the Washington University Genome Sequencing Center for sharing the sequences of related *Saccharomyces* species prior to publication. P. S. and N. S. thank all members of their respective laboratories for discussions and helpful suggestions.

REFERENCES

1. Hampsey, M. (1998) *Microbiol. Mol. Biol. Rev.* **62,** 465–503
2. Edwards, A. M., Kane, C. M., Young, R. A., and Kornberg, R. D. (1991) *J. Biol. Chem.* **266,** 71–75
3. Choder, M., and Young, R. A. (1993) *Mol. Cell. Biol.* **13,** 6984–6991
4. Kolodziej, P. A., Woychik, N., Liao, S. M., and Young, R. A. (1990) *Mol. Cell. Biol.* **10,** 1915–1920
5. Khazak, V., Estojak, J., Cho, H., Majors, J., Sonoda, G., Testa, J. R., and Golemis, E. A. (1998) *Mol. Cell. Biol.* **18,** 1935–1945
6. Sakurai, H., Mitsuzawa, H., Kimura, M., and Ishihama, A. (1999) *Mol. Cell. Biol.* **19,** 7511–7518
7. Larkin, R. M., and Guilfoyle, T. J. (1998) *J. Biol. Chem.* **273,** 5631–5637
8. Werner, F., Eloranta, J. J., and Weinzierl, R. O. (2000) *Nucleic Acids Res.* **28,** 4299–4305
9. Siaut, M., Zaros, C., Levivier, E., Ferri, M. L., Court, M., Werner, M., Callebaut, I., Thuriaux, P., Sentenac, A., and Conesa, C. (2003) *Mol. Cell. Biol.* **23,** 195–205
10. Peyroche, G., Levillain, E., Siaut, M., Callebaut, I., Schultz, P., Sentenac, A., Riva, M., and Carles, C. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 14670–14675
11. Shpakovski, G. V., Gadal, O., Labarre-Mariotte, S., Lebedenko, E. N., Miklos, I., Sakurai, H., Proshkin, S. A., Van Mullem, V., Ishihama, A., and Thuriaux, P. (2000) *J. Mol. Biol.* **295,** 1119–1127
12. Khazak, V., Sadhale, P. P., Woychik, N. A., Brent, R., and Golemis, E. A. (1995) *Mol. Biol. Cell* **6,** 759–775
13. Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Wheeler, D. L. (2003) *Nucleic Acids Res.* **31,** 23–27
14. Woychik, N. A., and Young, R. A. (1989) *Mol. Cell. Biol.* **9,** 2854–2859
15. Pillai, B., Verma, J., Abraham, A., Francis, P., Kumar, Y., Tatu, U., Brahmachari, S. K., and Sadhale, P. P. (2003) *J. Biol. Chem.* **278,** 3339–3346
16. Rosenheck, S., and Choder, M. (1998) *J. Bacteriol.* **180,** 6187–6192
17. Miyao, T., Barnett, J. D., and Woychik, N. A. (2001) *J. Biol. Chem.* **276,** 46408–46413
18. Bourbonnais, Y., Faucher, N., Pallotta, D., and Larouche, C. (2001) *Mol. Gen. Genet.* **264,** 763–772
19. Sharma, N., and Sadhale, P. P. (1999) *J. Genet.* **78,** 149–156
20. Sheffer, A., Varon, M., and Choder, M. (1999) *Mol. Cell. Biol.* **19,** 2672–2680
21. Tan, Q., Li, X., Sadhale, P. P., Miyao, T., and Woychik, N. A. (2000) *Mol. Cell. Biol.* **20,** 8124–8133
22. Maillet, I., Buhler, J. M., Sentenac, A., and Labarre, J. (1999) *J. Biol. Chem.* **274,** 22586–22590
23. Pillai, B., Sampath, V., Sharma, N., and Sadhale, P. (2001) *J. Biol. Chem.* **276,** 30641–30647
24. Ausubel, F. M., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J. A., and Struhl, K. (1987) in *Current Protocols in Molecular Biology*, pp. 13.0.1–13.13.9, John Wiley & Sons, New York, NY
25. Sherman, F., Fink, G. R., and Lawrence, C. W. (1983) *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26. Rose, M., and Botstein, D. (1983) *Methods Enzymol.* **101,** 167–180
27. Guarente, L. (1983) *Methods Enzymol.* **101,** 181–191
28. Armache, K. J., Kettenberger, H., and Cramer, P. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 6964–6968
29. Bushnell, D. A., and Kornberg, R. D. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 6969–6973
30. Todone, F., Brick, P., Werner, F., Weinzierl, R. O., and Onesti, S. (2001) *Mol. Cell* **8,** 1137–1143
31. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22,** 4673–4680
32. Bateman, A., Birney, E., Cerruti, L., Durbin, R., Etwiller, L., Eddy, S. R., Griffiths-Jones, S., Howe, K. L., Marshall, M., and Sonnhammer, E. L. (2002) *Nucleic Acids Res.* **30,** 276–280
33. Blundell, T., Carney, D., Gardner, S., Hayes, F., Howlin, B., Hubbard, T., Overington, J., Singh, D. A., Sibanda, B. L., and Sutcliffe, M. (1988) *Eur. J. Biochem.* **172,** 513–520
34. Srinivasan, N., and Blundell, T. L. (1993) *Protein Eng.* **6,** 501–512
35. Rekha, N., and Srinivasan, N. (2003) *BMC. Struct. Biol.* **3,** 4
36. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., and Bourne, P. E. (2000) *Nucleic Acids Res.* **28,** 235–242
37. Sadhale, P., Sharma, N., Pillai, B., Katoch, A., Acharya, N., and Singh, S. K. (1998) *J. Biosci.* **23,** 331–335
38. Ruet, A., Sentenac, A., Fromageot, P., Winsor, B., and Lacroute, F. (1980) *J. Biol. Chem.* **255,** 6450–6455
39. Tan, Q., Prysak, M. H., and Woychik, N. A. (2003) *Mol. Cell. Biol.* **23,** 3329–3338
40. Archambault, J., Jansma, D. B., and Friesen, J. D. (1996) *Genetics* **142,** 737–747
41. Bolotin-Fukuhara, M., Toffano-Nioche, C., Artiguenave, F., Duchateau-Nguyen, G., Lemaire, M., Marmeisse, R., Montrocher, R., Robert, C., Termier, M., Wincker, P., and Wésolowski-Louvel, M. (2000) *FEBS Lett.* **487,** 66–70
42. Cliften, P., Sudarsanam, P., Desikan, A., Fulton, L., Fulton, B., Majors, J., Waterston, R., Cohen, B. A., and Johnston, M. (2003) *Science* **301,** 71–76
43. Gouet, P., Courcelle, E., Stuart, D. I., and Metoz, F. (1999) *Bioinformatics* **15,** 305–308
44. Gietz, R. D., and Sugino, A. (1988) *Gene* (*Amst.*) **74,** 527–534
45. Scafe, C., Chao, D., Lopes, J., Hirsch, J. P., Henry, S., and Young, R. A. (1990) *Nature* **347,** 491–494
46. Slater, M. R., and Craig, E. A. (1987) *Mol. Cell. Biol.* **7,** 1906–1916