Whole Genome Expression Profiles of Yeast RNA Polymerase II Core Subunit, Rpb4, in Stress and Nonstress Conditions

Received for publication, December 20, 2001, and in revised form, October 17, 2002
Published, JBC Papers in Press, November 11, 2002, DOI 10.1074/jbc.M112180200

Beena Pillai‡, Jiyoti Verma‡, Anju Abraham‡, Princy Francis‡, Yadunanda Kumar‡, Utpal Tatu§, Samir K. Brahmacari§, and Parag P. Sadhale¶

From the Departments of §Microbiology and Cell Biology and ¶Biochemistry, Indian Institute of Science, Bangalore, 560 012, India and the ¶Institute for Genomics & Integrative Biology, New Delhi, 110007 India

Organisms respond to environmental stress by adopting changes in gene expression at the transcriptional level. Rpb4, a nonessential subunit of the core RNA polymerase II has been proposed to play a role in nonstress-specific transcription and in the regulation of stress response in yeast. We find that in addition to the temperature sensitivity of the null mutant of Rpb4, diploid null mutants are also compromised in sporulation and show morphological changes associated with nitrogen starvation. Using whole genome expression analysis, we report here the effects of Rpb4 on expression of genes during normal growth and following heat shock and nutritional starvation. Our analysis shows that Rpb4 affects expression of a small yet significant fraction of the genome in both stress and normal conditions. We found that genes involved in galactose metabolism were dependent on the presence of Rpb4 irrespective of the environmental condition. Rpb4 was also found to affect the expression of several other genes specifically in conditions of nutritional starvation. The general defect in the absence of Rpb4 is in the expression of metabolic genes, especially those involved in carbon metabolism and energy generation. We report that various stresses are affected by RPB4 and that on overexpression the stress-specific activators can partially rescue the corresponding defects.

The survival of a cell depends on its ability to respond rapidly to environmental changes. This involves sensing small changes in multiple parameters, integrating the signals together, and rapidly changing the expression profile. Temperature and nutrient levels are prone to frequent fluctuations in the environment and elicit rapid and transient genome-wide changes. Although transcriptional changes during stress response have been studied extensively, relatively little is known about the contribution of core RNA polymerase II in bringing about these changes in the transcriptional program of the cell.

The yeast RNA polymerase II is composed of 12 subunits, Rpb1–Rpb12. Rpb1, Rpb2, and Rpb3/Rpb11 are homologs of the bacterial core RNA polymerase subunits. Rpb5, Rpb6, Rpb8, Rpb10, and Rpb12 are shared between the three RNA polymerases, I, II, and III (1). Rpb1, Rpb2, and Rpb3/Rpb11 are homologs of the bacterial core RNA polymerase subunits. Rpb5, Rpb6, Rpb8, Rpb10, and Rpb12 are shared between the three RNA polymerases, I, II, and III (1). Rpb5 has been shown to have a role in transcriptional activation (2). Rpb4 and Rpb9 are nonessential for normal growth, but their deletion results in temperature sensitivity (3, 4). Rpb4 interacts with Rpb7, a smaller essential subunit, to form a subcomplex, which dissociates from the polymerase on mild denaturation (5). The stoichiometry of Rpb4 within the polymerase increases during the stationary phase (6).

Another interesting feature of the Rpb4 subunit is that in its absence cells exhibit a wide variety of phenotypes associated with stress conditions. An rpb4Δ mutant is unable to survive at extreme temperatures (> 34 °C and < -12 °C) and dies rapidly during a prolonged stationary phase (3, 6). Recently, several groups have shown that Rpb4 plays an important role in the activation of many genes (7, 8) but has a milder effect on the basal expression of these genes. Expression analysis of specific genes showed that RNA polymerase II from the mutant lacking Rpb4 cannot transcribe some genes (9). Whole genome expression profiles and two-dimensional gel electrophoresis of proteins have shown that in the absence of Rpb4, the polymerase is inactivated at high temperature (37 °C for 45 min to 1 h) (10, 11). Recently, we have reported the initial observations from whole genome expression analysis of rpb4Δ mutant before and after heat shock (12).

We report here that the stress-associated effects of Rpb4 extend beyond temperature sensitivity. Heat shock, a short and transient exposure to high temperature, reveals a transcriptional pattern in rpb4Δ that is strikingly different from that of wild type cells. We have studied whole genome expression profiles of haploid and diploid yeast cells, under conditions of normal growth and starvation. Comparison of expression profiles of rpb4Δ and wild type cells show that in the absence of Rpb4, some genes involved in specific pathways of stress response are down-regulated in the corresponding condition. In general, in the absence of Rpb4, the transcription of many genes involved in key physiological pathways like glycolysis and energy generation is affected. Nevertheless, Rpb4 is not an essential gene. Therefore, the aberrant stress response defects shown by yeast cells in the absence of RPB4 may be a consequence of an underlying defect in fine tuning the expression of metabolic genes.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—The rpb4Δ and wild type strains (SY10-1: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2-1, rpb4Δ::HIS3/pPS2; SY10-2: Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2, rpb4Δ::HIS3/pNS114) have been described before (8). The corresponding diploid rpb4Δ/rpb4Δ strain was generated by crossing SY10 (Mat a, his3Δ200, ura3-52, leu2-3, 112, lys2-1, rpb4Δ::HIS3) to its Mata sibling. The diploids were also transformed with pPS2 (vector) and pNS114 (pPS2 carrying RPB4 gene). The CGX19 (MATα/α, ura3-52/ura3-52, trp1-102/trp1-102, his3Δ200/his3Δ200, leu2-3/leu2-3, rps15Δ::HIS3) strain was a kind gift from Dr. Erica Golermis. PHD1 as well as IME4 open reading frames were PCR-amplified and expressed in yeast from multicopy plas-
Base without ammonium sulfate and amino acids, 0.05 mM ammonium water, and transferred to sterile SLAD medium (0.67% yeast nitrogen for RNA isolation.

The yeast cultures were grown until mid-log phase at 25 °C in YEPD (13). The cells were harvested, washed with sterile water, and transferred to sterile sporulation medium (1% potassium sulfate, 2% glucose). The cells were harvested after 12 h of incubation in sporulation medium for RNA isolation.

**Nitrogen Starvation**—Yeast cells were grown until mid-log phase at 25 °C in YEPD (13). The cells were harvested, washed with sterile water, and transferred to sterile SLAD medium (0.67% yeast nitrogen base without ammonium sulfate and amino acids, 0.05 mM ammonium sulfate, 2% glucose). The cells were harvested after 12 h of incubation in SLAD medium for RNA isolation.

**RNA Isolation and Microarray Analysis**—A detailed account of the methodology used is published (12). Briefly, the yeast whole genome microarray slides were procured from the Microarray Facility of the Ontario Cancer Centre. RNA was isolated using liquid nitrogen lysis protocol and labeled using a Micromax TSA kit (PerkinElmer Life Sciences).

**Glucose Estimation**—Yeast cells were inoculated from overnight cultures into synthetic medium at similar densities and grown in a shaker (250 rpm) at 25 °C (13). Sterile medium was used as control for the amount of glucose. The samples were recovered at appropriate times during the growth curve, the cells were removed by centrifugation, and the supernatant was used for glucose estimation. 100 μl of suitable dilution was mixed with 3.5-dinitrosalicylic acid (DNSA) reagent and boiled for 10 min. The tubes were cooled to room temperature, and the solutions were diluted to 1 ml. Absorbance at 540 nm was used to calculate the glucose concentration from a standard graph. The concentration of glucose at the time of inoculation was taken as 100%. The percentage glucose utilized at time t = [amount of glucose at t₀] – [amount of glucose at t] × 100/amount of glucose at t₀ and plotted against time in hours.

**RESULTS**

Rpb4 a nonessential subunit of the RNA polymerase II, is required for the survival of yeast cells at extreme temperatures above 34 °C and below 12 °C (3). At room temperature, the rpb4Δ mutant grows slowly and loses viability rapidly in stationary phase (3, 6). We constructed homozygous null mutants at the RPB4 locus to study the effect of Rpb4 on other stress responses. We found that the rpb4Δ/rpb4Δ strain was unable to sporulate efficiently compared with a rpb4Δ/rpb4Δ strain carrying a plasmid expressing RPB4 under the control of its own promoter (Fig. 1A). Overexpression of RPB4 resulted in a further increase in sporulation levels (Fig. 1B). We also observed that under nitrogen starvation conditions, rpb4Δ/rpb4Δ cells were more elongated than the wild type and bud in a unipolar budding pattern. This pattern resembles pseudohyphae formation. Expression of RPB4 restored the normal cell shape and budding pattern (Fig. 1C).

Previous studies on the various aspects of transcription in rpb4Δ cells have led to the suggestion that RNA polymerase II is unable to function effectively at high temperatures in the absence of Rpb4 (5, 9–11). To understand the effect of Rpb4 on transcription during normal and stress conditions, we determined whole genome expression patterns using microarray analyses under different stresses. The various strains and conditions used in each experiment are summarized in Table I. RNA was isolated from the null mutant of RPB4 carrying...
either a vector or a centromeric plasmid bearing RPB4 gene (under the control of its own promoter) grown under identical conditions. The data points which showed consistent results in duplicate spots and reciprocal experiments were used for further analyses. We normalized the intensity of the signal from each spot to the total intensity in each channel. Following normalization, the intensity of the spots corresponding to ACT1 gene (actin) in both channels was comparable. The genes, which showed more than 2-fold differences consistently in each condition, were compiled. All further analysis was done using this data set. The total number of genes up-regulated and down-regulated in the mutant as compared with the wild type are tabulated (Table II). Under normal growth conditions Rpb4 affects the expression of 120 and 121 genes, respectively in haploid and diploid yeast cells. The effect of Rpb4 is on a similar scale in other conditions of stress such as sporulation and nitrogen starvation. In all conditions, except heat shock, the genes affected by Rpb4 amounted to nearly 1.7% of the genome. Following a short duration of heat shock, the effect of Rpb4 is more pronounced, extending to 9.2% of the genome (589 genes: 237 up-regulated and 352 down-regulated).

We examined the effect of Rpb4 in various stress and non-stress conditions by comparing the overlap between the down-regulated genes in various conditions. The Venn diagrams in Fig. 2 show that the extent of overlap in the affected genes is minimal between stress and nonstress conditions. Overall, the transcriptional effects of RPB4 seem to be specific for the environmental condition because there are very few genes that are dependent on Rpb4 under all conditions. The overlap between the expression profiles under various stress conditions is marginally higher than that between nonstress and stress conditions.

We classified the known genes according to their functional roles as annotated in the Munich Information Centre for Protein Sequences data base (Table III). In all experiments, a substantial number of the affected genes were of unknown functions, and the largest number of genes affected by the absence of Rpb4 were involved in metabolism and energy generation. Hence, the general defect associated with rpb4Δ seems to be its inability to express metabolic genes properly. In addition to this defect in the expression of basic physiological pathways, specific defects associated with each condition studied are summarized below.

Heat Shock-specific Defects—Rpb4 has a pronounced effect on gene expression following heat shock. In comparison with other stress and nonstress conditions, a larger number of genes are affected following heat shock. Functional classification of these genes revealed that they are involved in basic metabolic pathways. A striking feature of the transcriptional profile was that rpb4Δ cells after heat shock showed higher levels of transcripts of genes involved in protein synthesis. These include genes, which code for 97 ribosomal proteins (of 132), proteins of the cap-binding complex, translation initiation factors, aminoacyl tRNA synthetases, components of the ribosome associated complex, and proteins involved in processing and transport of rRNA. It is well known that yeast cells transiently repress genes involved in protein synthesis following heat shock (20). The increase in expression of these genes is probably a reflection of a defect in this repression.

Sporulation-specific Defects—Wild type and rpb4Δ/rpb4Δ strains were grown in rich medium at permissive conditions until mid-log phase. The cells were then transferred to sporulation medium. Following 12 h of incubation in sporulation medium, 67 genes showed a more than 2-fold decrease in expression in rpb4Δ/rpb4Δ cells when compared with wild type cells under identical conditions. Interestingly, 22 of these 67 genes (33%) are on the right arm of the second chromosome. A majority of these genes are involved in carbon metabolism, as is the case in other conditions. RIM4, a regulatory gene, and SPS1, SPS2, SPS4, and SPS100 are down-regulated in rpb4Δ/rpb4Δ. The SPS genes are involved in spore wall synthesis and are usually induced during the late stages of sporulation (21). They require the master regulator of meiosis, Ime1, for their expression. This agrees well with our earlier observations, using 4,6-diamidino-2-phenylindole staining, electron microscopic analyses, and Northern analyses, that rpb4Δ/rpb4Δ cells are arrested in the early steps of meiosis and are unable to express early meiotic genes (data not shown). None of the 12 genes up-regulated under this condition is known to play any role in sporulation.

Defects Associated with Nitrogen Starvation Conditions—Despite the difference in morphology of rpb4Δ/rpb4Δ cells, we did not detect any general induction of genes known to be involved in pseudohyphae formation in these strains following nitrogen starvation. IRA1, BEM1, AXL1, and ADH1 are the only genes differentially expressed in rpb4Δ known to be important in pseudohyphae formation (22).

We clustered genes according to their ratio in the expression in mutant rpb4Δ (or rpb4Δ/rpb4Δ) to wild type in the five conditions tested (room temperature, heat shock, rich medium, sporulation condition, and pseudohyphal growth condition) as listed in Table I. Details of some interesting clusters are shown in Fig. 3. A more extensive table with the ratios of expression values of all of the differentially expressed genes is available as supplementary data (Table S1).

Many genes that code for hexose transporters of the major facilitator class were found to be down-regulated in the mutant lacking Rpb4. We therefore checked the rate of glucose uptake in these strains. We found that in agreement with the expression profile, haploid and diploid RPB4 deletion mutants consumed glucose slowly compared with the wild type. At mid-log phase, haploid and diploid wild type cells had exhausted 68 and
We observed that the sporulation defect was partially rescued when IME4 was overexpressed after transfer to sporulation medium. We found that there were gross changes in protein profiles reflecting the presence of Rpb4 resulted in altered transcriptional and protein profiles in normal and different stress conditions. We found that various unrelated genes are affected to different extents in rpb4Δ (8). On the other hand, the stress-related phenotypes of rpb4Δ cells like temperature sensitivity and lethality during the stationary phase point toward a stress-specific role for RPB4 in transcription. It was proposed that in the absence of Rpb4, the polymerase is unstable at 37 °C or above (1, 7, 9, 10). Instability of the polymerase lacking Rpb4 following exposure to high temperature is conceivable, but a similar mechanism may not fully explain the role of Rpb4 in other stresses. In addition to the known roles in survival at high temperature and stationary phase, we found that rpb4Δ cells were also defective in sporulation, a response to extreme starvation, and showed altered morphology associated with pseudohyphae formation.

There are many candidate transcriptional activators regulating pseudohyphae formation in Saccharomyces cerevisiae. We chose Phd1, a transcriptional activator that exaggerates pseudohyphae formation in strains predisposed to forming pseudohyphae (24). We overexpressed the Phd1 protein in the shr3-102/shr3-102 background (CGX19) as well as in rpb4Δ and the corresponding wild type strain. The overexpression in the CGX19 strain served as a control for overexpression of Phd1 because the CGX19 strain is also predisposed to pseudohyphae formation and shows an exaggerated pseudohyphal response (24). Overexpression of Phd1 resulted in exaggeration of pseudohyphae formation in rpb4Δ mutants as compared with wild type (Fig. 5C).

**TABLE II**

| Experiment | Ploidy/condition | Number of genes |
|------------|-----------------|-----------------|
|            |                 | Up-regulated | Down-regulated | Total | % of genome |
| 1          | Haploid, nonstress | 37 | 83 | 120 | 1.875 |
| 2          | Haploid, stress (temperature) | 237 | 352 | 589 | 9.20 |
| 3          | Diploid, nonstress | 46 | 75 | 121 | 1.89 |
| 4          | Diploid, stress (low nitrogen) | 11 | 108 | 119 | 1.85 |
| 5          | Diploid, stress (sporulation) | 12 | 67 | 79 | 1.23 |

**FIG. 2. Effect of Rpb4 on genome-wide transcription profile under various stress conditions.** The labeled cDNAs from wild type and the rpb4Δ haploid strain grown at room temperature or 39 °C for 30 min were used to probe S. cerevisiae whole genome microarrays. The Venn diagrams in the left and middle panels represent the overlap of number of genes down-regulated or up-regulated (at least 2-fold) in rpb4Δ strain under these conditions. The diploid homozygous rpb4Δ/rpb4Δ strain and the corresponding wild type strains were used to isolate RNA and detect differential expression of genes as described under “Experimental Procedures.” The Venn diagram in the right panel shows the overlap of genes down-regulated in diploid rpb4Δ/rpb4Δ strain in either rich medium, sporulation medium, or SLAD medium.

61% of the glucose provided in the medium, respectively. But in RPB4 deletion mutants these numbers dropped to 3 and 24%, respectively (Fig. 4). The homozygous diploid mutant compared with the haploid strain lacking Rpb4 seems to be less defective at high temperature and in glucose uptake, although the reasons for this difference are not clear. In normal and stress conditions rpb4Δ showed a distinctly different transcriptional profile compared with that of wild type cells. We compared protein profiles of the rpb4Δ cells to wild type under conditions in which we had found transcriptional differences. We found that there were gross changes in protein profiles reflecting the differences in transcription profiles (results not shown). Even under normal conditions of growth there are substantial differences in the levels of many proteins in rpb4Δ. Thus, the absence of Rpb4 resulted in altered transcriptional and protein profiles in normal and different stress conditions.

**Studies of Activator Overexpression in Rescue of Stress Response Phenotypes—**From previously reported studies and our results reported here, rpb4Δ mutants show: 1) defects in survival under extreme temperatures, 2) defects in sporulation, and 3) pseudohyphae-like morphology. We have previously reported that the activation defect of rpb4Δ strain can be partially rescued by overexpression of the cognate transcriptional activator (8). Msn2 (transcriptional activator in heat shock response) and some other proteins (that are not transcriptional activators) have been shown to partially rescue the temperature sensitivity of rpb4Δ cells (7). Because our focus in this manuscript is on other stress responses, we report here the effect of overexpression of specific transcriptional activators under each condition. We observed that in rpb4Δ cells the transcriptional activator IME4, of early meiosis gene IME1 (23), was not induced at all compared with the wild type cells, which showed a strong peak of induction at 0.5 h in sporulation medium (Fig. 5A). We used the inducible promoter P_CUP1 to overexpress IME4 at different times during pre growth in the rich medium and after transfer of cells in the sporulation medium. We observed that the sporulation defect was partially rescued when IME4 was overexpressed after transfer to sporulation medium (Fig. 5B).
genes detected show changes dependent on Rpb4. Only 9.5% of the genes dependent on Rpb4 after heat shock are similarly affected during normal growth. This implies that the role of Rpb4 in transcription immediately following heat shock is significantly different and more drastic from that in other stress conditions. It has a less conspicuous and probably different role during normal conditions of growth. Gasch et al. (25) and Causton et al. (26) have recently reported the genome-wide expression pattern during heat shock in yeast cells. Both reports show that genes involved in carbon metabolism, mitochondrial function, and glucose transport are up-regulated after heat shock, and genes involved in protein synthesis are down-regulated. In response to heat shock, rpb4Δ cells have lower transcript levels of genes involved in carbon metabolism and mitochondrial function and higher transcript levels of genes of ribosomal proteins as compared with wild type. It appears that rpb4Δ cells are incapable of adopting the normal transcriptional response to heat shock. Recently, genome-wide expression analyses done after exposing rpb4Δ cells to high temperatures for a relatively longer time than reported here has confirmed that most of the transcription in the cell is eventually shut down in rpb4Δ cells (10). Taken together, it appears that immediately following temperature stress, rpb4Δ cells do not adopt a normal heat shock-associated transcriptional response (repression of ribosomal proteins and up-regulation of mitochondrial genes, hexose transporters, and galactose metabolism genes). With prolonged incubation at high temperature, the polymerase lacking Rpb4 becomes incapable of transcribing 98% of the genome, the reach of this inactive polymerase being as wide as that of rpb1-1, a conditional mutant of the largest subunit of the polymerase (10).

The genes up-regulated and down-regulated after heat shock were grouped based on the factors (proteins, environmental factors, other inducers of gene expression) to which they are known to respond. These transcriptional regulators are probably either functionally correlated to Rpb4 or dependent on Rpb4 for their function. The maximum number of genes down-regulated in rpb4Δ after heat shock are known to be under the control of Msn2, the transcriptional activator of stress response element (STRE)-regulated genes (27). This agrees with the observation reported earlier that Msn2 overexpression can partially complement the temperature sensitivity of rpb4Δ cells (7). We tested the effect of overexpression of some of the transcriptional activators known to affect sporulation and pseudohyphae formation. It is difficult to choose the activators to be tested because the stress responses are complex phenotypes, and many regulators play important roles at various stages in the given response. We decided to test the effect of IME4 overexpression on sporulation because it is known to regulate IME1, which is one of the early positive regulators of meiotic genes. We have seen that rpb4Δ/rpb4Δ cells fail to induce endogenous IME4 under these conditions (Fig. 5A). Because the inducible CUP1 promoter is unaffected by the absence of Rpb4 (8), using the CUP1 promoter we induced the expression of IME4 in rpb4Δ/rpb4Δ cells in a manner similar to its expression in the wild type cells to overexpress IME4. The observation that the sporulation defect was rescued significantly only after induction of the activator supported our earlier observation. Similarly the overexpression of PHD1, a transcriptional activator known to enhance the pseudohyphal phenotype in strains predisposed to forming pseudohyphae, clearly enhanced the pseudohyphal morphology of rpb4Δ/rpb4Δ strain. This again supports the notion that the defect in transcriptional activation in rpb4Δ/rpb4Δ mutant is specific for certain sets of genes we proposed earlier (8).

The genes affected under heat shock conditions involved in

| Experiment | Up | Down |
|------------|----|------|
| Haploid/RT | 4  | 27   |
| Haploid/HIS| 228| 11   |
| Diploid/RT | 12 | 23   |
| Diploid/HIS| 29 | 17   |
| Diploid/Spor. | 12 | 11   |
| Genome     | 1063| 292  |

The classes with a substantially high number of genes are underlined. The total number of genes in each condition does not add up to the known genes detected because some genes that function in more than one class have been assigned to multiple groups.

### Functional classification of affected genes

| Experiment | Metabolism | Energy | Transport | Communication | Total |
|------------|------------|--------|-----------|---------------|-------|
| Haploid/RT | Up 4 | 14 | 10 | 1 | 29 |
| Haploid/HIS| Down 27 | 11 | 1 | 2 | 11 |
| Diploid/RT | Up 228 | 11 | 2 | 1 | 251 |
| Diploid/HIS| Down 1 | 1 | 1 | 1 | 1 |
| Diploid/Spor. | Up 12 | 1 | 1 | 1 | 16 |
| Genome     | 1063 | 292 | 11 | 1 | 5538 |
mitochondrial function were seen to be under the control of Hap4 and Gcn5 (28), both of which are also down-regulated in rpb4/H9004. Therefore the effect on mitochondrial function is probably due to Hap4 expression being compromised. A similar analysis of the up-regulated genes (during heat shock) revealed that most of these genes (47%) are co-regulated in the presence of rapamycin. This agrees well with previous reports from a high throughput screen that rpb4/H9004 is one of the mutations that confers rapamycin resistance (29). The deletion of RPB4 appears to have dual effects following heat shock: an immediate effect on many pathways critical to the physiology of the cell and an additional effect on the stability of the polymerase. In the absence of Rpb4, following prolonged exposure to high temperatures, the polymerase is rendered unstable leading to a defect in transcription as severe as in inactivation of the polymerase in an rpb1-1 mutant.

YGP1, a gene known to be highly induced in various stress responses (30) is among the most severely down-regulated genes in rpb4Δ/rpb4Δ under stress conditions. Mutants of IRA1 are known to constitutively express the cAMP-dependent PKA pathway, which regulates pseudohyphae formation (22). The down-regulation of IRA1 in rpb4Δ/rpb4Δ cells specifically following nitrogen depletion may be responsible for their tend-
The footprint of Rpb4 on the transcriptome in comparison with the other components of the transcription machinery

| Gene Symbol | Med6 | Gcn5 | Srb10a | Srb4 | Srb5 | Swi2 | Taf145 | Taf60 | Taf61 | Taf90 | Taf25 | Taf17 | Tsm1 | Tpk1 | Tpk2 | Tpk3 | Kin28 | Fcp1 |
|-------------|------|------|--------|------|------|------|--------|------|------|------|------|------|------|------|------|------|------|------|
| **RPB4**    | Up   | 14   | 78    | 168  | 29   | 59   | 200    | 54   | 49   | 97   | 153  | 169  | 52   | 153  | 138  | 149  | 138  | 37   | 103  |
|             | Down | 878  | 179   | 218  | 4755 | 675  | 116    | 1425 | 1736 | 633  | 490  | 1652 | 3488 | 4242 | 490  | 170  | 185  | 194  | 4658 | 195  |
| **RT**      | Up   | 37   | 1     | 2    | 1    | 2    | 1      | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
|             | Down | 83   | 13    | 3    | 14   | 3    | 6     | 5    | 9    | 8    | 8    | 11   | 4    | 1     | 3    | 5    | 4    | 55   | 4    | 55   | 4    |
| **HS**      | Up   | 237  | 4     | 2    | 4    | 2    | 4      | 3    | 5    | 3    | 5    | 3    | 5    | 3    | 5    | 3    | 5    | 3    | 5    | 3    | 5    |
|             | Down | 352  | 59    | 11   | 57   | 15   | 46     | 13   | 26   | 20   | 27   | 36   | 37   | 13    | 7    | 18   | 10   | 9    | 252  | 5    | 52   | 5    |
| **RI**      | Up   | 46   | 1     | 1    | 2    | 1    | 2      | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
|             | Down | 75   | 8     | 1    | 14   | 20   | 9      | 1    | 6    | 2    | 3    | 7    | 14   | 18    | 2    | 4    | 3    | 4    | 3    | 4    | 7    |
| **PS**      | Up   | 11   | 1     | 1    | 1    | 1    | 1      | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
|             | Down | 108  | 18    | 7    | 23   | 19   | 11     | 4    | 13   | 18   | 7    | 8    | 18    | 22    | 23   | 1    | 3    | 3    | 74   | 1    | 74   | 1    |
| **SP**      | Up   | 12   | 1     | 1    | 2    | 1    | 1      | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
|             | Down | 67   | 11    | 2    | 11   | 19   | 10     | 1    | 5    | 9    | 5    | 6    | 6     | 1     | 21   | 2    | 2    | 5    | 2    | 38   | 1    | 38   | 1    |

*a*Because Srb10 is a repressor; down-regulated genes in rpb4Δ have been compared with the genes up-regulated in absence of Srb10.

Acknowledgments—We hereby thank all members of our laboratories at the Indian Institute of Science and the Centre of Biochemical Technology. The microarray facility at Centre for Biochemical Technology was used for the work presented here. We especially acknowledge Dr. Ramachandran and his laboratory members (Centre of Biochemical Technology), Dr. Rambodhkar (Centre of Biochemical Technology), and Dr. Raja Mugasiamangalam (Genotypic Technologies) for help.

REFERENCES
1. Hampsey, M. (1998) Microbiol. Mol. Biol. Rev. 62, 465–503.
2. Miyas, T., and Woychik, N. A. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 15281–15286.
3. Woychik, N. A., and Young, R. A. (1989) Mol. Cell. Biol. 9, 2854–2859.
4. Woychik, N. A., Lane, W. S., and Young, R. A. (1991) J. Biol. Chem. 266, 19053–19055.
5. Edwards, A. M., Kane, C. M., Young, R. A., and Kornberg, R. D. (1991) J. Biol. Chem. 266, 71–75.
6. Choder, M., and Young, R. A. (1993) Mol. Cell. Biol. 13, 6984–6991.
7. Tan, Q. Li, X., Sadhale, P. P., Miyao, T., and Woychik, N. A. (2000) Mol. Cell. Biol. 20, 8124–8133.
8. Pillai, B., Sampath, V., Sharma, N., and Sadhale, P. P. (2001) J. Biol. Chem. 276, 30641–30647.
9. Rosenbeck, S., and Choder, M. (1998) J. Bacteriol. 170, 6187–6192.
10. Miyas, T., Barnett, J. D., and Woychik, N. A. (2001) J. Biol. Chem. 276, 46408–46413.
11. Maillet, I., Buhler, J. M., Sentenac, A., and Labarre, J. (1999) J. Biol. Chem. 274, 22586–22590.
12. Pillai, B., Brahmachari, S. K., and Sadhale, P. P. (2001) Curr. Sci. 81, 574–578.
13. Ausubel, F. M., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J. A., and Struhl, K. (1994) Current Protocols in Molecular Biology, John Wiley & Sons, New York.
14. Mewes, H. W., Albermann, K., Heumann, K., Liebl, S., and Pfeiffer, F. (1997) Nucleic Acids Res. 25, 26–30.
15. Cherry, J. M., Ball, C., Dolinski, K., Dwight, S., Harris, M., Matese, J. C., Sherlock, G., Binkley, G., Jin, H., Weng, S., and Botstein, D. (2001) Saccharomyces Genome Database [http://genome-www.stanford.edu/Saccharomyces].
16. Costanzo, M. C., Crawford, M. E., Hirschman, J. E., Kranz, J. E., Olsen, P., Robertson, L. S., Skrzypek, M. S., Braun, B. H., Hopkins, K. L., Kondu, P., Lenschea, C., Lew-Smith, J. E., Tillberg, M., and Garrels, J. I. (2001) Nucleic Acids Res. 29, 75–79.
17. Bailey, T. L., and Elkan, C. (1994) in: Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology 26, 28–36.
18. Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 14863–14868.
19. Marc, P., Devaux, F., and Jaeg, C. (2001) Nucleic Acids Res. 29, E633–E63.
20. Warner, J. R. (1999) Trends Biochem. Sci. 24, 437–440.
21. Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O., and Herskowitz, I. (1998) Science 282, 909–915.
22. Palecek, S. P., Parikh, A. S., and Korn, S. J. (2000) Genetics 156, 1005–1023.
23. Shah J. C., and Clancy, M. J. (1992) J. Biol. Chem. 267, 2112–2118.
24. Giumeno, C. J., and Fink, G. R. (1994) Mol. Cell. Biol. 14, 2100–2112.
25. Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B.,
    Storz, G., Botstein, D., and Brown, P. O. (2000) Mol. Biol. Cell 11,
    4241–4257
26. Causton, H. C., Ren, B., Koh, S. S., Harbison, C. T., Kanin, E., Jennings, E. G.,
    Lee, T. I., True, H. L., Lander, E. S., and Young, R. A. (2001) Mol. Biol. Cell
    12, 323–337
27. Moskvina, E., Imre, E. M., and Ruis, H. (1999) Mol. Microbiol. 32,
    1283–1272
28. Georgakopoulos, T., and Thireos G. (1992) EMBO J. 11, 4145–4152
29. Chan, T. F., Carvalho, J., Riles, L., and Zheng, X. F. (2000) Proc. Natl. Acad.
    Sci. U. S. A. 97, 13227–13232
30. Werner-Washburne, M., Braun, E. L., Crawford, M. E., and Peck, V. M. (1996)
    Mol. Microbiol. 19, 1159–1166
31. Entian, K. D., Schuster, T., Hogemann, J. H., Becher, D., Feldmann, H.,
    Guldener, U., Gotz, R., Hansen, M., Hollenberg, C. P., Jansen, G., Kramer,
    W., Klein, S., Kotter, P., Kricke, J., Launhardt, H., Mannhaupt, G., Maierl,
    A., Meyer, P., Mewes, W., Munder, T., Niedenthal, R. K., Rad, M. R.,
    Rohner, A., Romer, A., Rose, M., Schafer, B., Siegler, M. L., Vetter, J.,
    Wilhelm, N., Wolf, K., Zimmermann, F. K., Zollner, A., and Hinnen, A.
    (1999) Mol. Gen. Genet. 262, 683–702
32. Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J.,
    Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) Cell. 95,
    717–728
33. Kimura, M., Suzuki, H., and Ishihama, A. (2002) Mol. Cell. Biol. 22,
    1577–1588