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Rpb4, a Subunit of RNA Polymerase II, Enables the Enzyme To Transcribe at Temperature Extremes In Vitro

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Rpb4 is a subunit of *Saccharomyces cerevisiae* RNA polymerase II (Pol II). It associates with the polymerase preferentially in stationary phase and is essential for some stress responses. Using the promoter-independent initiation and chain elongation assay, we monitored Pol II enzymatic activity in cell extracts. We show here that Rpb4 is required for the polymerase activity at temperature extremes (10 and 35°C). In contrast, at moderate temperature (23°C) Pol II activity is independent of Rpb4. These results are consistent with the role previously attributed to Rpb4 as a subunit whose association with Pol II helps Pol II to transcribe during extreme temperatures. The enzymatic inactivation of Pol II lacking Rpb4 at the nonoptimal temperature was prevented by the addition of recombinant Rpb4 produced in *Escherichia coli* prior to the in vitro reaction assay. This finding suggests that modification of Rpb4 is not required for its functional association with the other Pol II subunits. Sucrose gradient and immunoprecipitation experiments demonstrated that Rpb4 is present in the cell in excess over the Pol II complex during all growth phases. Nevertheless, the rescue of Pol II activity at the nonoptimal temperature by Rpb4 is possible only when cell extracts are obtained from postlogarithmic cells, not from logarithmically growing cells. This result suggests that Pol II molecules should be modified in order to recruit Rpb4; the portion of the modified Pol II molecules is small during logarithmic phase and becomes predominant in stationary phase.

Although changes in transcription are a hallmark of stress responses (12), little is known about the mechanisms that permit the transcription apparatus itself to tolerate stress. Several observations have led us to investigate the possibility that Rpb4, a yeast RNA polymerase II (Pol II) subunit, plays a critical role in enabling Pol II to transcribe during some stress conditions. The yeast *Saccharomyces cerevisiae* Pol II is composed of 12 subunits (18). Rpb4 exhibits some unique features distinguishing it from the other subunits. As for Rpb7 (15a) but unlike the case for other subunits, the stoichiometry of Rpb4 is dependent on growth conditions. In optimally growing cells, the fraction of Pol II molecules containing Rpb4 is about 20% (5, 11), and it gradually increases following the shift to postlogarithmic phases. Thus, in stationary phase virtually all Pol II molecules contain Rpb4 (5), and these molecules, unlike Pol II molecules obtained from logarithmically growing cells, can form high-quality two-dimensional crystals (2, 8). *RPB4* is not essential for cell viability (17). Under optimal growth conditions at moderate temperatures (18 to 22°C), cells lacking *RPB4* (designated herein *rpb4*$^-$ cells) grow indistinguishably from their wild-type counterparts (5). Consistently, under these conditions, the global transcriptional activity in *rpb4*$^-$ cells is comparable to that in the wild-type strains. However *rpb4*$^-$ cells rapidly lose the capacity for efficient growth and global transcription as they experience higher or lower temperatures. In addition to the requirement for Rpb4 at temperature extremes, this subunit is required for efficient transcription in post-logarithmic phases (at moderate temperatures). Normally, as yeast cells sense that nutrients are being depleted, they alter their pattern of gene expression and briefly stop growth; this event is termed the diauxic shift (9, 14). Following the diauxic shift, cells continue to grow for one to three generations at a slower, albeit exponential, rate. Cells lacking *RPB4* grow more slowly than wild-type cells during the second growth phase, exhibit a substantial decline in mRNA synthesis relative to wild-type cells, do not enter stationary phase normally, and rapidly lose viability during starvation (5). Interestingly, the normal level of Rpb4 limits growth rate after but not before the diauxic shift. Thus, whereas cells overexpressing *RPB4* grow indistinguishably from wild-type cells during log phase, they grow substantially faster than wild-type cells during post-diauxic shift growth phase (4).

The pattern of *RPB4* expression differs from the pattern of expression of the other Pol II subunit genes. Whereas mRNA and protein levels of other subunits decline following the shift from log to post-log phases, *RPB4* mRNA and protein remain constitutively high (3–5). Furthermore, in stationary phase, but not during optimal growth conditions, Rpb4 protein level is regulated posttranscriptionally. Thus, under optimal growth conditions, when Rpb4 is dispensable, the Rpb4 protein level is directly proportional to the *RPB4* mRNA level. However, in stationary phase, when Rpb4 is essential for maintaining viability, Rpb4 protein level is little affected by artificial changes in its mRNA level (4). Taken together, the unusual phenotype of *rpb4*$^-$ cells and the pattern of *RPB4* expression indicate that Rpb4 plays a vital role specifically during some stress conditions.

Rpb4 is known to interact with an essential Pol II subunit, Rpb7 (6, 10, 11, 18). The association of Rpb7 with Pol II is influenced by Rpb4, and it seems that they both interact with the polymerase as a heterodimer, called Rpb4/7. However, the functions of these subunits are not necessarily coupled under all circumstances. First, *RPB7* but not *RPB4* is essential for viability (18), indicating that Rpb7 can function in the absence of Rpb4. Second, overexpression of *RPB7* but not *RPB4* can influence cell morphology and induce pseudohyphal growth (10). Furthermore, deletion of *RPB4* has no effect on pseudohy-

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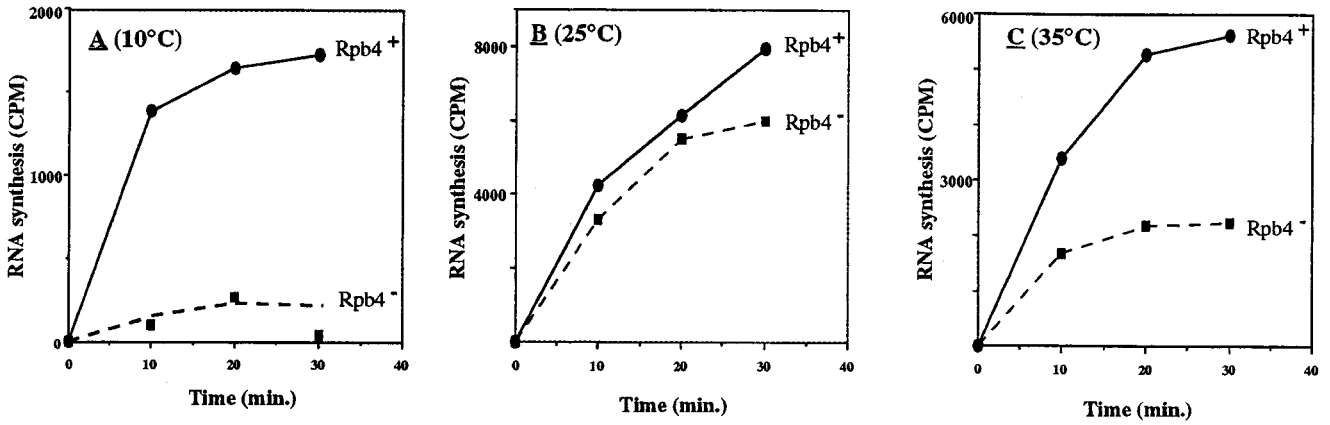

FIG. 1. Rpb4 is required for Pol II activity at high and low, but not moderate, temperatures. Wild-type and rpb4$^-$ cells were grown in rich medium (YPD) at 26°C to stationary phase. Cell extracts were obtained as described in Materials and Methods. Pol II activity was tested at the indicated temperatures in the promoter-independent assay (see Materials and Methods). Rpb4$^+$ and Rpb4$^-$ represent incorporation kinetics for extracts from wild-type and rpb4$^-$ cells, respectively. The reactions at 25 and 35°C (B and C) were done in the same experimental setup. The reactions at 10°C (A) were done in a different experiment, and therefore the extent of incorporation should not be compared to those in panels B and C. All reactions were done at least three times with at least three different batches of cell extracts. The extent of incorporation varied between the batches. However, the relative kinetics of wild-type Pol II versus pol IIΔ4 at each specific temperature was highly reproducible.

phal growth (6a). It is also worth noting that the human homolog of Rpb7 can form a stable homodimer (1) and may interact as a homodimer with Pol II independently of Rpb4. Taken together, the results suggest that Rpb4 and Rpb7 have distinct functions independent of each other, in addition to their role as a heterodimer.

Here we show that the interaction of Rpb4 with Pol II in vitro permits the enzyme to transcribe at temperature extremes. Results presented here are consistent with the transcriptional phenotype of cells lacking *RPB4* and support the role suggested for Rpb4 as a Pol II subunit essential for transcription under nonoptimal temperatures.

MATERIALS AND METHODS

Yeast strains and medium. The wild-type strain SUB62 and its isogenic *rpb4Δ1* (designated here *rpb4*$^-$) strain (MC11-1) were described previously (5). Z277, carrying epitope-tagged *RPB3*, was described previously (11). RS420 (*MAT***a** *ura3-53 his4 trp1 leu2-3,112 rpb1-1*) was a generous gift from R. Sternglanz. Cells were grown in a YPD medium (2% Bacto Peptone, 1% yeast extract [Difco Laboratories], 2% dextrose) at 25°C. For all experiments, the inoculum came from cell cultures that had been growing in log phase for at least seven generations.

Antibodies and Western analysis. Affinity-purified anti-Rpb4 and anti-Rpb2 antibodies were a generous gift from A. Sentenac (7). The anti-Rpb1 C-terminal domain monoclonal antibody 8WG16 was a generous gift from Nancy Thompson and Richard Burgess (16). Western analysis was done as described previously (5).

Protein extraction. Whole-cell protein extraction was done essentially as described elsewhere (5). Briefly, proteins were extracted from 1×10^9 to 3×10^9 cells in 0.5 ml of PEB (50 mM Tris HCl [pH 7.9], 10 mM $MgCl_2$, 0.3 M ammonium sulfate, 1 mM dithiothreitol, 0.5 mM EDTA, 10% glycerol, 20 μg each of aprotinin, antipain, and leupeptin per ml, 1 mM each phenylmethylsulfonyl fluoride and pepstatin A, 50 μg of Nα-*p*-tosyl-L-lysine chloromethyl ketone [TLCK] per ml [all protein inhibitors from Sigma]), using the glass beads (350 μl) procedure. Protein concentrations determined by the Bradford assay (Bio-Rad) were 3 to 11 mg/ml.

Transcription assay. Nonspecific initiation and chain elongation with poly(rC) as the template was assayed as described elsewhere (15). After preincubation of cell extracts at the desired temperature for 3 min, prewarmed transcription mixture (15) was added and mixed by pipetting up and down while the tube was in the water bath. To keep the reaction temperature constant, tubes were not removed from the water bath during any manipulations. Immediately following addition of the transcription mixture, one half of the reaction mixture was transferred into a prewarmed tube containing α-amanitin (Sigma). The drug (final concentration, 50 μg/ml) was mixed by pipetting up and down while the tube was in the water bath. To terminate transcription, samples were spotted onto 3MM paper which had been soaked in 10 mM EDTA and then air dried. The 3MM paper containing all samples was subjected to trichloroacetic acid (TCA) precipitation on ice as follows: 30 min of incubation in 0.2 M $Na_4P_2O_7$–10% TCA and three washes for several hours in 0.1 M $Na_4P_2O_7$–5% TCA, followed by washes with ethanol and then acetone. The paper was dried, and radioactivity was measured in a scintillation counter. Pol II-specific incorporation is calculated as the difference between the TCA-incorporated radioactivity (catalyzed by Pol I, Pol II, and Pol III) and that obtained in the parallel reaction containing α-amanitin (catalyzed by Pol I and Pol III).

RESULTS AND DISCUSSION

Rpb4 is required for efficient transcription by Pol II only at temperature extremes. Previously it was shown that *RPB4* is required for growth and efficient transcription at temperature extremes in vivo (5, 17). In contrast, under optimal growth conditions at moderate temperatures, Rpb4 was shown to be dispensable for Pol II activity, as cells lacking *RPB4* were able to grow indistinguishably from wild-type cells under these conditions (5). These results raised two alternative but not mutually exclusive explanations, for the function of Rpb4. (i) Rpb4 affects the expression of a subpopulation of genes (e.g., some specific heat shock genes) needed for transcription of other genes under some stress conditions, and (ii) Rpb4 affects the enzymatic activity of pol II in a direct and promoter-independent manner. According to the latter possibility, the involvement of Rpb4 is dispensable for Pol II activity under optimal conditions but essential for its enzymatic activity under some nonoptimal conditions. To test the hypothesis that Rpb4 is directly required for Pol II activity at temperature extremes, we monitored Pol II activity in extracts from *RPB4*$^+$ and *rpb4*$^-$ cells at various temperatures. To monitor Pol II activity per se, independently of other factors, we used the promoter-independent initiation and chain elongation assay developed by Ruet et al. (15). It was previously shown that the removal of Rpb4 had little effect on Pol II activity at moderate temperatures in this assay (6). Results shown in Fig. 1B led to the same conclusion. Rpb4 has little effect on Pol II activity within a temperature window ranging between 20 and 28°C (Fig. 1B and results not shown). In contrast with the activity at moderate temperatures, Rpb4 is shown here to be required for efficient activity of Pol II at high or low temperatures. Figure 1A demonstrates that extract from *RPB4*$^+$ cells can support efficient Pol II transcription at 10°C, whereas Pol II activity in extract from *rpb4*$^-$ cells is severely impaired at this temperature. Similarly, extract from *RPB4*$^+$ cells can support efficient Pol II

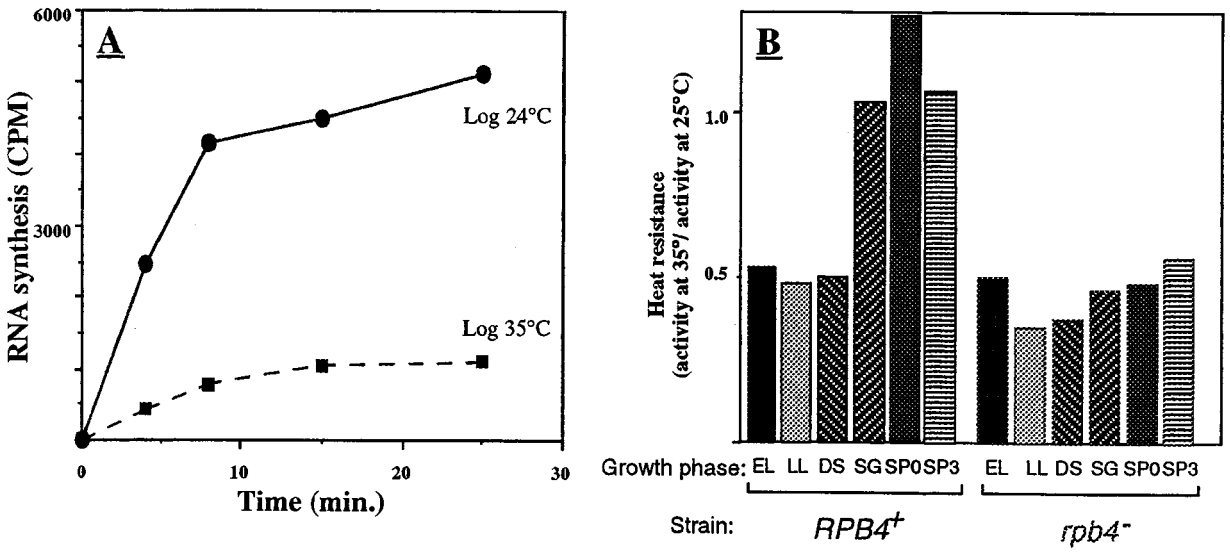

FIG. 2. Rescue of Pol II activity at high temperature is dependent on the growth phase of the cells used to extract the proteins. Wild-type (*RPB4*$^+$) and rpb4$^-$ cells were grown in rich medium (YPD) at 26°C. Equal amounts of cells were harvested at various growth phases. Cell extracts were prepared and Pol II activity was assayed as for Fig. 1. A. Incorporation kinetics for extracts prepared from *RPB4*$^+$ cells harvested at early log phase was determined at 24 and 35°C. (B) Heat resistance of Pol II as a function of growth phase of the cells used as a source of protein extract. Heat resistance is defined as the ratio between the Pol II-specific radioactivity incorporated at 35°C during 30 min and that incorporated at 25°C during 30 min. EL, early log; LL, late log; DS, diauxic shift; SG, slow-growth phase; SP0, beginning of stationary phase; SP3, 3 days into stationary phase.

transcription at 35°C, whereas Pol II activity in extract from *rpb4*$^-$ cells is severely impaired at this temperature. The enzyme lacking Rpb4 exhibits some initial activity at 35°C and only after a few minutes loses this activity completely. This late inactivation suggests that in vitro, some early stages of the initiation process are less dependent on Rpb4 at this nonoptimal temperature. At 10°C, the Pol II extracted from *rbp4*$^-$ cells (Pol IIΔ4) is so poorly active that this late effect can hardly be observed. These results are in agreement with the in vivo results, favoring our suggestion that Rpb4 is essential for the appropriate function of Pol II under temperature stresses.

Experiments described in Fig. 1 were carried out with extracts from stationary-phase cells, during which Pol II molecules carry stoichiometric amounts of Rpb4 (5). Therefore, the effect of Rpb4 on enzymatic activity could easily be tested. Figure 2A shows that Pol II activity in extract from logarithmically growing wild-type cells, in which most Pol II molecules do not contain Rpb4 (5), is heat sensitive. Figure 2B shows that the shift from heat-sensitive to heat-tolerant Pol II occurs following the shift from logarithmic to post-logarithmic phases. Specifically, Pol II extracted from optimally and logarithmically growing cells exhibited heat sensitivity similarly to the Pol II extracted from *rpb4*$^-$ cells (Fig. 2B; compare columns EL for *RPB4*$^+$ and *rpb4*$^-$); however, Pol II extracted from post-logarithmic wild-type (but not *rpb4*$^-$) cells became heat tolerant. During the diauxic shift, the transition phase between logarithmic and slow growth (see the introduction), Pol II was still heat sensitive. These results are in good correlation with the stoichiometry of Rpb4 found previously in vivo. In logarithmic phase and during the diauxic shift, most Pol II molecules do not contain Rpb4 (5) and the in vitro activity of Pol II is heat sensitive (Fig. 2A). Following the diauxic shift, most Pol II molecules contain Rpb4 (5) and the in vitro activity of Pol II is heat tolerant (Fig. 1C and 2B). Interestingly, Pol II extracted from cells which had been growing logarithmically at 37°C carry substoichiometric amounts of Rpb4 (5). Consistently, the in vitro activity of this Pol II molecules is heat sensitive (results not shown).

Rpb4 is present in excess over Rpb1, Rpb2, and Rpb3. Previous results from in vivo experiments and the present results obtained in vitro (Fig. 2) demonstrate that interaction of Rpb4 with the Pol II complex is influenced by nutritional conditions. We sought to identify what determines the interaction between Rpb4 and the other Pol II subunits. First, we examined whether Rpb4 level is a limiting factor by performing two sets of experiments. Results of an immunoprecipitation experiment using antibodies directed against the C-terminal domain of Rpb1 (Fig. 3A) demonstrated that most Rpb4 molecules did not precipitate with the Pol II complex whereas Rpb1 and Rpb2 subunits precipitated quite efficiently (compare the levels of individual subunits in lanes P and S). Thus, whereas Rpb4 is much higher in lanes S than in lanes P, the inverse is observed for Rpb1 and Rpb2. This differential immunoprecipitation was observed in extracts from both log-phase and stationary-phase cells (Fig. 3A). Sedimentation through a sucrose gradient (Fig. 3B) revealed that most Rpb4 molecules do not cosediment with the Pol II complex and Pol II activity (fractions 5 to 7) but instead sediment more slowly (fractions 7 to 12). This result indicates that most Rpb4 molecules do not associate in a stable complex with Pol II. Excess of free Rpb4 over Pol II-associated Rpb4 is observed by sedimenting extracts from both logarithmically growing (Fig. 3B) and from stationary-phase (results not shown) cells. Taken together, these observations demonstrate that Rpb4 is in excess over Rpb1, Rpb2, and Rpb3, suggesting that Rpb4 is not a limiting factor. Thus, changes in Rpb4 level are not the main determinants of the extent of Rpb4 interaction with the Pol II complex. Indeed, the Rpb4 level does not increase following the shift from log to post-log phase but remains close to a constant value during all growth phases (4). These results focused our attention to the posttranslational modifications of either Rpb4 or other Pol II subunits as the cause for the differential inter-

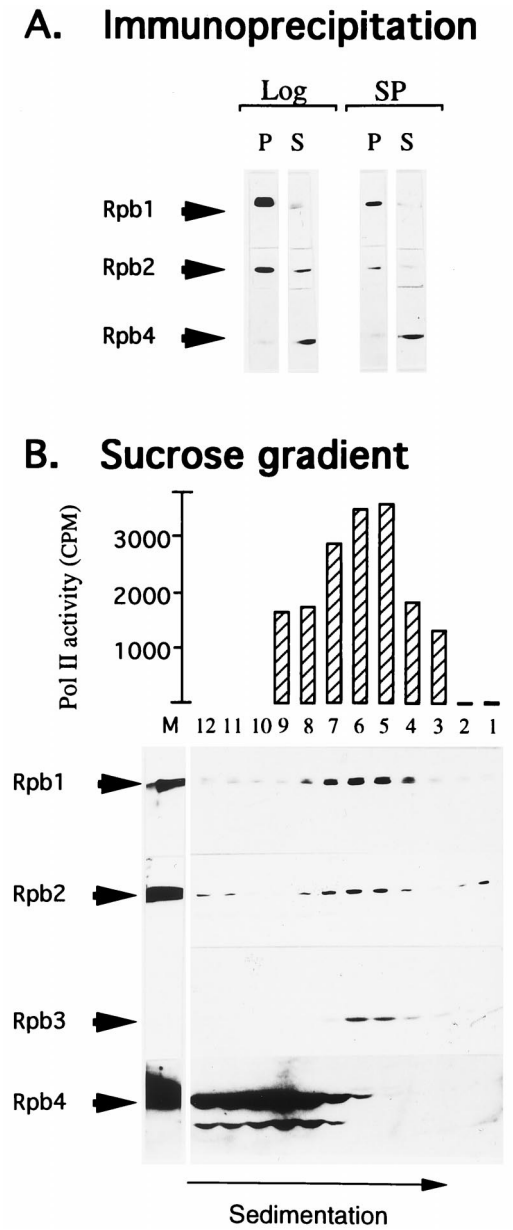

FIG. 3. Pol II-free Rpb4 is present in excess over Pol II-associated Rpb4. (A) Immunoprecipitation experiment. Pol II was immunoprecipitated from extract obtained from logarithmically growing cells (Log) or from stationary-phase cells (SP). Immunoprecipitation, using a monoclonal antibody against the C-terminal domain of Rpb1 (8WG16), was carried out as described previously (5). Following immunoprecipitation, both the immunoprecipitated material (lanes P) and one half of the unprecipitated supernatant (lanes S) were electrophoresed, then electrotransferred onto a nitrocellulose filter, and probed with antibodies against the indicated Pol II subunits (see Materials and Methods). Antibody 8WG16 was used to detect Rpb1, affinity-purified rabbit anti-Rpb2 polyclonal antibodies were used to detect Rpb2, and affinity-purified rabbit anti-Rpb4 polyclonal antibodies were used to detect Rpb4. (B) Sucrose gradient. Protein extract (270 μg) obtained from Z277, a strain carrying hemagglutinin epitope-tagged Rpb3 (11), was sedimented through a 5 to 20% (wt/wt) sucrose gradient, using an SW60 rotor at 60,000 rpm (485,000 × *g*) for 2.5 h at 4°C. Gradient was fractionated into 12 fractions, and Pol II activity in 9 fractions was monitored at 24°C as described in Materials and Methods (upper panel). Fifty microliters from each fraction was added to 1× Laemli sample buffer and boiled for 3 min, and samples were electrophoresed. Following electrophoresis, proteins were electrotransferred onto nitrocellulose filters and probed with antibodies against the indicated subunits as described previously (5). Rpb1, Rpb2, and Rpb4 were detected by the antibodies used for panel A. To detect the epitope-tagged-Rpb3, antibody 12CA5 was used. Lane M, purified Pol II (carrying wild-type Rpb3, which is not detected by antibody 12CA5).

action of Rpb4 with the Pol II complex in log and stationary phases.

Growth phase-dependent modification of the remainder of Pol II is required for its interaction with Rpb4. To determine whether Rpb4 must be modified to functionally interact with the Pol II complex, we examined the possibility that recombinant Rpb4, produced in *E. coli*, can rescue the activity of Pol IIΔ4 at high temperature. Results in Fig. 4A demonstrate that the addition of a recombinant Rpb4 prior to transcription initiation restored full activity of Pol IIΔ4 at high temperature. The results suggest that eukaryotic cell-specific posttranslational modification is not required for the functional interaction of Rpb4 with the Pol II complex. To determine whether Pol II subunits other than Rpb4 must be modified to interact with Rpb4, we carried out mixing experiments in which extracts from *rpb4*$^-$ cells were mixed with extracts from *rpb1-1* cells which had been heated at 42°C for 18 min. Pol II in a prewarmed extract from the *rpb1-1* strain is completely inactive, due to a defect in its Rpb1 (6, 13) (Fig. 4B). As shown in Fig. 1C, 2B, and 4A, Pol II in the *rpb4*Δ extract is inactive at 35°C due to the absence of Rpb4. Thus, each extract alone cannot support efficient RNA synthesis in the promoter-independent assay at 35°C. Activity at 35°C can be restored if the Rpb4, present in excess over Pol II complex in the extract from the *rpb1-1* strain, can functionally interact with the Pol IIΔ4 extracted from the *rpb4Δ1* cells. Note that in these mixing experiments the main source of Rpb4 is the unbound (Pol II-free) Rpb4 which is present in a large excess over the Pol II-associated Rpb4 both during the logarithmic growth phase and in stationary phase (Fig. 3). In the mixing experiments, Rpb4 was diluted only twofold, as equal volumes of extracts were mixed, and it remained in excess over Pol II complexes. As shown in Fig. 4B, when preheated *rpb1-1* extract was mixed with extract from logarithmically growing *rpb4*$^-$ cells, no complementation could be observed; Pol II was active at 25°C but inactive at 35°C. Thus, although unbound Rpb4 molecules were present in excess over Pol II complexes, they could not interact with Pol II and restore its activity at 35°C. However, when preheated extract from *rpb1-1* cells was mixed with an extract from stationary *rpb4*$^-$ cells, Pol II regained activity at the high temperature. Thus, Rpb4 could interact with Pol II present in the stationary-phase extract but not with Pol II present in the log-phase extract. The possibility that the largest subunit can change from Pol IIΔ4 to the heat-inactivated polymerase is unlikely; dissociation of this subunit from the polymerase under transcription conditions has never been observed, nor has dissociation of the homologous subunit from the *E. coli* enzyme (6). To summarize, results in Fig. 4 demonstrate that Pol II extracted from stationary cells can be rescued from heat inactivation by Rpb4, whereas Pol II extracted from logarithmically growing cells cannot. The source of Rpb4, whether *E. coli* (Fig. 4A), or log-phase (Fig. 4B) or stationary (data not shown) yeast cells, is not important for its ability to render Pol II heat resistant. Therefore, we suggest that modification of the Pol II complex, not Rpb4, is required to recruit Rpb4 and that this modification occurs predominantly following the shift from log to post-log phases. The identification of the Pol II subunit(s) that becomes modified and the nature of this modification remain to be determined.

Does the ability of Rpb4 to render Pol II resistant to temperature extremes in vitro correlate with its known function in vivo? We summarize below our present results and previous results obtained in vivo (3, 4). First, under optimal growth conditions at moderate temperatures (18 to 23°C), cells lacking *RPB4* can grow and transcribe genes almost indistinguishably from their wild-type counterparts. Similarly, in the test tube at

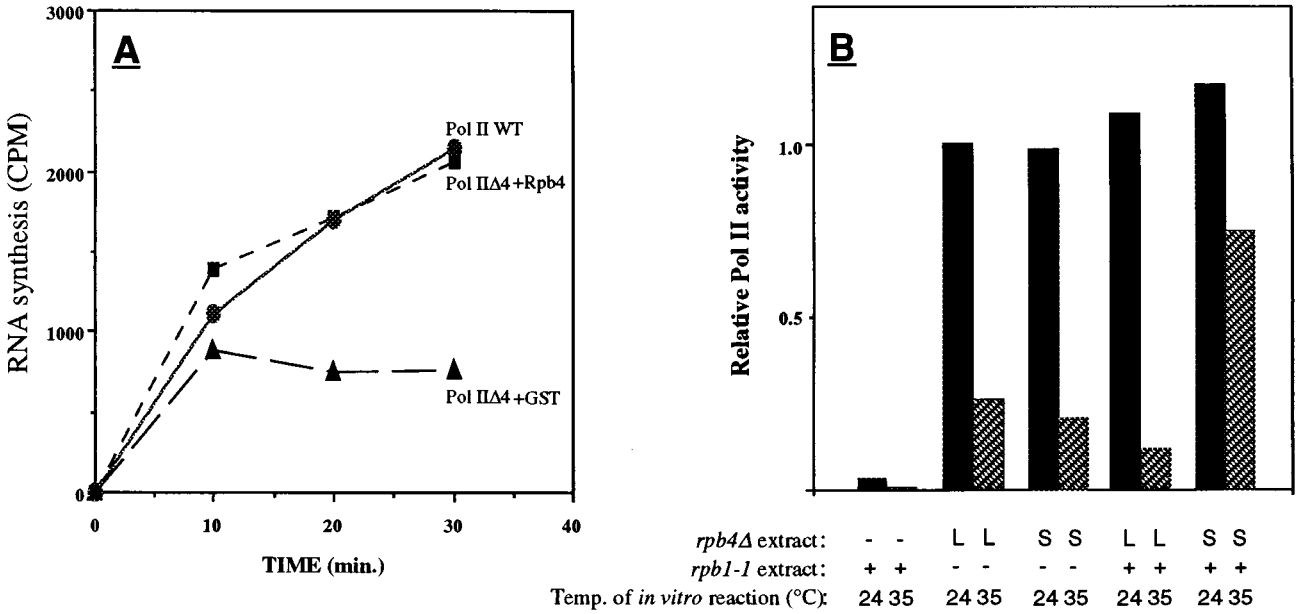

FIG. 4. Activity at high temperature of Pol IIΔ4 extracted from post-log-phase but not from logarithmically growing cells can be rescued by Rpb4. (A) Reconstitution of Pol IIΔ4 activity at high temperature with recombinant Rpb4 produced in *E. coli*. Rpb4-GST fusion protein was expressed in *E. coli* by using plasmid pGEX-2T (Pharmacia) followed by purification on a glutathione-Sepharose column, as instructed by the manufacturer. The fusion protein was cleaved with thrombin, and the release of free Rpb4 was ascertained by Western analysis (not shown). Reactions were carried out at 35°C as described in Materials and Methods. Pol IIΔ4+Rpb4 (squares), the thrombin digest (0.5 μg) was preincubated with 33 μg of extract from stationary *rpb4*$^-$ cells at 30°C for 15 min followed by 10 min at 25°C and 1 min at 35°C before the reaction commenced; Pol IIΔ4+GST (triangles), 33 μg of extract from stationary *rpb4*$^-$ cells preincubated with 0.5 μg of GST as described above; Pol II WT (circles), 33 μg of extract from stationary wild-type (*RPB4*$^+$) cells. (B) In vitro complementation between *rpb4*$^-$ and heat-treated *rpb1-1* extracts. Cell were grown at 25°C in YPD and harvested at the indicated growth phase, and their proteins were extracted, as described in Materials and Methods. Extracts were prepared from logarithmically growing (L) *rpb1-1* cells or from logarithmically growing or stationary (S) *rpb4*$^-$ cells, and the protein concentration in each extract was brought to 2.5 mg/ml in PEB (see Materials and Methods). The *rpb1-1* extract was preheated at 42°C for 18 min to inactivate Pol II. Equal volumes of extracts, containing equal amounts of protein, were mixed at various combinations, specified below the columns, before transcription reactions were initiated. In the reactions containing only one extract, an equal volume of PEB was added (-). Note that in these mixtures the concentration of the bulk protein is lower than that in the other mixtures. However, preliminary experiments demonstrated that this difference had no significant effect on Pol II activity. The various mixtures were incubated at 30°C for 15 min, then at 23°C for 45 min, and finally for at 38°C for 1 min. After cooling in ice, each mixture was divided into two equal samples and transcription was assayed at 24 or 35°C as described in Materials and Methods. Relative Pol II activity was calculated with respect to the activity in extract from the logarithmically growing *rpb4*$^-$ cells at 24°C (defined arbitrarily as 1).

moderate temperatures, Pol II lacking Rpb4 is as active as Pol II containing Rpb4. Second, *rpb4*$^-$ cells cannot grow at temperature extremes (below 13°C and above 32°C); furthermore, when these mutant cells are shifted from moderate to high temperature, their Pol II activity is rapidly lost. Similarly, in the test tube, Pol II requires Rpb4 for its activity at temperature extremes. Third, in log phase only a minor subpopulation of Pol II molecules contain Rpb4 (stoichiometry of ~0.2). Consistently, Pol II molecules extracted from logarithmically growing wild-type cells are heat sensitive, as are those extracted from *rpb4*$^-$ cells. Fourth, in stationary phase, stoichiometric amounts of Rpb4 are found associated with the Pol II. Consistently, Pol II molecules extracted from stationary-phase cells are heat resistant. Thus, results obtained in vitro and described in this paper are in accord with results found previously in vivo and are likely to be biologically relevant.

The specific requirement for Rpb4 at temperature extremes shown in this study favors a model in which a major, but not necessarily the sole, role of this subunit is to permit the enzyme to function under nonoptimal conditions. It is worth noting that the requirement for Rpb4 at temperature extremes can potentially be an indirect effect. For example, it is possible that Rpb4 is required for recruiting yet another factor (e.g., Rpb7) which helps Pol II to transcribe at temperature extremes.

Rpb4/7 was suggested to play a role other than those related directly to the transcriptional response to stress. Pol II lacking Rpb4/7 was shown to be deficient in selective transcription initiation in vitro (6). Recently, electron crystallography of Pol II molecules extracted from stationary-phase cells revealed that the molecules containing Rpb4/7 differ in conformation from those lacking Rpb4/7 (2, 8). These results were interpreted in terms of open and closed conformations that the enzyme undergoes during the initial phases of transcription initiation process. Jensen et al. (8) have also proposed that Rpb4/7 heterodimer stabilizes the paused Pol II located downstream of heat shock promoters. Our results, demonstrating that Rpb4 is required for Pol II activity at temperature extremes, support a model in which Rpb4 plays a global role during stress, in a promoter-independent fashion, rather than a specific role restricted to the transcription of heat shock genes (see first paragraph in Results). It is possible, then, that the addition of Rpb4/7 and the specific conformational change that it elicits (2, 8) become critical for the global Pol II activity especially during some stress conditions. Alternatively, it is possible that the biochemical process leading to this conformational change is important under all conditions. Yet, during some nonstress conditions it can be carried out by a factor other than Rpb4, and only during stress is Rpb4 irreplaceable.

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