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

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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). 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 *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ and wild type strains (SY10-1: *Mat a, his3*Δ*-200, ura3-52, leu2-3, 112, lys2-1, rpb4*Δ*::HIS3*/pPS2; SY10-2: *Mat a, his3*Δ*-200, ura3-52, leu2-3, 112, lys2, rpb4*Δ*::HIS3*/pNS114) have been described before (8). The corresponding diploid *rpb4*Δ/*rpb4*Δ strain was generated by crossing SY10 (*Mat a, his3*Δ*-200, ura3-52, leu2-3, 112, lys2-1, rpb4*Δ*::HIS3*) to its Mat*α* sibling. The diploids were also transformed with pPS2 (vector) and pNS114 (pPS2 carrying *RPB4* gene). The CGX19 (*MATa*/*α, ura 3-52*/*ura 3-52, shr3-102*/*shr3-102*) strain was a kind gift from Dr. Erica Golemis. *PHD1* as well as *IME4* open reading frames were PCR-amplified and expressed in yeast from multicopy plas-

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[S] The on-line version of this article (available at *http://www.jbc.org*) contains a supplemental table (Table SI).

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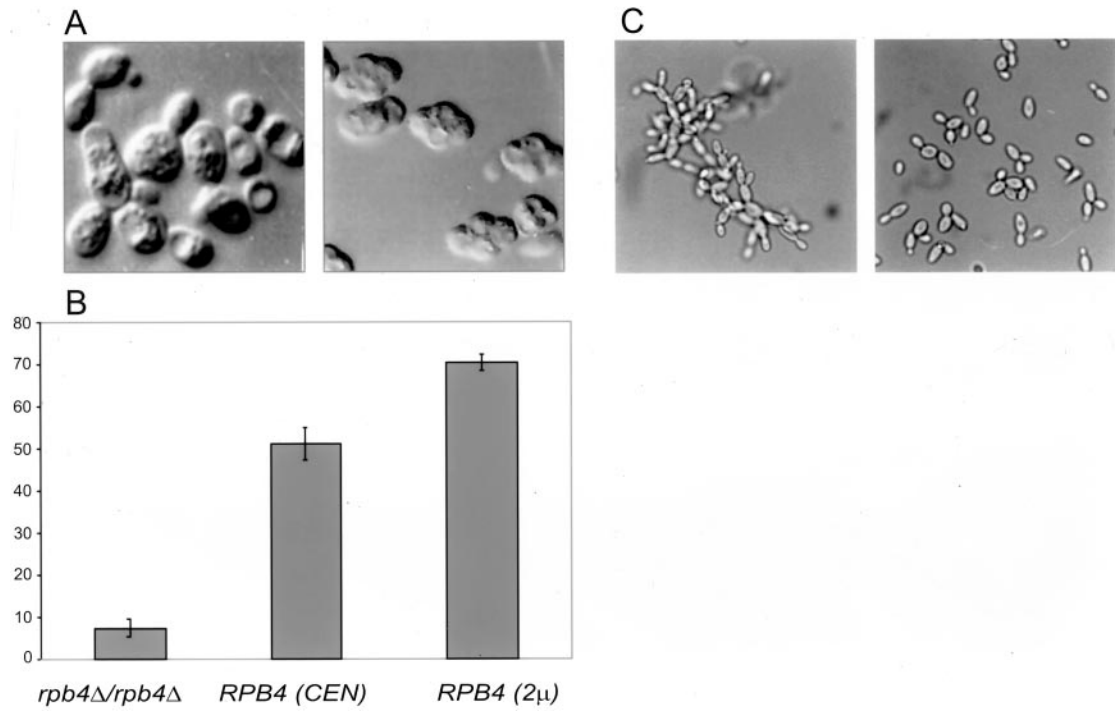

FIG. 1. **Diploid cells lacking Rpb4 are defective in sporulation but predisposed to pseudohyphae formation.** *A*, the *rpb4D/rpb4*Δ strain transformed with a vector (*left panel*) or a *RPB4* gene containing plasmid (*right panel*) was grown in rich medium and transferred to sporulation medium. The cells were photographed (at 800×) after 4 days. *B*, sporulation percentage after 4 days was measured and plotted as a percentage of total cell numbers (at least 500 cells/strain). *C*, the two strains used in *A* were grown in SLAD medium and photographed (at 400×) after 18 h. The *left panel* shows *rpb4D/rpb4*Δ cells with vector, whereas the *right panel* shows *rpb4D/rpb4*Δ cells with the *RPB4* (CEN) plasmid.

mids. The PCR primers used for *IME4* amplification were IME4f (5′-CGG GAA TTC AAT AAA AGT TGT AAG CAG GC-3′) and IME4r (5′-CCC GCT GCA GTC TTT TTT ATG ACC A-3′), and those for *PHD1* were PHD1f (5′-AGC AGA TCT ATA TGT ACC ATG TCC C-3′) and PHD1r (5′-CCC GAA TTC ATG ATA ATT CAT TTT TTG C-3′). PCR amplification was carried out by routine methods as described (13). The media used for routine growth and manipulation of yeast cultures were made as described (13).

Sporulation—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 acetate). Sporulation counts were performed using a hemocytometer after 4 days and are expressed as percentages of total cells (number of tetrads × 100/number of cells); at least 500 cells/sample were counted. The cells were harvested after 12 h of incubation in sporulation medium for RNA isolation.

Nitrogen Starvation—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 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 t_0 − amount of glucose at t] × 100/amount of glucose at t_0 and plotted against time in hours.

Data Bases and Software Used—Data from the Munich Information Centre for Protein Sequences data base, the *Saccharomyces* Genome Data Base, and the Yeast Proteome Data Base were used to classify genes according to their function (14–16). SCPD (*Saccharomyces cerevisiae* Promoter Database) was used to retrieve promoter sequences, and MEME (Multiple Em for Motif Elicitation) was used to analyze them (17). Clustering of gene expression data was carried out using CLUSTER (18). Data from published microarray experiments used to compare with data generated in our experiments was retrieved using yMGV (Yeast Microarray Global Viewer) (19).

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 *rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ strain was unable to sporulate efficiently compared with a *rpb4*Δ/*rpb4*Δ strain carrying a plasmid expressing *RPB4* under the control of its own promoter (Fig. 1*A*). Overexpression of *RPB4* resulted in a further increase in sporulation levels (Fig. 1*B*). We also observed that under nitrogen starvation conditions, *rpb4*Δ/*rpb4*Δ 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. 1*C*).

Previous studies on the various aspects of transcription in *rpb4*Δ 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

TABLE I
Summary of strain backgrounds and conditions in each experiment
In experiments 2, 4, and 5 the cultures were pregrown in YEPD for 12 h at 25 °C before being subjected to the stress

Experiment	Strains compared	Conditions			
		Growth medium	Temperature	Time	Ploidy
			°C		
1	*rpb4*Δ + vector *rpb4*Δ + *RPB4*	YPD	25	12 h	*n*
2	*rpb4*Δ + vector *rpb4*Δ + *RPB4*	YPD	39	30 min	*n*
3	*rpb4*Δ/*rpb4*Δ + vector *rpb4*Δ/*rpb4*Δ + *RPB4*	YPD	25	12 h	2*n*
4	*rpb4*Δ/*rpb4*Δ + vector *rpb4*Δ/*rpb4*Δ + *RPB4*	SLAD	25	12 h	2*n*
5	*rpb4*Δ/*rpb4*Δ + vector *rpb4*Δ/*rpb4*Δ + *RPB4*	1% potassium acetate	25	12 h	2*n*

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 nonstress 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 *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ. 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 *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ known to be important in pseudohyphae formation (22).

We clustered genes according to the ratio of their expression in mutant *rpb4*Δ (or *rpb4*Δ/*rpb4*Δ) 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 SI).

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

TABLE II
Summary of genes affected in rpb4Δ mutant in all experiments

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

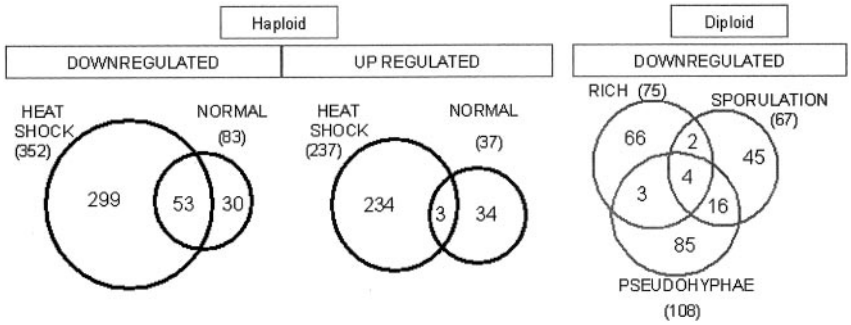

FIG. 2. **Effect of Rpb4 on genome-wide transcription profile under various stress conditions.** The labeled cDNAs from wild type and the *rpb4*Δ haploid strain grown at room temperature or 39 °C for 30 min were used to probe *S. cerevisiae* whole genome microarrays. The Venn diagrams in the *left* and *middle panels* represent the overlap of number of genes down-regulated or up-regulated (at least 2-fold) in *rpb4*Δ strain under these conditions. The diploid homozygous *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ showed a distinctly different transcriptional profile compared with that of wild type cells. We compared protein profiles of the *rpb4*Δ 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 *rpb4*Δ. 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, *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ 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. 5*A*). 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. 5*B*).

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 *rpb4*Δ 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 *rpb4*Δ mutants as compared with wild type (Fig. 5*C*).

DISCUSSION

Previous studies have conclusively shown that Rpb4 can affect the transcription of many promoters *in vitro* (5, 7, 9). Studies using promoter reporter constructs have also shown that various unrelated genes are affected to different extents in *rpb4*Δ (8). On the other hand, the stress-related phenotypes of *rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ cells were also defective in sporulation, a response to extreme starvation, and showed altered morphology associated with pseudohyphae formation.

We observed that Rpb4 affects (up-regulated and down-regulated) 1.9% of the total number of genes whose expression pattern was detected under normal conditions. Consistent with our previous report using promoter-reporter fusions, we see that the endogenous expression of *GAL1* and *INO1* genes is compromised in *rpb4*Δ cells (12). Interestingly, following a short exposure to nonpermissive temperature, 9.2% of the total

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, *rpb4*Δ 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 *rpb4*Δ cells are incapable of adopting the normal transcriptional response to heat shock. Recently, genome-wide expression analyses done after exposing *rpb4*Δ 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 *rpb4*Δ cells (10). Taken together, it appears that immediately following temperature stress, *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ cells fail to induce endogenous *IME4* under these conditions (Fig. 5*A*). Because the inducible *CUP1* promoter is unaffected by the absence of Rpb4 (8), using the *CUP1* promoter we induced the expression of *IME4* in *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ strain. This again supports the notion that the defect in transcriptional activation in *rpb4*Δ/*rpb4*Δ mutant is specific for certain sets of genes we proposed earlier (8).

The genes affected under heat shock conditions involved in

TABLE III
Functional classification of affected genes

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. RT, room temperature; HS, heat shock; Spor, sporulation.

Experiment		Metabolism	Energy	Cell growth/ division	Transcription	Protein synthesis	Protein destination	Transport facilitation	Cellular transport	Cellular biogenesis	Cellular communication	Cell rescue/ death	Ionic homeostasis	Total
Haploid/RT	Up	4	0	5	4	2	4	3	2	2	1	2	0	29
	Down	27	14	3	4	2	5	1	1	1	0	6	0	64
Haploid/HS	Up	42	6	18	20	111	13	6	8	7	4	9	0	244
	Down	103	52	29	28	8	22	19	21	8	8	26	8	332
Diploid/rich	Up	8	2	7	6	0	3	5	4	0	1	6	0	42
	Down	25	15	6	3	2	2	4	4	3	1	6	2	73
Diploid/pseudohyphae	Up	1	0	4	1	0	2	2	1	1	0	3	1	16
	Down	42	23	9	12	0	8	3	8	3	4	18	2	132
Diploid/spor.	Up	4	0	2	3	0	1	0	1	0	0	2	0	13
	Down	29	7	11	7	1	6	10	12	1	0	6	3	93
Genome		1065	252	836	793	359	589	311	498	206	135	369	125	5538

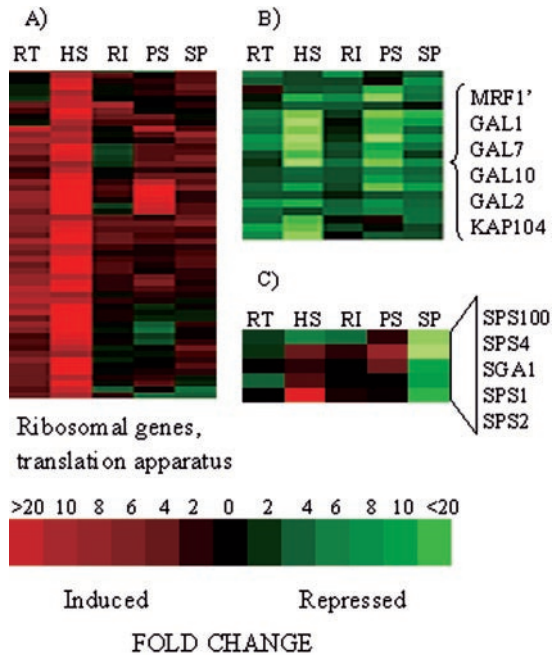

FIG. 3. **Clustering analysis shows classes of genes, which show Rpb4-dependent effects in normal and stress conditions.** *A*, ribosomal proteins and other proteins of the translation machinery are expressed in *rpb4*Δ at higher levels than in the wild type strain following heat shock. *B*, the cluster of genes showing dependence on Rpb4 irrespective of the environmental condition include the group of genes at the Gal1–Gal10 locus (*GAL1*, *GAL10*, *GAL7*, and *KAP104*). Only some of the genes in this cluster have been mentioned in the figure. *C*, genes in this cluster were specifically down-regulated in sporulation conditions. These genes are known to be induced during late stages of sporulation and play a role in spore wall maturation. In *rpb4*Δ/*rpb4*Δ these genes are down-regulated in keeping with its inability to sporulate. *RT*, room temperature; *HS*, heat shock; *RI*, rich medium; *PS*, pseudohyphae-inducing medium; *SP*, sporulation medium.

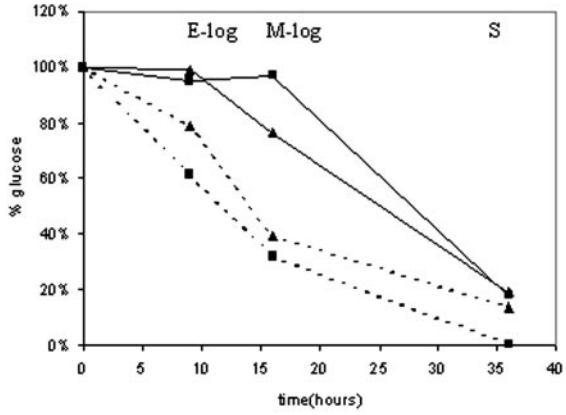

FIG. 4. **The microarray results are corroborated by biochemical phenotypes.** Glucose uptake is affected in *rpb4*Δ strain. The graph represents the percentage of glucose left in the supernatant medium during growth of the wild type (*dotted lines*) and the *rpb4*Δ mutant (*solid lines*). The haploid (■) and diploid (▲) lines are marked. The growth phases: lag, early log (*E-log*), mid-log (*M-log*), and stationary (*S*) phases are indicated at the *top* of the graph.

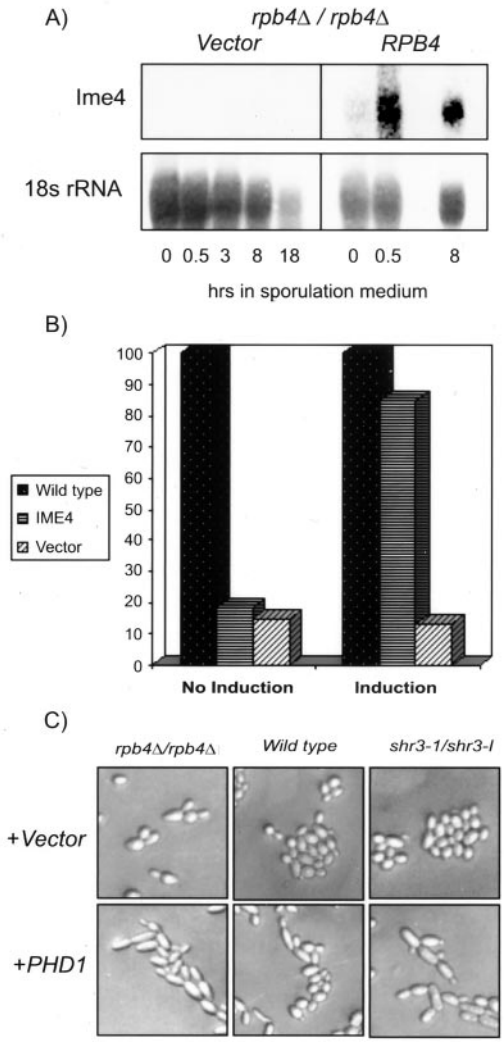

FIG. 5. **The overexpression of transcriptional activators rescues stress responses.** *A*, the Ime4 transcriptional activator is not induced in sporulation conditions in absence of *RPB4*. The Northern blot of RNA isolated from *rpb4* and *RPB4* cells incubated in sporulation medium was probed with IME4 gene. The *lower panel* shows the same blot probed with 18 S RNA probe as control for equal loading of RNA. The *numbers* below the *panels* indicate the numbers of hours of incubation in sporulation medium. *B*, the transcriptional activator *IME4* on overexpression rescues the sporulation defect. The *bar graph* shows percentage sporulation after 4 days in the three strains tested. The results of induction at half an hour post-transfer into the sporulation medium are shown. *C*, overexpression of transcriptional activator *PHD1* enhances pseudohyphal morphology. The *top panel* shows the cells containing the vector, whereas the *lower panel* shows the cells carrying the above vector with *PHD1* open reading frame under *GAL1* promoter. The photos were taken after 3 days of incubation on SLAGR medium (SLAGR medium contains 2% galactose and 1% raffinose instead of dextrose in the SLAD medium).

mitochondrial function were seen to be under the control of Hap4 and Gcn5 (28), both of which are also down-regulated in *rpb4*Δ. 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*Δ 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 *rpb4*Δ/*rpb4*Δ 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 *rpb4*Δ/*rpb4*Δ cells specifically following nitrogen depletion may be responsible for their tend-

TABLE IV

The footprint of Rpb4 on the transcriptome in comparison with the other components of the transcription machinery

The genes that are up- or down-regulated more than 2-fold in mutants of other components of transcription apparatus were compared with the genes that undergo similar changes in *rpb4*Δ under various conditions. RT, experiment 1 (normal haploid); HS, experiment 2 (heat shock); RI, experiment 3 (normal diploid); PS, experiment 4 (nitrogen starvation); SP, experiment 4 (sporulation) in Table I. Thus 13 common genes were down-regulated (>2-fold) in the absence of Med6 or Rpb4.

		RPB4	*MED6*	*GCN5*	*SRB10a*	*SRB4*	*SRB5*	*SWI2*	*TAF145*	*TAF60*	*TAF61*	*TAF90*	*TAF25*	*TAF17*	*TFA1*	*TSM1*	*TPK1*	*TPK2*	*TPK3*	*KIN28*	*FCP1*
	Up	14	78	168	29	59	200	54	49	97	153	103	169	52	153	138	149	138	37	103
	Down	878	179	9	4765	675	116	1425	1736	633	490	1652	3488	4242	490	170	185	194	4658	195
RT	Up	37		1			2	1			3	2		1			1	1	1		
	Down	83	13	3	14	3	8	6	5	9	9	8	8	11	4	1	3	5	4	55	4
HS	Up	237					2	4			3	5	1	3	1	1					1
	Down	352	59	11	57	15	46	13	26	20	27	36	37		13	7	8	10	9	252	5
RI	Up	46						1					1	2					2		1
	Down	75	8	1	14	20	9	1	6	8	2	3	7	14	18	2	4	3	4	47	
PS	Up	11																			
	Down	108	18	7	23	19	11	4	13	18	7	8	18	22	23	1	3	2	3	74	1
SP	Up	12											2	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

[a]Because Srb10 is a repressor; down-regulated genes in *rpb4*Δ have been compared with the genes up-regulated in absence of Srb10.

ency to form pseudohyphae. During sporulation, as mentioned above, the spore wall synthesis genes *SPS1*, *SPS2*, *SPS4*, and *SPS100* were all down-regulated in *rpb4*Δ/*rpb4*Δ cells at 12 h post-induction of sporulation. These "late" genes are normally induced in wild type at this stage during sporulation (21). Ime1, the master regulator of sporulation, regulates all four genes. Ime4, a positive regulator of sporulation, which is required for Ime1 expression, is not induced in *rpb4*Δ/*rpb4*Δ cells (Fig. 5*A*). The inability to initiate transcription of early meiotic genes agrees with the down-regulation of downstream late genes.

It is evident from our analysis that a few genes are dependent on Rpb4 for their expression irrespective of stress. These are mainly involved in galactose metabolism and glucose uptake. Interestingly, Kap104, a β-karyopherin required for survival at high temperatures is also consistently affected (31). *KAP104* is present adjacent to *GAL7* on the genome and shows an expression pattern similar to the GAL gene cluster. Promoter analysis of these genes using MEME software revealed the presence of a putative regulatory site upstream of these genes. The motif (C/T)GGAG(A/C/G)(A/C)CTG(C/T)(T/C)G(A/C)(C/G)CG, which is 60% similar to the Gal4-binding site, is present in all of the galactose-regulated genes as expected. In addition to this site, a 15-nucleotide-long T-rich segment is also present upstream of all these genes. When the entire intergenic region between *KAP104* and *GAL7* (~700 bp) was considered, an additional element (TGC(C/G)(A/T)(T/C/G)(T/G)(A/C/T)(G/C/T)T(T/C)TT(T/G)(T/G)A(A/G)(A/C)(C/G/T)(T/C/T)(A/T)T(T/A)(T/A/C)(C/T)T(G/C)G(G/T)(G/A/T)(G/T)(A/C/T)(A/C)(T/C/G)(T/A)(A/C/G)(A/T)T(C/G)A(G/A)CG(A/G)AG(C/G/T)(G/C)) present in the *GAL* gene cluster and *KAP104* was identified. The significance of these elements is being studied.

We have compared the transcriptional defects of Rpb4 with other subunits of the transcription machinery. If the genes affected by Rpb4 form a significant subset of genes affected by some other component of the holoenzyme, we might gain insights into the mechanism by which Rpb4 regulates transcription. Because most other components of the holoenzyme are essential, their transcriptional effects have been studied using conditional mutants at restrictive temperatures (32). Under these conditions Rpb4 affects 9.2% of the genome; this forms a distinct subset with minimal overlaps with the transcriptome of some components of the holoenzyme like Srb4, Med6, etc. Table IV summarizes the extent of overlap between the footprints of Rpb4 and all other well studied components of the holoenzyme on the transcriptome. More than 50% of the genes affected in *rpb4*Δ in each condition constitute a subset of genes affected by the CTD kinase, Kin28. Recently, the *Schizosaccharomyces pombe* homolog of Rpb4 has been shown to interact with Fcp1, a CTD phosphatase (33). Thus, the mechanism of transcriptional regulation by Rpb4 may be linked to CTD phosphorylation. In conclusion, Rpb4 affects central physiological processes like glucose uptake and carbon and energy metabolism, which in turn can regulate various phenotypes. Thus, the diverse stress response defects seen in *rpb4*Δ strains may be a consequence of a general defect in optimal expression of basic metabolic pathways.

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