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Rpb1 foot mutations demonstrate a major role of Rpb4 in mRNA stability during stress situations in yeast

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ABSTRACT

The *RPB1* mutants in the foot region of RNA polymerase II affect the assembly of the complex by altering the correct association of both the Rpb6 and the Rpb4/7 dimer. Assembly defects alter both transcriptional activity as well as the amount of enzyme associated with genes. Here, we show that the global transcriptional analysis of foot mutants reveals the activation of an environmental stress response (ESR), which occurs at a permissive temperature under optimal growth conditions. Our data indicate that the ESR that occurs in foot mutants depends mostly on a global post-transcriptional regulation mechanism which, in turn, depends on Rpb4–mRNA imprinting. Under optimal growth conditions, we propose that Rpb4 serves as a key to globally modulate mRNA stability as well as to coordinate transcription and decay. Overall, our results imply that post-transcriptional regulation plays a major role in controlling the ESR at both the transcription and mRNA decay levels.

1. Introduction

Transcription is the most extensively studied step in gene expression. Recent studies have provided new insights into the coordination of the mRNA life cycle, from transcription to mRNA stability, as well as other important elements of this process [1–6]. Some regulatory factors have been called "mRNA coordinators" as they have been described to regulate the entire life of mRNAs from synthesis to decay [7,8]. RNA polymerase II (RNA pol II) is a highly conserved 12-subunit enzyme responsible for the transcription of all mRNAs and many non-coding RNAs [9]. Pol II consists of a 10-polypeptide catalytic core and the heterodimeric Rpb4/7 complex, which can dissociate from the core enzyme [10,11]. Rpb7 is essential for cell viability, whereas Rpb4 is dispensable under optimal growth conditions [12,13]. The crystal structure shows that Rpb7 interacts with the large subunit Rpb1 through the Rpb7 conserved N-terminal region [11], while the N-terminal extension of Rpb4 (amino acids 1–46) interacts with the rest of the RNA pol II through Rpb2 [11,14], and may regulate the translation and degradation of some transcripts by co-transcriptionally loading the Rpb4/7 subcomplex onto these mRNAs [7,15–17].

The role of Rpb4/7 in transcription and the mRNA life cycle has been widely studied. It has been proposed that Rpb4 is essential for transcription during heat shock and nutrient deprivation, and also for survival under these stress conditions [18–20]. However, other authors have reported that loss of Rpb4 perturbs several cellular functions that contribute to the inappropriate stress response of *rpb4*Δ yeast cells, although Rpb4 is not specifically involved in stress response [21]. Thus the global expression analysis of the *rpb4* null mutant shows an overall drop in mRNA levels after a temperature shift, but also before it, which is not limited to heat shock or to other stress proteins [20,22].

The Rpb4/7 heterodimer has been related to some gene expression steps, such as promoter-directed transcription initiation [10] and transcription elongation [14,23,24], although the Rpb4/7 dissociation during transcription elongation contradicts its proposed role [25]. A post-transcriptional role for Rpb4/7 has been proposed, based on its interaction with RNA pol II transcripts [15,26]. However, recent data have provided conflictive evidence that Rpb4 functions mainly in

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mRNA synthesis in the nucleus [24]. Despite excess Rpb4/7 over RNA pol II, the interaction between Rpb4/7 and mRNAs can occur only in the RNA pol II context. Specifically, Rpb4/7 remains associated with mRNAs throughout its life, and regulates processes such as export, translation, movement out of P-bodies, and 5′ to 3′ decay and 3′ to 5′ decay pathways [3,7,15,27,28]. It has been suggested that the co-transcriptional loading of the Rpb4/7 subcomplex onto mRNAs can regulate their translation and degradation [7,15,16]. Moreover, Rpb4/7 imprinted mRNAs are better able to recruit general decay machinery [15,27,29].

Proper RNA pol II assembly is crucial for maintaining correct transcriptional activity [30,31]. Rpb6 and its bacterial homolog are important for the proper assembly and stability of RNA polymerases [30, 32–34]. Accordingly, the *rpb6Q100R* mutant causes the loss of both the Rpb4/7 dimer and RNA pol II activity at high temperature [31] in the same way as the *rpb1C67S,C70S* mutant does, which affects any contact with Rpb7 [7,35]. All this suggests that reduced ability to recruit Rpb4/7 to the RNA pol II core results in impaired production and decay for selected genes [3,15].

In this work, we investigated the consequences of the mutants that alter RNA pol II assembly in transcription and mRNA decay. For this, we used two different *rpb1* mutants which affected the correct association of the Rpb4/7 dimer and Rpb6 to RNA pol II [30], as well as an *rpb4*Δ mutant. We also investigated the consequences in transcription and mRNA decay. Our data support the idea that under optimal growth conditions, Rpb4 serves as a general coordinator to modulate mRNA stability and also to coordinate transcription and mRNA decay of most, or perhaps all, genes and not only to modulate the mRNA stability of some groups of genes, such as those that encode ribosomal proteins or translational factors (protein biosynthetic factors: PBF mRNA), as previously shown [29]. Notably, this coordination is mediated by global Rpb4–mRNA imprinting. We also demonstrated that the environmental stress response (ESR), which occurs at a permissive temperature under optimal growth conditions in RNA pol II foot mutants, is more complex than the mechanism established at the transcription initiation level. This regulation acts on the ESR-up-regulated genes only at the mRNA stabilization level, while the effect on the ESR-down-regulated genes requires the transcriptional repression of RP and RiBi (Ribosome Biogenesis) genes in order to compensate for generally increased mRNA stability.

2. Materials and methods

2.1. Yeast strains, plasmids, genetic manipulations, media, and genetic analysis

Common yeast media, growth conditions, and genetic techniques were used as described elsewhere [36].

The yeast strains, plasmids, and primers are listed in Supplementary Tables S1–3, respectively. Yeast strain YFN469 was generated by replacement of the *HSP26* and the *HSP12* promoters with the *GAL1* promoter [37], performed as previously described [37] using plasmids pFA6a–KanMX6–pGAL1 and pFA6a–TRP1–pGAL1 (Table S2), and primers F4HSP12, R2HSP12, F4HSP26 and R2HSP26 (Table S3), to amplify the transformation modules.

Centromeric vector pCM189-*RPB4* is a derivative pCM190-*RPB4* vector [30] constructed by the PCR amplification of *RPB4* under the control of the *TETоff* promoter from pCM190-*RPB4* with oligonucleotides *pCM-Debut* and *pCM-Fin* (Table S3), which was introduced into pCM189 by homologous recombination.

Centromeric YCplac33-*MSN2-GFP* vector, which contains *URA3* marker, is a derivative pADH1Msn2-GFP vector [38] constructed by the PCR amplification of *MSN-GFP* under the control of the *ADH1* promoter from pADH1Msn2-GFP with oligonucleotides *M13-forward* and *M13-reverse* (Supplementary Table S3), which was introduced into YCplac33 by homologous recombination.

2.2. Chromatin immunoprecipitation

For Rpb1 immunoprecipitation, 8WG16 or y-80 antibodies (Santa Cruz Biotechnology) against the C or N-terminal region were used, respectively.

Chromatin immunoprecipitation was performed as previously described [39]. For real-time PCR, a dilution of 1:100 was used for INPUT, and one of 1:2 dilution was employed for the immunoprecipitated samples.

Genes were analyzed by quantitative real-time PCR in triplicate with at least three independent biological replicates using SYBR premix EX Taq (Takara).

The values found for the immunoprecipitated products were compared to those of the total input, and the ratio was calculated for the value of each PCR product of the transcribed genes *vs.* the value of a non-transcribed region of Chromosome V. Table S3 lists the oligonucleotides used.

2.3. mRNA extraction and reverse transcription.

The total RNA from yeast cells was prepared and quantified as previously described [30]. For the genome-wide analyses of the *rpb4* and foot mutant strains and their wild types, the exact concentration of total RNA in the cytoplasm was determined by the repeated extraction of three independent cell aliquots, which contained a known number of cells, and using the average cell volume of each strain, as described elsewhere [1].

First-strand cDNA was synthesized using 1 μg of RNA with the iScript cDNA synthesis kit (Bio-Rad) following the manufacturer's protocol. Each sample was subjected to the same reaction without reverse transcriptase as a negative control for genomic DNA contamination.

The proportion of [mRNA:total RNA] for each strain was estimated according to a protocol based on an Experion device (Biorad), as described in [1]. This ratio was calculated from the electropherograms of total RNA as the percentage of the area between the 5S and 18S rRNA peaks in relation to the total area. Alternatively, the [mRNA:total RNA] ratio was determined by a dot-blot procedure, as described later under subheading 2.6.

2.4. Real-time quantitative PCR (RT-qPCR)

Real-time PCR was performed in a CFX-384 Real-Time PCR instrument (BioRad) with the EvaGreen detection system "SsoFast™ EvaGreen® Supermix" (BioRad). Reactions were performed in 10 μl of total volume that contained the cDNA, which corresponded to 0.1 ng of total RNA. Each PCR reaction was performed at least 3 times with three independent biological replicates in order to have a representative average. The 18S rRNA gene was used as a normalizer. Table S3 lists the oligonucleotides used.

2.5. Fluorescence microscopy

For p-body localization, strains were transformed with the centromeric plasmid that expressed a Dcp2-Gfp fusion protein. For the co-localization of Rpb4 in p-bodies, strains were transformed with the centromeric plasmids that expressed fusion proteins Gfp-Rpb4 and Dcp2-Rfp (see Table S2 for plasmids). Cells were grown at 30 °C in SD medium which lacked the corresponding amino acids (OD_{600} ~ 0.5–0.7). Slides were covered with Vectashield (Vector Laboratories) mounting solution that contained DAPI.

Fluorescence intensity was scored with a fluorescence microscope (Olympus BX51).

2.6. mRNA stability analyses.

For the mRNA stability analysis, cells were grown in SD (with requirements) to an OD_{600} ~ 0.5. At that time, cells were treated with

5 μg/ml of thiolutin. Cell samples were taken at different times after thiolutin addition (up to 120 min), and were pelleted and frozen. Total RNA was isolated from these samples and the mRNA stability (half-lives, HL) for the selected genes was analyzed following the decay curves in a Northern blot, as described in [40] or by RT-qPCR with specific primers (see Table S3).

For the global mRNA stability analysis, the RNA from different strains (BY4741, *rpb4*Δ, *rpb4*Δ + *pCM189-RPB4*) was extracted as described above. In this case, sampling times were: 0 (before thiolutin addition) and 10, 20, 30, 45 min after thiolutin addition. Total RNA was printed by using a BioGrid robot on a nylon membrane in three different amounts per spot (15, 7.5 and 3.75 ng). For each sampling time, two biological and two technical replicates were considered. The mRNA proportion was estimated by hybridizing membranes with a γ-^{32}P radiolabeled oligo $d(T)_{40}$. Phosphorimager images were quantified as in [41]. Relative values of the mRNA:RNA ratios to the respective wild-type strain were calculated. Decay values were represented and an averaged mRNA half-life was calculated from two separate experiments for each strain.

For the analysis of the mRNA stability of stress genes *HSP26* and *HSP12*, their promoters were replaced with the *GAL1* promoter indicated in [37], and mRNA stability was analyzed by shifting the cells that grew exponentially (OD_{600} ~ 0.5–0.6) from SD-galactose to SD-glucose in order to stop transcription. Cell samples were collected at different times after glucose addition (10 min, 20 min, 30 min, and 45 min). RNA extraction was performed as described above, and mRNA stability was analyzed by RT-qPCR with specific primers for the corresponding genes (see Table S3).

2.7. Isolation of the mRNA-associated proteins

The crosslinking of mRNA was carried out as described in [42] with some modifications. Briefly, cells grown in 500 ml of SD media (with requirements) until an OD_{600} ~ 0.6–0.8 was reached were harvested by centrifugation at 4000 rpm for 5 min at room temperature, resuspended with 25 ml of PBS supplemented with 0.005% of Nonidet-P40, and transferred to a 150-mm plate disk. Plate disks were exposed to 1200 mJ/cm^2 of 254 nm UV in a UV crosslinker (Biolink Shortwave 254 nm) in three steps of 400 mJ/cm^2 with two 2-min breaks on ice and gentle mixing [42]. Then cells were harvested by centrifugation at 4000 rpm for 5 min at 4 °C and resuspended in 1.5 ml of lysis buffer (20 mM Tris pH 7.5, 0.5 M NaCl, 1 mM EDTA, 1× protease inhibitor cocktail [Complete; Roche]). After the addition of 900 μl of glass beads (425–600 μm, Sigma), cells were broken by vortexing for 15 min at 4 °C at the highest power. Glass beads were pelleted by centrifugation at 5000 rpm for 5 min and the lysate was passed 3–5 times through a needle using a 1–2 ml syringe to shear DNA. The lysate was clarified by a 10-min centrifugation at 14,000 rpm and 4 °C, and 50 μl was kept as a control.

Next 150 μl of oligo $(dT)_{25}$ cellulose beads (New England BioLabs, cat no. S1408S) were equilibrated with 500 μl of loading buffer (20 mM Tris–HCl pH 7.5, 0.5 M NaCl, 1 mM EDTA), spun, and mixed with the lysate. The mixture was incubated at room temperature for 15 min with gentle stirring. Then oligo $(dT)_{25}$ cellulose beads were washed 5 times with 500 μl of loading buffer and once with 500 μl of low salt buffer (10 mM Tris–HCl pH 7.5, 0.1 M NaCl, 1 mM EDTA). Elution was performed by adding 250 μl of elution buffer (20 mM Tris–HCl pH 7.5; pre-warmed at 70 °C) to the beads and incubating at room temperature for 5 min with gentle agitation. Elution was carried out twice and the two supernatants were mixed, lyophilized, and resuspended in 35 μl of miliQ H_2O. This sample was used for the mRNA quantification at 260 nm, SDS-PAGE and Western blot, and equal amounts of mRNA were loaded.

For western-blotting purposes, the anti-Rpb4 (Pol II RPB4 (2Y14), Santa Cruz Biotechnology), anti-Pgk1 (3-PGK; Invitrogen), anti-H3 (ab1791; Abcam), anti-Pab1 (gift from T.H. Jensen; [43]), anti-Rpb1 (8WG16; Santa Cruz Biotechnology), and anti-Rpl1 (gift from F. Lacroute; [44]) antibodies were used.

2.8. Global expression analyses

For all the genome experiments, *Saccharomyces cerevisiae* DNA macrochips in nylon membranes [45] were used. For the transcriptome analysis (RA data) of all the wild type and mutants, 40 μg of total RNA was used to synthesize radiolabeled cDNA using 500 ng of Oligo dT (T_{15}VN) as in [46]. The RA data of the *rpb4* experiment were normalized by a total mRNA concentration, determined as described in Section 2.3 for the *rpb4* experiment. For the experiments with foot mutants, no correction by total mRNA concentration was made because only relative changes in RA compared with the wild type were analyzed. For the transcription rates (SR data), a Genomic Run-On experiment was conducted as previously described [1]. The HL data were obtained from the RA and SR data as described in Ref. [1]. Three replicates of each strain were used. Scanning, statistical and bioinformatics analyses were run as in Ref. [41].

The GEO accession numbers for the transcriptomic data are GSE57467 for the *rpb4*Δ data and GSE65283 for the *rpo21–4* and *rpb1–84* ft mutant strains.

3. Results

3.1. The RPB1 mutations that affect RNA pol II assembly lead to a global stress response at the permissive temperature

To investigate the consequences of the mutations that affected RNA pol II assembly in mRNA expression, two strains containing the mutations in *RPB1* in the region that corresponded to the foot domain were used. These mutations, called *rpo21–4* and *rpb1–84*, caused an RNA pol II integrity defect that altered the correct association of Rpb6 and the Rpb4/7 dimer [30]. We ran a global expression analysis of the *rpo21–4* and *rpb1–84* mutants grown at 30 °C in rich medium with radiolabeled cDNA [45]. By analyzing those genes with expression differences higher than 2-fold in each mutant compared to the wild-type strain, we found a similar response for both mutant strains (Fig. 1A right-hand panel and 2A), although the *rpb1–84* mutant gave a larger number of genes for which expression was altered (see Fig. 2A). The global expression analysis results (Table 1) were corroborated by a quantitative real-time PCR (RT-qPCR) of the mRNA amount of the different genes whose mRNA accumulation had changed (Fig. 1B). An increase in the *CIT2* gene expression, and a decrease in the expression of genes *MFα2*, *URA2*, *UTP2*, and *STE3*, occurred in both mutants. However, for the *SPP2* gene, a decrease was found, while an increase occurred in the global expression analysis. By calculating the RNA content and the total mRNA:total RNA ratios in mutants *rpo21–4* and *rpb1–84* compared with the wild-type strain, we confirmed that the changes shown as relative in Fig. 1A also corresponded to absolute changes (not shown).

To further explore the effect of the incorrect RNA pol II assembly in mRNA expression, we ran a functional categories (Gene Ontology, GO) analysis with the genes that were differentially expressed more than 2-fold in foot mutants compared with the wild-type strain (Table 1). For both mutants, this analysis showed groups of genes related to a stress response, characterized by the alteration of more than 700 genes in a wild-type strain [47]. To confirm the stress response of mutants *rpo21-4* and *rpb1-84*, we used Venn diagrams to represent the genes whose expression increased or decreased by more than 2-fold in both mutants compared with the wild-type strains, and those belonging to the aforementioned ESR [47]. The overlap shown in Fig. 2A between the two mutant responses and the ESR was statistically significant. The number of common genes between the two mutants was larger than with the ESR, which probably reflects other common altered ESR-independent processes. The analysis of the transcription factors that were putatively associated with all the genes which are

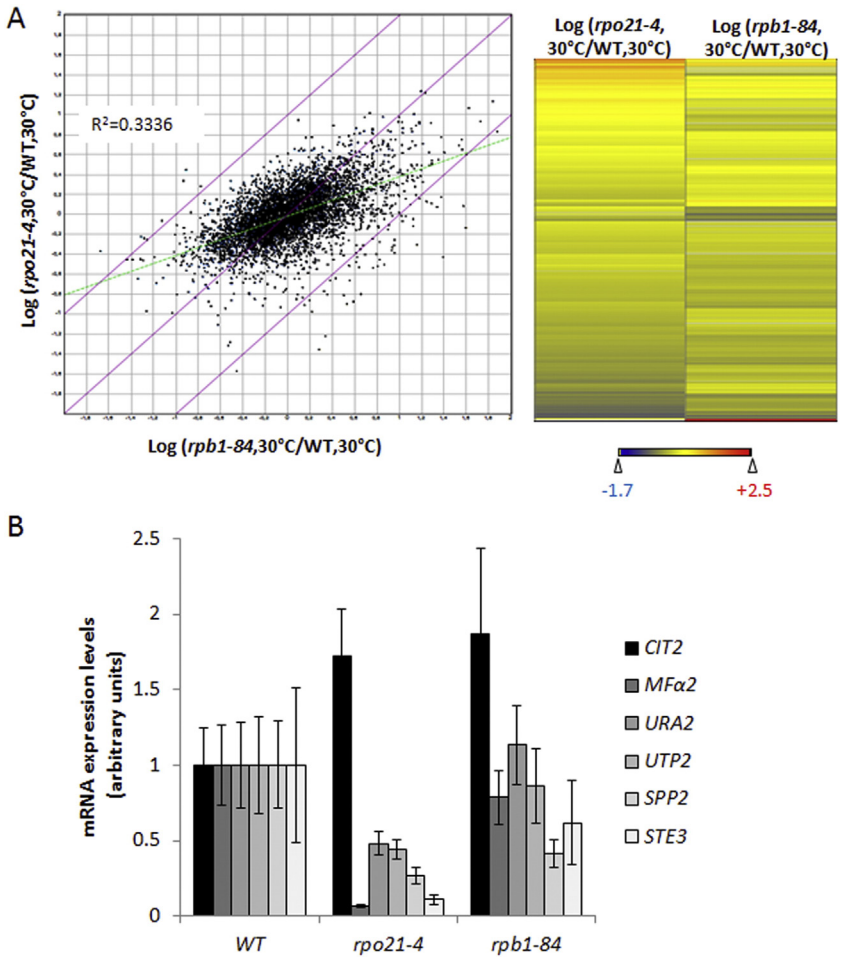

Fig. 1. Transcriptomic global expression analysis of foot mutants *rpo21-4* and *rpb1-84*. A) The relative mRNA abundance profile of mutants *rpo21-4* and *rpb1-84* at the permissive temperature, compared with a wild-type strain (YFN116), by DNA macroarray analysis [45]. The analyses of the results were performed with the DNAstar software. The left-hand graph shows the plot for all the individual mRNAs. Pearson r correlation coefficient is shown. The right-hand panel shows a heat map of all the individual mRNAs shown in the left-hand graph. B) The mRNA levels for genes *CIT2*, *MFα2*, *URA2*, *UTP2*, *SPP2*, *and STE3* in the foot mutants and wild-type strains measured by RT-qPCR at 30 °C. rRNA *18S* was used as a normalizer. Data are shown as the average and standard deviation (SD) of the results of at least three independent experiments.

commonly altered in foot mutants, analyzed by a YEASTRACT platform (http://www.yeastract.com/index.php; [48]), corresponded mainly to those related to stress (Table 2). These mutants also showed a transcriptome feature of a slow-growth phenotype, which has been described as being parallel to the ESR, and had a strength signature of about 17 units for both mutants according to the algorithm described in [49].

To further investigate this stress response, we analyzed the mRNA expression levels of some stress genes at the permissive temperature of 30 °C and after a 37 °C shift for 30 min. At the permissive temperature, foot mutants showed higher mRNA expression levels for all the genes analyzed (see Fig. 2B). After a shift to 37 °C for 30 min, however, their mRNA levels in general reached lower levels than the average wild-type ones (Fig. 2B), and the ratio of the mRNA levels at 37 °C *vs.* 30 °C was lower. These data suggest a defect in repression at the permissive temperature. However, we cannot rule out that the activation of these genes at 37 °C could also be affected (Fig. 2C).

It has been reported that p-bodies are important for cellular stress and that they accumulate during stress responses [50]. Hence we analyzed the p-body accumulation in the foot mutants that contained a plasmid expressing p-body marker Dcp2 fused to a GFP protein [51]. At the permissive temperature of 30 °C, foot mutants showed higher levels of p-bodies than did the wild-type strain (see Fig. 2D).

All these data suggest that foot mutants display a constitutively activated stress response under optimal growth conditions at a permissive temperature and, therefore, show a reduced stress response after a change in temperature.

3.2. Stress response under optimal growth conditions at a permissive temperature is dependent on Rpb4

Foot mutants partially alter the association of the Rpb4/7 dimer from RNA pol II [30]. To investigate whether the stress response under optimal growth conditions at a permissive temperature depended on Rpb4/7, we performed an enrichment analysis of the functional categories (GO), by using the FASTICAN software [52] with published data on the mRNA levels from the *rpb4*Δ mutant grown at 30 °C [24]. Notably, the *rpb4*Δ mutant showed an ESR signature and a slow-growth-phenotype index of about 21 units, as described by [49], which was slightly higher than that shown by foot mutants (see above). After analyzing some HSP genes (*HSP26*, *HSP12*, and *HSP104*) by RT-qPCR in our *rpb4*Δ mutant strain (Supplementary Fig. S1), grown at a permissive temperature, their amount of mRNA increased compared with the wild-type strain.

All these data suggest that a deletion of *RPB4* behaves similarly to foot mutants and that Rpb4 might be directly related to the ESR under optimal growth conditions at a permissive temperature.

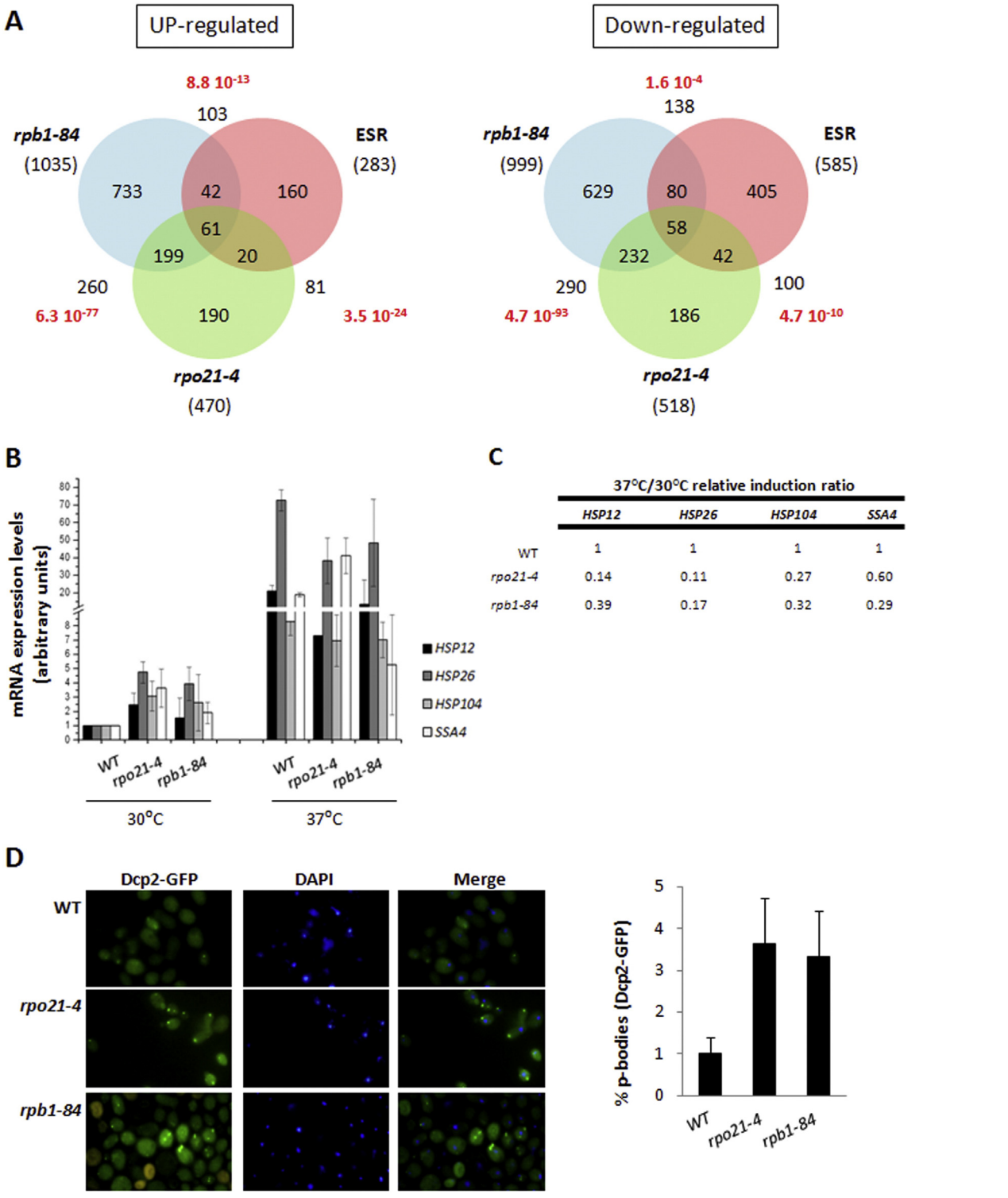

Fig. 2. Mutants *rpo21-4* and *rpb1-84* activate an environmental stress response (ESR) under optimal growth conditions at the permissive temperature. A) Venn diagrams representing the differentially expressed genes (more than 2-fold) in foot mutants compared with a wild-type strain (YFN116), and those altered in an ESR response after treatment for 60 min at 37 °C [47]. B) The mRNA amounts for the *HSP12*, *HSP26*, *HSP104*, and *SSA4* genes in the foot mutants and wild-type (YFN116) strains measured by RT-qPCR at 30 °C and 37 °C. rRNA *18S* was used as a normalizer. Data are the results or at least three independent experiments (see Fig. 1 for details). C) 37 °C/30 °C relative induction ratios of the genes analyzed in B, which shows the activation for these stress-response genes. The induction ratio of the wild type (YFN116) was set arbitrarily at 1 and the induction of the mutants is shown in relation to it. D) Left: Dcp2-GFP localization as a marker of the p-body in the foot mutants and wild-type (YFN116) strains transformed with an empty vector. Right: Quantification of p-body accumulation shown in the picture on the left. The wild-type p-body level was set at 1 and the mutant levels are shown in relation to it. One hundred cells of each strain were analyzed.

3.3. Restoring correct RNA pol II assembly suppresses the stress response of foot mutants

The partial dissociation of Rpb4/7 from RNA pol II in foot mutants is overcome by *RPB6* overexpression [30]. Hence we studied whether the stress response in these mutants could be associated with incorrect RNA pol II assembly, and thus with Rpb4 dissociation. To do this, we analyzed the mRNA expression levels of the stress genes under *RPB6* overexpression using a pCM185-*RPB6* plasmid [30]. As shown in Fig. 3A (compared to Fig. 2B), the expression levels of the stress genes at

Table 1
Functional categories of the differentially expressed genes in foot mutants compared with a wild-type strain. The analysis was performed using the FatiScan software [52].

rpo21-4/wt		*rpb1-84*/wt	
Functional categories	p-Value	Functional categories	p-Value
Up-regulated		Up-regulated	
Cytoplasm	5.0 e–10	Heat-shock protein activity	6.8 e–12
Heat-shock protein activity	3.3 e–5	Catalytic activity	1.0 e–10
Proteasome complex (*sensu eukarya*)	4.7 e–5	Carbohydrate metabolism	1.6 e–8
		Alcohol metabolism	3.6 e–7
		Protein folding	3.1 e–6
		Cell	4.7 e–5
Down-regulated		Down-regulated	
Cytosolic ribosome (*sensu eukarya*)	<1.00 e–15	Ribosome	< 1.0 e–15
Cytosolic large ribosomal subunit (*sensu eukarya*)	1.1 e–09	Ribonucleoprotein complex	< 1.0 e–15
Ribonucleoprotein complex	3.1 e–08	Cytosolic ribosome (*sensu eukarya*)	< 1.0 e–15
Ribosome biogenesis and assembly	3.8 e–08	Structural constituent of ribosome	< 1.0 e–15
Ribosome biogenesis	3.4 e–07	Cytosolic large ribosomal subunit (*sensu eukarya*)	3.2 e–12
rRNA processing	3.9 e–07	Large ribosomal subunit	1.5 e–11
rRNA metabolism	6.1 e–07	Structural molecule activity	8.2 e–11
Nucleolus	1.1 e–06	Protein biosynthesis	1.8 e–05
Ribosome	1.8 e–06	Small ribosomal subunit	2.0 e–05
Structural constituent of ribosome	8.2 e–06	Cytosolic small ribosomal subunit (*sensu eukarya*)	5.0 e–05
Nucleobase, nucleoside, nucleotide, and nucleic acid metabolism	5.0 e–05	Cellular component unknown	5.0 e–05

30 °C were partially restored under the *RPB6* overexpression for the *rpo21-4* mutant and, to a lesser extent, for the *rpb1-84* mutant. After a shift to 37 °C for 30 min, the mRNA expression levels were comparable in foot mutants and wild-type strains. The ratios between the expression of the stress genes at 37 °C *vs.* 30 °C under *RPB6* overexpression were similar for the *rpo21-4* mutant and the wild-type strain, but remained lower for the *rpb1-84* mutant (Fig. 3B, compared to Fig. 2C). The higher levels of p-bodies in foot mutants were recovered under *RPB6* overexpression (Fig. 3C), a situation in which the RNA pol II assembly defect was suppressed.

According to these data, we propose that the dissociation of the Rpb4/7 dimer in foot mutants from the rest of RNA pol II is responsible for the stress response. In any case, we cannot rule out a partial contribution of the specific *RPB1* foot mutations.

3.4. Chromatin immunoprecipitation of RNA pol II indicates a general effect on the mRNA stabilization in mutants rpo21-4 and rpb1-84

After characterizing the direct effect of the *RPB1* foot mutations, we investigated the level at which they participate. We first analyzed RNA pol II occupancy by performing chromatin immunoprecipitation (ChIP) on the wild-type and foot mutants using the 8WG16 antibody against the C-terminal domain of Rpb1, and we analyzed its occupancy at the promoters and ORFs of stress genes *HSP12*, *HSP26*, *HSP104*, and *SSA4*. RNA pol II occupancy seemed to vary little on average in foot mutants compared with the wild-type strain (see Fig. 4A). Conversely, occupancy strongly diminished for other non-stress genes, such as *PMA1*, *PYK1*, and *CIT2* (Fig. 4B), as in our previous report [30]. These data suggest that the global relative amount of RNA pol II is biased toward the stress genes in these mutants compared with the wild-type strain.

In an attempt to correlate the mRNA expression and RNA pol II occupancy for stress genes, we compared the Rpb1 occupancy data to some stress genes in the wild-type strain, and the *rpo21–4* and *rpb1–84* mutants to those of the mRNA levels for the same genes. The relative amount of mRNA in foot mutants *vs.* the wild type was larger (3× on average) than for Rpb1 recruitment (Figs. 4C and S2). Similarly for the non-stress genes (Figs. 4D and S2), although the mRNA levels were lower than in the wild type, Rpb1 occupancy was much lower (half on average). These data suggest that foot mutations cause a general mRNA stabilization effect.

Table 2
Percentage of the genes putatively regulated by each transcription factor among those in which expression changed more than 2-fold. The analysis was performed by the YEASTRACT program [48].

Transcription factor	% genes	
	rpo21-4, *rpb1-84*/WT (30 °C) (>/<2 fold-change)	WT ESR [47]
Sfp1	71.5	92.9
Ste12	61.9	64.8
Msn2	60.1	84.7
Rap1	48.3	43.8
Msn4	47.3	72.9
Gcn4	45.3	66.2
Yap1	40.2	58.4
Sok2	39.9	72.9
Rpn4	38.1	60.8
Hsf1	35.7	54.4
Arr1	29.9	44.1
Aft1	26.5	38.8
Fhl1	21.8	30.6
Pdr3	20.4	39.1

3.5. Altering correct RNA pol II assembly leads to global mRNA stabilization

To confirm the increased mRNA half-lives in foot mutants, we analyzed mRNA decay for some transcripts of the non-stress genes. For this purpose, the wild-type and mutant cells were treated with 5 μg/ml of thiolutin to block transcription, and total RNA was extracted at different times after thiolutin addition. We analyzed mRNA decay by Northern blot, where we used specific probes against transcripts *ACT1*, *RPL17*, *RPB6*, *HHF1*, and *YKE2* (Supplementary Fig. S3). Foot mutants clearly increased the mRNA half-lives of all the tested genes *vs.* the half-lives in the wild-type strain (Table 3), except *ACT1* for the *rpo21-4* mutant. The increase in the mRNA half-lives was generally more marked for the *rpb1-84* mutant. These results were corroborated by analyzing the mRNA half-lives for genes *ACT1*, *RPL27*, *HHF1*, and *YKE2* in the wild-type and the *rpb1–84* mutant strains by RT-qPCR (Fig. S4).

Thiolutin has been shown to induce stress genes, such as *HSP82* (5- to 10-fold), *SSA4* (more than 25-fold) and *HSP26* (more than 50-fold), under the conditions in which the transcription of the non-heat-shock genes was blocked [53], and its effect on transcription and mRNA stability depended on its concentration [40]. Therefore, it cannot be used for mRNA half-life determinations in these genes [2,54]. To analyze the

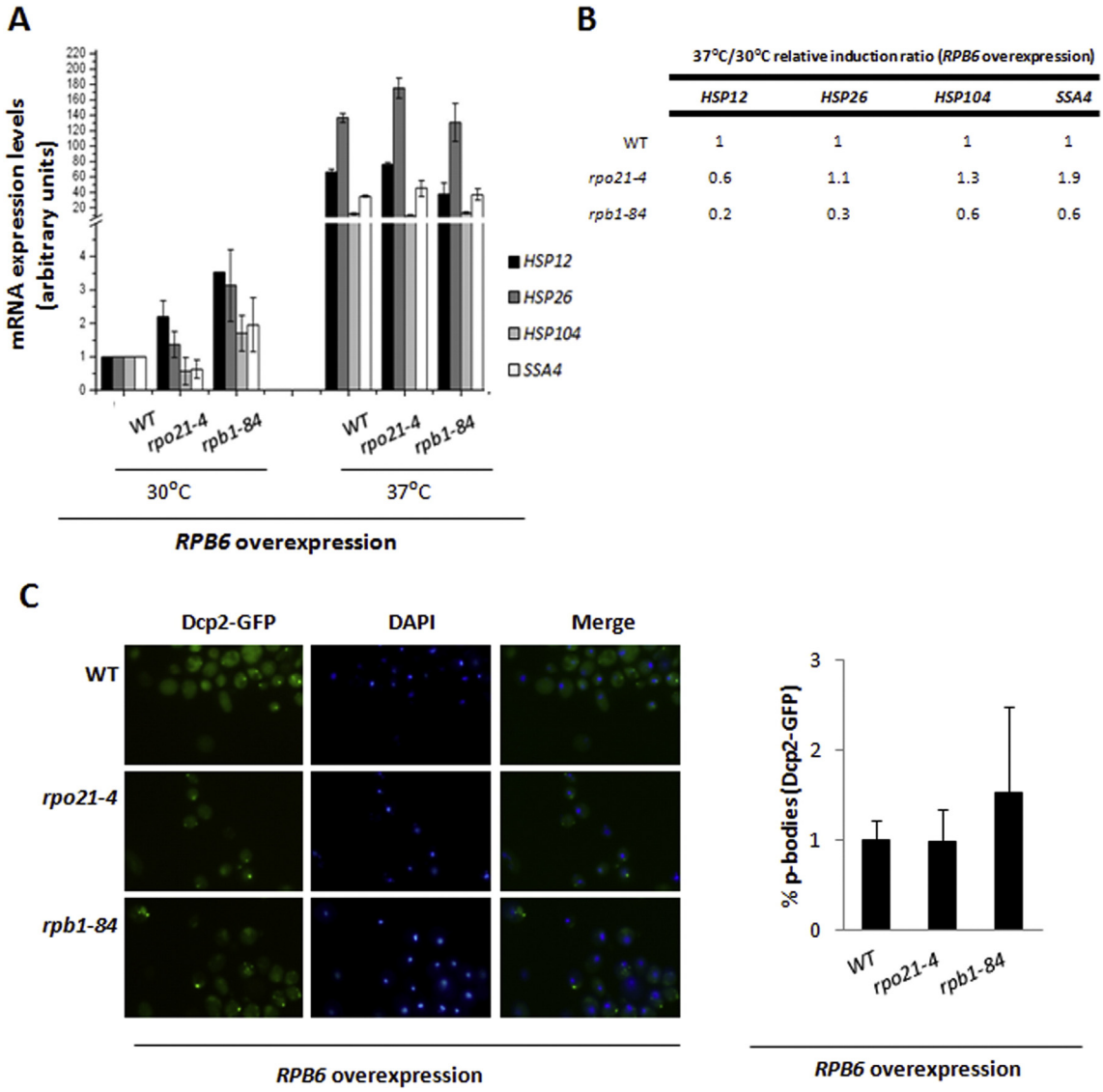

Fig. 3. Correction of the assembly of RNA pol II by *RPB6* overexpression in foot mutants partially suppresses the stress response (ESR) under optimal growth conditions at the permissive temperature. A) mRNA accumulation for the *HSP12*, *HSP26*, *HSP104*, and *SSA4* genes in the foot mutants and wild-type (YFN116) strains measured by RT-qPCR at 30 °C and 37 °C under *RPB6* overexpression. rRNA *18S* was used as a normalizer. Data are the results of at least three independent experiments (see Fig. 1 for details). B) 37 °C /30 °C relative induction ratios of the genes analyzed in A, which shows the activation for these stress-response genes under *RPB6* overexpression. The induction ratio of the wild type (YFN116) was set arbitrarily at 1 and the induction of the mutants is shown in relation to it. C) Left: Dcp2-GFP localization as a marker of p-bodies in the foot mutants and wild-type (YFN116) strains transformed with a vector that overexpresses *RPB6*. Right: Quantification of the p-body accumulation shown in the left-hand frame. The wild-type p-body level was set at 1 and the mutant levels are shown in relation to it.

mRNA half-lives of stress genes, we simultaneously replaced the promoters of both *HSP26* and *HSP12* genes with the *GAL1* promoter indicated in [37] in a wild-type strain, and also in mutants *rpo21-4* and *rpb1-84*. We analyzed the mRNA half-lives by RT-qPCR using the RNA extracted at different times after blocking transcription by adding glucose to the cells that grew exponentially in SD-galactose (Fig. 5A). As shown in Table 3, mRNA stability increased by about 50% in foot mutants compared to the wild-type strains for genes *HSP26* and *HSP12*, and also for the *GAL1* gene used as a control. These results confirm the inference of the previously proposed general mRNA stabilization.

Collectively, all these data indicate that altering correct RNA pol II assembly leads to global mRNA stabilization, which could be the result of Rpb4/7 dissociation, and agrees with the proposed role of the Rpb4/7 dimer in mRNA stability [1,3,7,15,17,27,29]. These two Rpb1 alleles extend the current list of mutants which disrupt interactions with the Rpb4/7 heterodimer, and which display defects in mRNA degradation. This scenario reveals that this is a general feature of the mutants that lack Rpb4/7 assembly, such as *rpb4*Δ [20,22,24], mutants *rpb6Q100R* and *rpb1C67S*, *C70S* [55] and the *RPB1* foot mutants (in this paper).

3.6. Rpb4–mRNA interaction diminishes in foot mutants

Rpb7 interacts with the emerging RNA pol II transcript *in vitro* [26], and Rpb4 has been shown to associate with some mRNA transcripts *in vivo* in the RNA pol II elongation context [15]. Rpb4/7 remains associated with mRNAs throughout its life, and regulates processes such as export, translation, and decay pathways [15,16,27,29,55]. It has recently been proposed that the reduced ability to recruit Rpb4/7 to core RNA pol II results in impaired production and decay for selected genes [3]. These data suggest that the RNA pol II assembly defect could be responsible for global mRNA stabilization in foot mutants by reducing the amount of imprinted mRNAs. This is in fact true of mutants *rpb6Q100R* and *rpb1C67S*,*C70S* [3,15,55], at least for the PBF mRNA family.

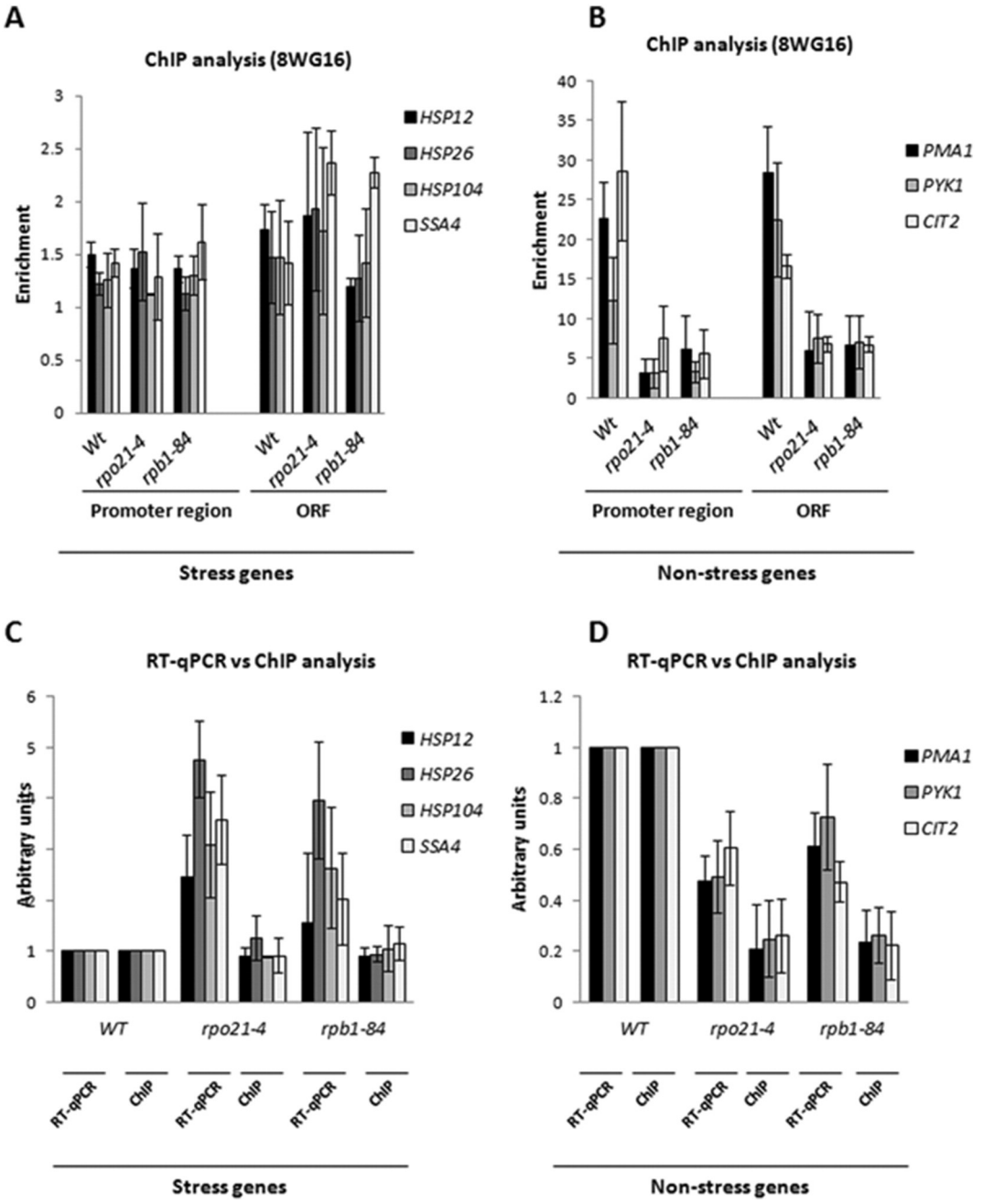

Fig. 4. Foot mutations suggest increased mRNA stability under optimal growth conditions at the permissive temperature. A) Rpb1 occupancy in stress-response genes in foot mutants and wild-type (YFN116) strains using the 8WG16 antibody at both the promoters and open reading frames (ORFs). B) A ChIP analysis of different non-stress genes in the wild type (YFN116) and foot mutants using the 8WG16 antibody at both the promoters and ORFs. C) Comparison of the mRNA levels, measured by RT-qPCR (from Fig. 3), and the RNA pol II occupancy for the stress-response genes determined by ChIP (from panel A). D) Comparison of the mRNA levels, measured by RT-qPCR (from Fig. 1), and the RNA pol II occupancy for the non-stress-response genes determined by ChIP (from panel B). The ChIP results corresponded to the promoter region. Similar results were found in the 3′ coding region (Fig. S2).

To decipher whether the RNA pol II assembly defect in foot mutants was responsible for global mRNA stabilization, we analyzed the association of Rpb4 with mRNAs by Western blot after mRNA–protein cross-linking by UV irradiation at 254 nm. First, to demonstrate the validity of the technique used, we analyzed the association of Pab1 with the mRNAs in the wild-type cells either treated or not treated with UV irradiation (Fig. S5). As shown, UV-irradiation increased the amount of Pab1 associated with mRNA. To demonstrate that no major genomic or cytoplasmic contaminants were associated with the resin, we analyzed the presence of Histone H3 and Pgk1 proteins in the samples by using specific antibodies. As shown, no genomic or cytoplasmic contamination was detected. Similarly, no significant ribosomal protein contamination was found, as demonstrated by using an antibody against the Rpl1 ribosomal protein. Rpb4 associated with the mRNA under UV irradiation, while no association of the largest subunit of the RNA pol II, Rpb1, was observed.

As shown in Fig. 6A, the Rpb4 association with mRNA was poorer in foot mutants than in the wild-type strain. The Rpb4–mRNA association was specific, given that no cross-reaction was detected when using the antibodies against histone H3 and Pgk1 as negative controls, this ruling out chromatin or cytoplasmic contamination in the poly(A) samples.

These data support the hypothesis that lower levels of Rpb4-imprinted mRNAs in foot mutants can cause the observed increase in global mRNA stability.

Table 3
mRNA stability under optimal growth conditions at the permissive temperature. The mRNA half-lives for non-stress genes calculated from the Northern-blot experiments represented in Fig. S2. The mRNA half-lives in foot mutants were compared to those of the wild-type (YFN116) strain at the indicated time after stopping transcription with thiolutin. Time 0 corresponded to the cells grown in the absence of thiolutin. The mRNA half-lives for stress genes *HSP12* and *HSP26* under the control of the *pGAL1* promoter, calculated from the RT-qPCR experiments represented in Fig. 5. The mRNA half-lives in foot mutants are compared to those of the wild-type (YFN116) strain at the indicated time after stopping transcription with glucose in the cells that harbored an empty vector (upper panel) or a vector that overexpressed *RPB6* (lower panel). Time 0 corresponded to the cells grown in the presence of galactose as the carbon source.* The *GAL1* gene was used as a control of the cells grown in the presence of galactose as the carbon source.

Non-stress genes

	ACT1	*RPL27*	*RPB6*	*HHF1* (*H4*)	*YKE2*
WT	1	1	1	1	1
rpo21-4	0.8	1.2	1.1	1.2	1.2
rpb1-84	1.7	1.5	2.0	1.7	2

Stress genes

	HSP12	*HSP26*	*GAL1*
Empty vector			
WT	1	1	1
rpo21-4	1.8	1.7	1.5
rpb1-84	1.3	1.8	1.4
RPB6 overexpression			
WT	1	1	1
rpo21–4	1	1.1	1.2
rpb1–84	1.1	1.1	1.2

3.7. Overexpression of Rpb6 suppresses mRNA stability alterations and restores the Rpb4–mRNA association

Global mRNA stability in foot mutants depends on the Rpb4/7 dissociation from RNA pol II. To investigate this possibility, we analyzed the mRNA half-lives of *HSP12*, *HSP26*, and *GAL1* mRNAs under *RPB6* overexpression, where the assembly defect was overcome. As shown in Fig. 5B, favoring RNA pol II assembly by *RPB6* overexpression corrected the increase in mRNA stability and led to *HSP12*, *HSP26*, and *GAL1* mRNA half-lives similar to that in a wild-type strain (compare with Table 3).

The correction of the RNA pol II assembly defect by *RPB6* overexpression led to higher levels of Rpb4-imprinted mRNAs in foot mutants, with similar values to those found in a wild-type strain (Fig. 6B).

These data confirm that incorrect RNA pol II assembly is responsible for the decreased mRNA imprinting and the increased mRNA stabilization in foot mutants. Although we cannot rule out a role for Rpb7 in this process, our data suggest that this phenomenon globally depends on the Rpb4–mRNA association.

3.8. Rpb4 globally modulates mRNA stability

To confirm that a lack of Rpb4 causes global mRNA stability, we independently performed a genome-wide analysis in an *rpb4*Δ mutant (BY4741 background), which differed from that described in [24], given that we checked to ensure that it was not aneuploid. We found that in this mutant the mRNA general synthesis rate (SR) slowed down compared with its wild-type strain under optimal growth conditions. Notably, as predicted, the data also showed a longer mRNA half-life (HL) (Fig. 7A) similar to that of the mutant described in [24].

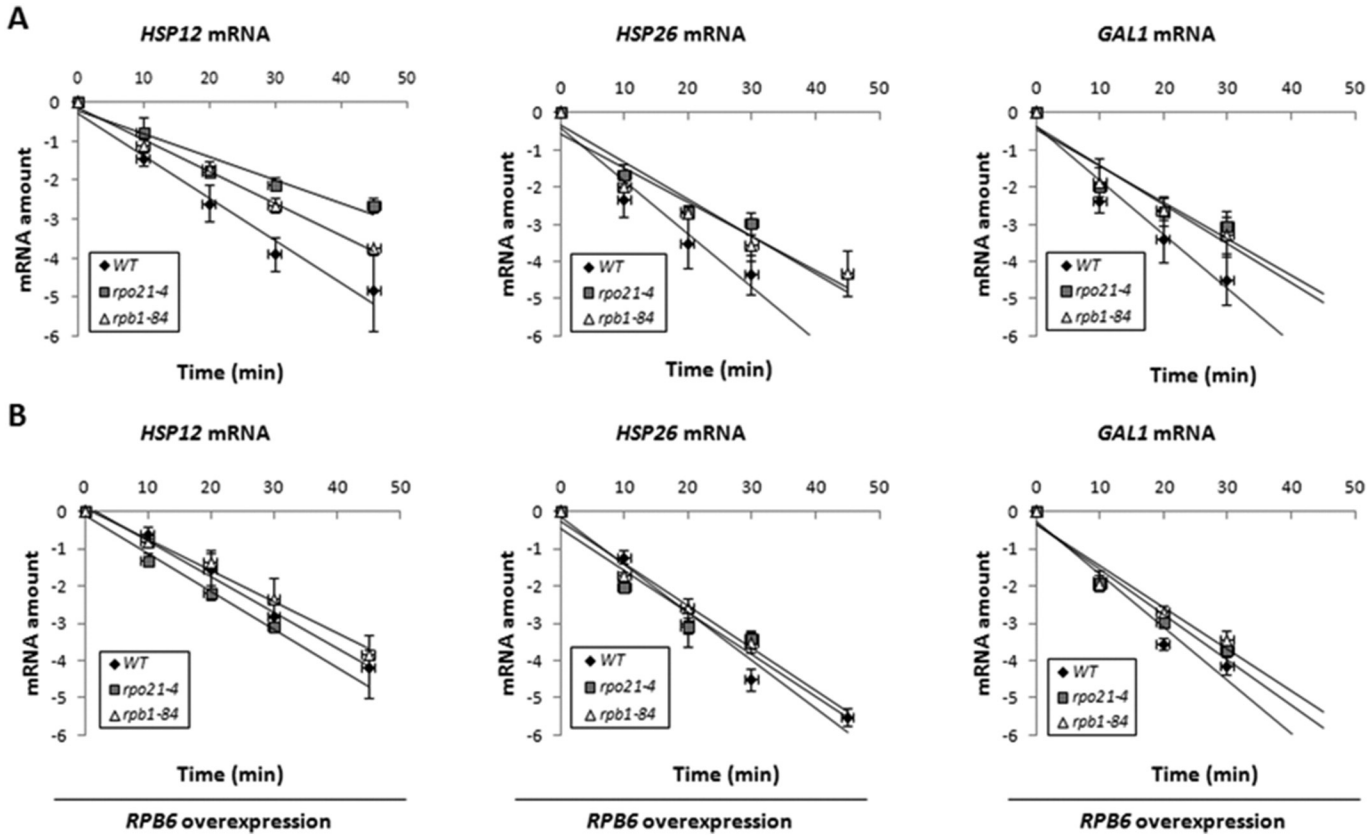

Fig. 5. Global mRNA stability under optimal growth conditions at the permissive temperature depends on the Rpb4 association with mRNA. A) mRNA levels, measured by RT-qPCR, for stress-response genes *HSP12* and *HSP26* under the control of the *pGAL1* promoter and the *GAL1* gene in both foot mutants and a wild-type strain (YFN482) at the indicated time after stopping transcription with glucose. Time 0 corresponded to the cells that grew in the presence of galactose as the carbon source. A drop in the mRNA levels after shutoff at different times (10, 22, 30, and 45 min) is represented on a natural logarithmic scale. B) Same as in A), but for the foot mutants and wild-type (YFN482) strains transformed with a vector that overexpresses *RPB6*. rRNA *18S* was used as a normalizer.

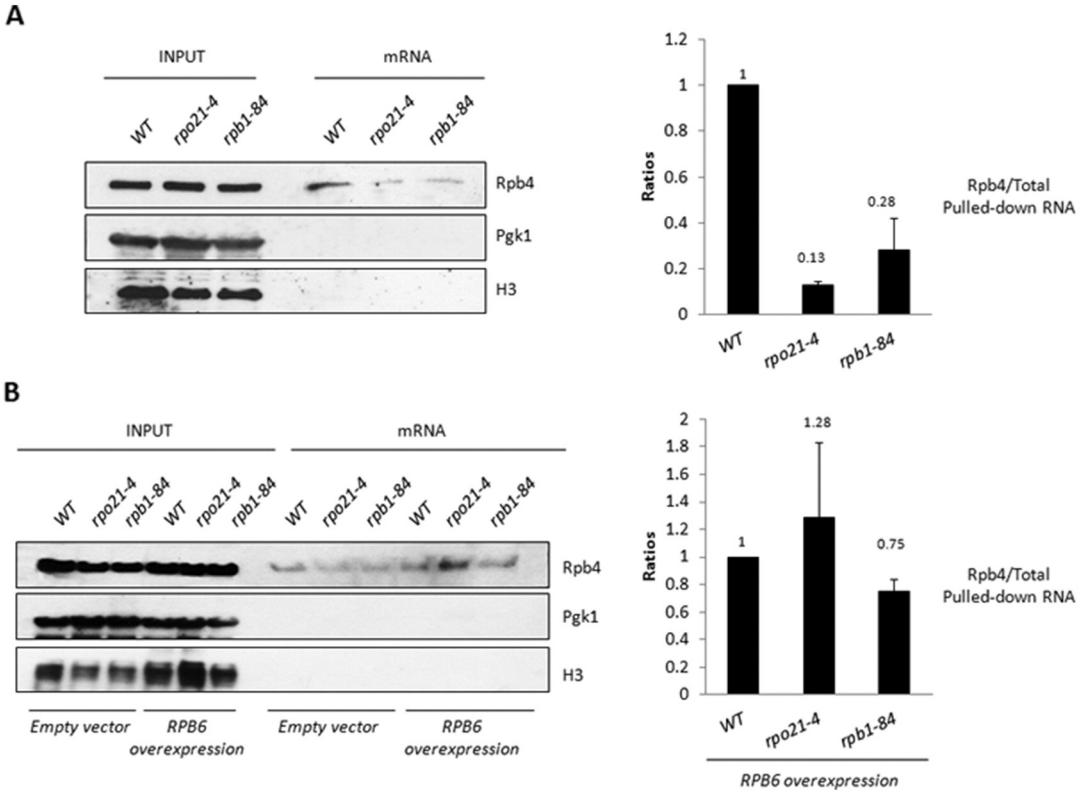

Fig. 6. Rpb4 association with mRNA is altered in foot mutants. A) Left panel, Western blot of Rpb4 in both whole-cell free extracts and oligo-dT purified mRNAs after exposure to 1200 mJ/cm2 of 254 nm UV (6 min). Antibodies anti-H3 and anti-Pgk1 were used as negative controls. Right panel, Rpb4 quantification of the Western blot signal represented as being relative to the total "pull-downed" mRNA amount in mutants *rpo21–4* and *rpb1–84* compared to a wild-type strain (YFN116). B) Same as in A), but for the foot mutants and wild-type (YFN116) strains transformed with an empty vector or with a vector that overexpresses *RPB6*.

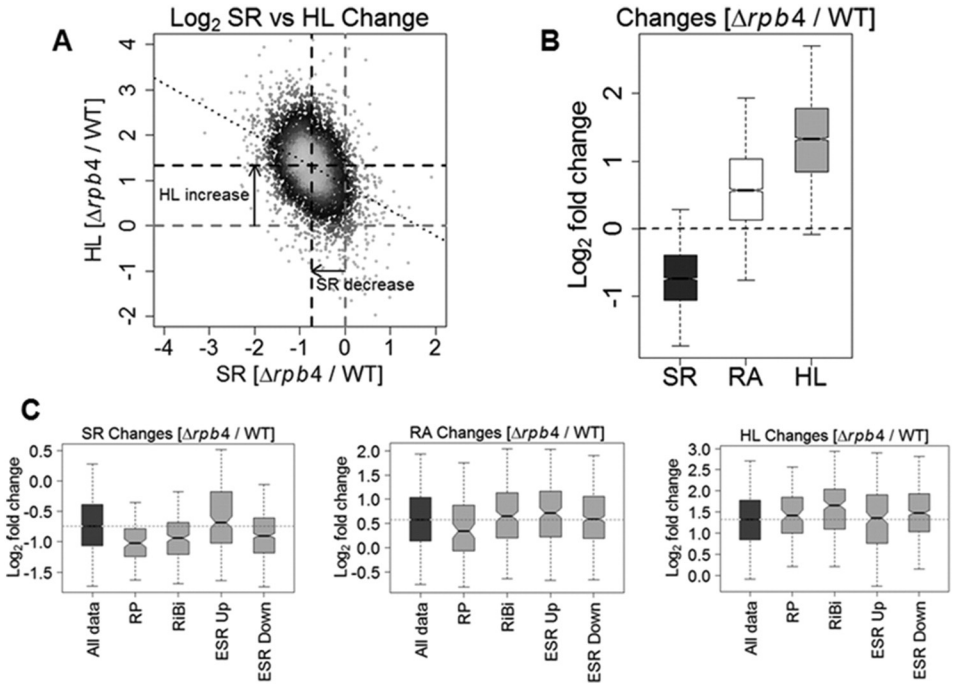

Fig. 7. Genome-wide analyses of the *rpb4*Δ mutant. A) Comparison made between the mRNA synthesis rates (SR) and the mRNA half-life (HL) in the *rpb4*Δ mutant *vs.* a wild-type (BY4741) strain by the GRO method [41]. Arrows indicate the average displacement observed in the *rpb4* mutant compared with the wild type. B) The fold change of the SR, mRNA amounts (RA) and HL in the *rpb4*Δ mutant *vs.* a wild-type (BY4741) strain. C) Same as in B), but for specific functional categories (RP: ribosomal proteins; RiBi: ribosome biogenesis; ESR down: environmental stress response down-regulated genes; ESR up: environmental stress response up-regulated genes). Note that a dotted line marks the global average for comparison purposes.

This effect was comparable to that of foot mutants, although it appeared to be quantitatively increased. Thus the consequence of a more marked HL increase than the SR decrease, resulting in a less marked rise in the mRNA amounts (RA) (Fig. 7B), indicated partial compensatory buffering [1,56]. The analyses performed with the *rpb4*Δ mutant data described in [24] have shown results qualitatively similar to our own: general mRNA stabilization and a clear tendency of mRNAs with higher stabilization to be those with the most pronounced decreases in the SR (see Fig. 1C and D in [24]).

In the present study, the analysis of several functional groups showed that RP and RiBi (and all the ESR down-response genes in general) were more affected (in both the SR and HL) in this particular *rpb4* mutant, whereas the SR of the ESR up-group increased more, but stabilized to the average value, causing a relative increase in RA (Fig. 7C).

To corroborate that Rpb4 function is, in fact, based on these phenomena and that no other suppressor mutations in the *rpb4*Δ mutant strain could influence the observed data, we constructed and introduced a centromeric pCM189-*RPB4* vector into the *rpb4*Δ mutant strain and analyzed growth. As seen in Supplementary Fig. S6 (panel A), *RPB4* expression overcame the temperature sensitivity of the *rpb4*Δ mutant strain. We also analyzed global mRNA stability in the wild-type and mutant cells that contained the centromeric pCM189-*RPB4* vector upon thiolutin addition to block transcription. The global mRNA decay determined from a dot-blot experiment, as described in M&M, showed that *RPB4* expression mostly corrected the increase in mRNA stability provoked by lack of Rpb4 (Supplementary Fig. S6).

Collectively, these data demonstrate that the transcription and stability for most mRNAs depend on the presence of Rpb4, and that this effect is greater in the main components of the down-regulated ESR response, the PBF genes.

4. Discussion

Mutations in *RPB1*, in the region corresponding to the foot of RNA pol II, alter the integrity or stability of the complex by promoting the partial dissociation of both Rpb6 and the Rpb4/7 dimer, which affects the amount of enzyme associated with genes and transcriptional activity [30]. In this work, a previous observation made of foot mutations, which affect transcriptional activity, is extended with a global transcriptional analysis to explore the consequences of the Rpb4/7 dissociation. We show that Rpb4 is key to globally modulate mRNA levels by influencing both transcription and mRNA decay, and that the constitutively activated stress response of these mutants depends on post-transcriptional regulation.

The stress response in foot mutants occurs under optimal growth conditions and may depend on a defect in Rpb4 association, which suggests that the lack of Rpb4 mRNA imprinting, and probably of Rpb4/7, plays a major role in the ESR at the permissive temperature. In agreement with this, Rpb4 plays a role in the heat-shock response [18–20,57]. The previously published *rpb4*Δ mutant [24] shows an ESR response [47] and the corresponding slow-growth phenotype [49] under optimal growth conditions, as we determined by a GO-enrichment analysis. This finding suggests that Rpb4 contributes to stress response under these conditions. In agreement with these observations, the experiments we performed with a different *rpb4*Δ mutant demonstrated higher mRNA levels for some HSP genes (Fig. S1) and the activation of ESR-inducible genes (Fig. 7, RA panel) under optimal growth conditions at the permissive temperature compared with the wild-type strain.

The contribution of Rpb4 to the ESR could correlate with the greater cytoplasmic accumulation of Rpb4 in the foot *rpb1-84* mutant compared to *rpo21-4* that we have previously reported [30], since Rpb4 translocation to the cytoplasm has been associated with stress conditions [58], and our transcriptomic analysis showed a stronger ESR response for the *rpb1–84* mutant.

Our data clearly demonstrate that correct Rpb4/7 association with RNA pol II is necessary to modulate mRNA decay and transcription under optimal growth conditions. In fact, either the dissociation of Rpb4/7 in foot mutants, a phenomenon that is overcome by overexpressing *RPB6* [30], or lack of Rpb4 in a *rpb4*Δ strain, prompts a global increase in mRNA stability under optimal growth conditions. Partially similar results have been reported by Schulz et al. [24] when these authors followed a different transcriptomic methodology. mRNA decay may depend on Rpb4, since complementing a *rpb4*Δ strain with a plasmid that harbors *RPB4* globally restores wild-type levels of mRNA stability. Our conclusion, however, partially disagrees with the interpretation of Schulz et al. [24], who argued that Rpb4 affects only transcription and not mRNA stability. These authors used an Rpb2–Rpb4 fusion to tether Rpb4 to the nucleus. This construction, however, only partially restored wild-type features, leaving open the possibility that Rpb4 deletion causes changes in mRNA stability by more than one mechanism. Moreover, the involvement of Rpb4/7 in mRNA stability mediated by direct binding to mRNAs has been previously demonstrated by other groups [3,7,15,17,27,29]. We speculate that Rpb4 also serves in the co-transcriptional recruitment of other factors needed to modulate mRNA stability in a coordinated way. As the present study also demonstrates, there is a correlation between global reduction in mRNA decay and the global binding of Rpb4 to poly(A) mRNA (Rpb4 mRNA imprinting), since the *RPB6* overexpression that suppresses the Rpb4/7 dissociation corrects the defects in mRNA decay and the decrease in the amount of Rpb4 associated with mRNAs. In line with this, it has been proposed that the reduced ability to recruit Rpb4/7 to core RNA pol II results in impaired production and decay for some selected genes [3]. Furthermore, reducing the amount of imprinted mRNAs could account for mRNA stabilization, at least for the PBF mRNA family, in mutants *rpb6Q100R* and *rpb1C67S,C70S* [3,15,56].

Surprisingly, the *rpb6Q100R* mutant showed neither a transcriptome feature of a slow growth phenotype nor an ESR response under optimal growth conditions, as determined by a previously published GO-enrichment analysis [3]. In agreement with this, and unlike the *rpb4*Δ mutant, *rpb6Q100R* did not show higher mRNA levels for some *HSP* genes under optimal growth conditions at the permissive temperature (not shown). We were unable to determine the reason for the inconsistency of the *rpb6Q100R* mutant, but we have evidence that the *rpb6Q100R* mutation in other genetic backgrounds than those mentioned by other groups [3,15] shows no growth phenotype, which agrees with our current results. These data suggest that the *rpb6Q100R* mutant used by these other groups [3,15] contained some genetic abnormality, which would account for its thermosensitive phenotype.

Our data also confirmed the special association of Rpb4 with PBF mRNAs [15,29], but indicated a more general association of Rpb4 with mRNAs than was previously noted. Thus our data extend and complement previous works which have demonstrated that Rpb4/7 affects the cytoplasmic degradation of some specific transcripts in yeast, such as those involved in protein biosynthesis, and which encode ribosomal proteins or translational factors [29]. We also revealed that the Rpb4 defect provoked a specific decrease in transcription for the PBF genes (Fig. 7C). In fact, despite the fact that the role of Rpb4/7 in the synthesis/degradation of PBF and other stress-dependent transcripts have been clearly established [3,7,15,24,29], its role in the general synthesis/degradation of mRNA remains unclear. As indicated above, the defect in Rpb4 imprinting in the mRNAs in the *RPB1* foot mutants generally led to lower synthesis rates and longer mRNA half-lives (Fig. 7A, B, ref. [24]). This global effect is in addition to the specific effect on PBF mRNAs. Notably, our data also introduce a new function for Rpb4 in transcription and mRNA decay under optimal growth conditions, unlike previous statements which have suggested that Rpb4 does not serve as a key coordinator between transcription and decay under optimal growth conditions [29].

Our data also suggested that the constitutive ESR response in these mutants resulted from acting in this atypical situation: up-regulated genes (such as HSP genes) displayed no activated transcription, and down-regulated genes (RPs and RiBi) were over-repressed to compensate the globally greater mRNA stability. We conclude that the ESR is not only the result of a transcriptional response, but may also act through the coordinated regulation of both transcriptional and post-transcriptional mechanisms. We previously reported that in external stress situations, the yeast ESR is composed of transcriptional and post-transcriptional mechanisms, although the former are quantitatively more important [58]. In fact, the idea of mRNA stability participating in the ESR was originally proposed very soon after the discovery of the ESR [59]. Then, the work of Grigull et al. [54] showed that the mRNA stability of PBF mRNAs was reduced during stress responses. However, unlike previous works, we show here that posttranscriptional mechanisms modulate the constitutive stress response by provoking a global increase in mRNA stability compensated for by a significant lowering of the TR. Thus, we extend our previous results by showing that in some constitutively stressed mutants the post-transcriptional arm of the ESR could be the main, or even the only, mechanism to act. Moreover, given the compensation of mRNA stability by over-repressing RP and RiBi genes, we conclude that the only way to strike a balance between the two arms of the ESR is by the occurrence of permanent cross-talk and coordination between mRNA synthesis and decay [1]. This scenario could account for the unchanged mRNA levels in foot mutants compared with a wild-type strain under optimal growth conditions.

What regulatory pathways might be behind this atypical ESR? Msn2/4 proteins have been shown to promote the transcription of stress-induced genes. In the absence of stress, Msn2/4 reside in the cytoplasm, while they translocate to the nucleus under stress to promote the transcription of ESR-induced genes [60]. However, since Msn2-Gfp localization in foot mutants is similar to that in wild-type (Supplementary Fig. S8), and simultaneous deletion of both *MSN2* and *MSN4* does not alter the global mRNA stability increase in the *rpb1–84* mutant (G–M,J; P–O, J.E.; G–G, AI and N, F. not shown), our data suggest that that a full Msn2/4-dependent response is not activated in foot mutants.

The greater p-body accumulation in foot mutants could result from the ESR response [50], but is also indicative of defective cytoplasmic mRNA degradation [29]. Accordingly, p-body accumulation also increases for mutants *rpb4*Δ and *rpb7–26* [7,29]. It is tempting to speculate that Rpb4-free polyA-mRNAs accumulate in p-bodies, which would increase their stability. We cannot rule out that part of free Rpb4 could be localized in p-bodies, although we detected no co-localization of Rpb4 and Dcp2 in p-bodies (Fig. S7), in agreement with previous data indicating that Rpb4-containing P-bodies are short-lived and, hence, difficult to detect [29].

Finally, foot mutants constitute a new class of thermosensitive mutants of RNA pol II, where the dissociation of the Rpb4/7 heterodimer affects global mRNA stability under optimal growth conditions. However, our data do not rule out a more general role for Rpb7 in mRNA decay compared with Rpb4, as previously suggested [55].

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagrm.2016.03.008.

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Transparency document

The Transparency document associated with this article can be found, in online version.

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