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The RNA polymerase II Rpb4/7 subcomplex regulates cellular lifespan through an mRNA decay process

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ABSTRACT

In budding yeast, a highly conserved heterodimeric protein complex that is composed of the Rpb4 and Rpb7 proteins within RNA polymerase II shuttles between the nucleus and cytoplasm where it coordinates various steps of gene expression by associating with mRNAs. Although distinct stages of gene expression potentially contribute to the regulation of cellular lifespan, little is known about the underlying mechanisms. Here, we addressed the role of the dissociable Rpb4/7 heterodimeric protein complex in the regulation of replicative lifespan during various stages of gene expression in the yeast *Saccharomyces cerevisiae*. We observed that the loss of Rpb4 resulted in a shortened lifespan. In contrast, we found that defects in the dissociation of Rpb4/7 from the RNA polymerase core complex and in translation initiation steps affected by Rpb4/7 did not impact lifespan. Tandem affinity purification experiments demonstrated that Rpb7 physically associates with Tpk2 and Pat1, which are both implicated in mRNA degradation. Consistent with this data, the loss of the mRNA decay regulators Pat1 and Dhh1 reduced the cellular lifespan. In summary, our findings further reinforce the pivotal role of Rpb4/7 in the coordination of distinct steps of gene expression and suggest that among the many stages of gene expression, mRNA decay is a critical process that is required for normal replicative lifespan.

1. Introduction

Various stages of gene expression in prokaryotes are closely coupled within the protoplasm, where transcription and translation occur simultaneously. In eukaryotes, the nuclear membrane separates these two key processes both spatially and temporally: transcription occurs in the nucleus, whereas translation initiates only after mRNA transcripts have been exported to the cytoplasm. mRNAs that are not engaged in translation eventually accumulate within processing bodies (P-bodies), in which components involved in translation repression and mRNA decay facilitate mRNA degradation (for review, see [1]). In particular, the decapping activators Pat1 and Dhh1 have been shown to function in mRNA decay [2], translation repression, and P-body formation [3].

Recently, several lines of evidence have indicated that crosstalk exists between transcription, mRNA decay, and translation in *S accharomyces cerevisiae* [4,5]. These data challenge the long-standing dogma regarding the functional separation of these processes during gene expression in eukaryotes. The surprising coupling between transcription and translation in yeast *S. cerevisiae* shown by Harel-Sharvit et al. was referred to as the "remote controlling" of translation by the transcription apparatus [5,6]. The key factor that is responsible for this remote control is the highly conserved heterodimeric protein complex composed of Rpb4 and Rpb7 (Rpb4/7), which are subunits of RNA polymerase II (RNApII) [5]. These subunits associate loosely with the decameric core of RNApII [7] and can shuttle between the nucleus and cytoplasm [8]. Within the context of RNApII, Rpb4/7 has roles in transcription initiation [9,10] and in efficient transcription during stress conditions [11]. Ejection of the dissociable Rpb4/7 heterodimer from the rest of the subunits occurs during transcription elongation [12] through the Asr1-mediated ubiquitylation of Rpb1 and Rpb2 [13]. Rpb4/7 binds to nascent transcripts cotranscriptionally [14] and is then exported from the nucleus into the cytoplasm [8] where it plays a post-transcriptional role. Rpb4/7 has been found to interact physically and functionally with Hcr1 and Nip1 [5], which are components of the eukaryotic initiation factor 3 (eIF3) that provide a platform for the assembly of all of the initiation factors into polysomes [6,15].

In addition to the indispensable role of Rpb4/7 in translation, this heterodimer is also engaged in mRNA degradation through the association with cytoplasmic P-bodies and mRNA decay factors [16,17]. Both proteins are required for stimulating the processes of deadenylation and post-deadenylation within the mRNA decay pathway [16,17]. Of note, the non-essential protein Rpb4 specifically mediates the decay of mRNAs that encode protein

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biosynthetic factors through decapping and 5′ to 3′ degradation [16]. In contrast, the essential protein Rpb7 has a general role in mRNA decay and is involved in both 5′ to 3′ and 3′ to 5′ degradation pathways [17]. Furthermore, there is some evidence that the coupling of transcription with translation and mRNA decay by the Rpb4/7 heterodimer relies strictly on the correct recruitment of Rpb4/7 to the RNApII complex in the nucleus [4,5]. It has therefore been proposed that Rpb4/7 remains associated with mRNAs throughout the mRNA lifecycle to act as an "mRNA coordinator" by integrating various stages of gene expression [5].

Many studies have shown that replicative lifespan, which is defined as the number of daughter cells produced by a mother cell prior to senescence [18], is controlled by various cellular mechanisms in yeast cells [19,20]. Importantly, a drastic reduction in longevity was demonstrated in the *sir2Δ* mutant, which has a deficiency in transcriptional repression at the mating type loci, telomeres, and rDNA [21–23]. However, emerging evidence also indicates that distinct stages of gene expression may also regulate cellular lifespan. The reduction in 60S ribosomal subunit levels extends lifespan in yeast through the induction of the nutrient-responsive transcription factor Gcn4 by a Sir2-independent mechanism [24]. AUF1 is an mRNA decay factor in mice and its deficiency results in striking telomere erosion, pronounced cellular senescence, and rapid premature aging [25]. Thus, these reports raise the possibility that the indispensable function of the Rpb4/7 heterodimer in various stages of gene expression may be involved in the regulation of replicative lifespan.

In the present study, we investigated the role of Rpb4/7 in the regulation of replicative lifespan in yeast. We found that the loss of Rpb4 resulted in a shortened lifespan due to a defect in mRNA decay but not due to dissociation from RNApII or deficient translation initiation. Our observations suggest that even though Rpb4/7 acts as an "mRNA coordinator" by interacting with multiple factors and integrating various steps of gene expression, mRNA decay appears to be the process that is required for normal lifespan.

2. Materials and methods

2.1. Yeast strains

All of the deletion strains were derived from BY4741 (Euroscarf) and confirmed by PCR using gene-specific and deletion cassette-specific primers. For the tandem affinity purification (TAP) of Rpb7, the TAP-tagged Rpb7 strain in the BY4741 background (Euroscarf) was used.

2.2. Replicative lifespan analysis

The replicative lifespans of the yeast strains were determined as previously described [21]. A total of 50 virgin daughter cells were isolated from mother cells and subjected to lifespan analysis. To assess the significance of the lifespan differences, a Wilcoxon rank-sum test (the "ranksum" function in MATLAB) was performed with a cut-off of $p = 0.05$. The mean lifespan and p values obtained from these analyses are listed in Supplementary Table S1.

2.3. Tandem affinity purification and mass spectrometry

TAP purification was performed as previously described [26]. Rpb7-TAP tagged cells were grown at 30 °C in 2 L of YPD (1% yeast extract, 2% peptone, and 2% glucose) and lysed by two passages through a French press (Sim-Aminco) at 8.27 MPa. IgG-Sepharose beads (GE healthcare) and recombinant TEV protease (Invitrogen) were used to immunoprecipitate and cleave the Rpb7-TAP protein, respectively. The eluate was then agitated with calmodulin beads (GE healthcare) and fractionally eluted. Proteins in the final eluate were separated by 12% SDS–PAGE and then stained with silver using a silver stain kit (GE Healthcare). Mass spectrometry analysis was performed as previously reported [27]. In-gel tryptic digests were analyzed using an Ultraflex matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) mass spectrometer (Bruker Daltonics). To process the obtained spectra, the Flex Analysis 2.0 and Flex Control 2.0 software tools (Bruker Daltonics) were used with peptide masses in the range of 700–4000 Da. Mass data of all of the peptides and their fragmentation patterns were analyzed with the ProFound website (http://prowl.rockefeller.edu). Protein identifications were ranked according to peptide coverage, and the list of proteins identified in the Rpb7-TAP purification is shown in Fig. 3.

3. Results

3.1. Yeast cells lacking Rpb4 subunit are short-lived

Age-related changes in gene expression were identified during the aging process in yeast cells [28]. As introduced earlier, factors that are involved in distinct stages of gene expression are associated with the regulation of cellular lifespan [24,25]. To determine whether the Rpb4/7 heterodimer is involved in lifespan regulation, we performed replicative lifespan analyses. Cells lacking Rpb4, which is not essential for cell viability [29], displayed a lifespan that was shortened by approximately 20% in comparison to the wild-type (WT) cells (Fig. 1). Because both Rpb4 and Rpb7 are absent from the RNApII complex in the *rpb4Δ* mutant [30], the decrease in lifespan in *rpb4Δ* cells may be because of the role of Rpb4/7 as an "mRNA coordinator" in various stages of gene expression. As expected, the loss of the non-essential RNApII subunit Rpb9, which is not involved in coupling transcription with translation [5], led to a more drastic decrease in lifespan than in the *rpb4Δ* mutant (Fig. 1). In summary, the results show that the non-essential RNApII subunit Rpb4 is required for a normal lifespan.

3.2. Dissociation of Rpb4/7 from the RNApII core complex has no effect on lifespan

To determine whether a defect in the dissociation of Rpb4/7 from the rest of the RNApII subunits affects the cellular lifespan, we measured replicative lifespan in *asr1Δ* cells. We observed that deletion of Asf1 failed to affect lifespan and resulted in a lifespan that was indistinguishable from that of the WT cells. To further determine the effect of ubiquitylation of the RNApII subunits on aging, we analyzed the replicative lifespan of cells lacking the E3 ubiquitin ligases that are required for proteolytic degradation in

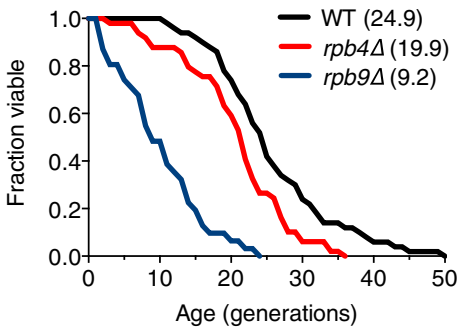


Fig. 1. Loss of Rpb4 subunit results in a shortened lifespan. The average replicative lifespans of BY4741 (WT), *rpb4Δ*, and *rpb9Δ* strains were determined by counting the total number of daughter cells that were produced by each mother cell. The numbers in parentheses denote the mean lifespan of the corresponding strains.

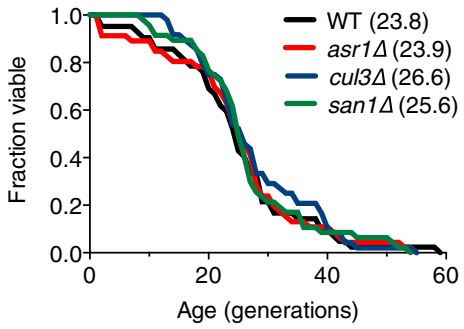


Fig. 2. Yeast cells lacking the nuclear E3 ubiquitin ligases Asr1, Cul3, or San1 do not exhibit defects in replicative lifespan. Replicative lifespan analyses were performed in parallel with the BY4741 (WT), *asr1*Δ, *cul3*Δ and *san1*Δ strains. The mean lifespans are shown in parentheses as in Fig. 1.

the nucleus. Cells deficient in Cul3, which is a ubiquitin ligase that functions with Elc1 to produce poly-ubiquitylated Rpb1 and trigger proteolysis [31], had a lifespan only marginally longer than that of the WT cells (Fig. 2). Additionally, a similar median lifespan was observed in *san1*Δ cells. Unlike RNApII subunit-ubiquitylating proteins Asr1 and Elc1, San1 targets aberrant nuclear proteins for ubiquitylation and degradation [32]. In summary, our results show that the ubiquitylation-triggered non-proteolytic ejection of Rpb4/7 and the proteolytic degradation of Rpb1 in the nucleus had little effect on the regulation of cellular aging.

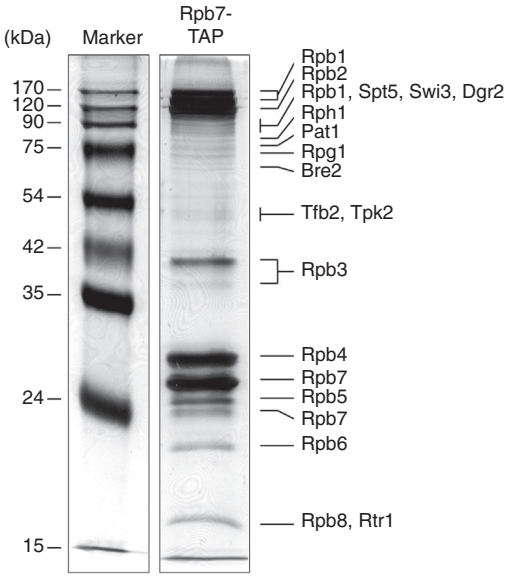
3.3. Rpb7 physically associates with proteins involved in various steps of gene expression

Because the Rpb4/7 subunits of RNApII have a role in integrating various steps of gene expression [5] and the *rpb4*Δ mutant decreased lifespan (Fig. 1), we next performed tandem affinity purification and MALDI-TOF mass spectrometry to identify Rpb4/7-associated proteins that function in various stages of gene expression. Purification of TAP-tagged Rpb7 from yeast extracts showed the co-purification of multiple proteins (Fig. 3). In agreement with previous findings, Rpb7 physically interacted with several RNApII subunits, including Rpb1, Rpb2, Rpb3, Rpb4, Rpb5, Rpb6, and Rpb8 [12]. Rpb7 also interacted with a number of transcription regulators, such as Rtr1 and Spt5, which are involved in transcription elongation [33]. As described earlier, Rbp4/7 has an indispensable role in stimulating efficient translation initiation by interacting physically and functionally with the eIF3 components Nip1 and Hcr1 [5]. We also found that Rpb7 physically associated with the eIF3 component Rpg1, which was previously reported to physically interact with Rpb4 [5].

In accordance with the idea that the Rpb4/7 heterodimer is required for key mRNA decay pathways in the cytoplasm [16,17], Tpk2 and Pat1, which are both implicated in mRNA degradation, were found to physically associate with Rpb7. Tpk2 is a cAMP-dependent protein kinase that participates in the cyclic AMP-protein kinase A pathway in yeast and associates with P-bodies where mRNA decay takes place [34]. Pat1 serves as a decapping activator that functions in both transcription repression and P-body formation [3]. Apart from these factors, several proteins that were not previously found to associate with Rpb7 were also observed, including the SWI/SNF chromatin remodeling subunit Swi3 [35], the COMPASS complex subunit Bre2 [36], the histone H3 lysine 36 demethylase Rph1, [37], and the basal transcription factor TFIIH subunit Tfb2 [38]. In summary, our results show that the RNApII subunit Rpb7 associates with proteins involved in several steps of gene expression, which supports the idea that Rpb4/7 plays a significant role in coordinating various steps of gene expression through regulation of the mRNA lifecycle.

3.4. mRNA decay regulators are required for normal lifespan

Having shown that cellular aging was not influenced by Asr1-mediated Rpb4/7 exclusion from the RNApII complex (Fig. 2), we then hypothesized that the shortened lifespan of *rpb4*Δ cells (Fig. 1) may be due to defects in translation initiation or mRNA decay. To explore whether Rpb4/7-associated regulators that are in-



Protein	Coverage	Description
Rpb7	72%	RNA polymerase II
Rpb4	62%	RNA polymerase II
Rpb5	59%	RNA polymerase II
Rpb6	43%	RNA polymerase II
Rpb8	42%	RNA polymerase II
Rpb3	35%	RNA polymerase II
Rtr1	30%	CTD phosphatase
Rpb1	25%	RNA polymerase II
Dgr2	24%	uncharacterized
Spt5	23%	Spt4/5 complex
Swi3	22%	SWI/SNF chromatin remodeling complex
Rpb2	21%	RNA polymerase II
Bre2	21%	COMPASS complex
Tfb2	19%	TFIIH
Tpk2	16%	cAMP-dependent protein kinase
Rph1	11%	histone demethylase
Pat1	9%	mRNA-decapping factor
Rpg1	9%	eIF3

Fig. 3. Affinity purification of TAP-tagged Rpb7-associated proteins. Rpb7 purified by tandem affinity purification was separated by 12% SDS–PAGE gel and was followed by silver staining to allow for visualization of interacting proteins. The molecular weights of the protein markers in kilodaltons are shown on the left panel. Proteins that co-purified with Rpb7 are shown on the right panel and are ranked by the percent coverage of all of the amino acids in each protein sequence. Functional descriptions of each protein are also presented.

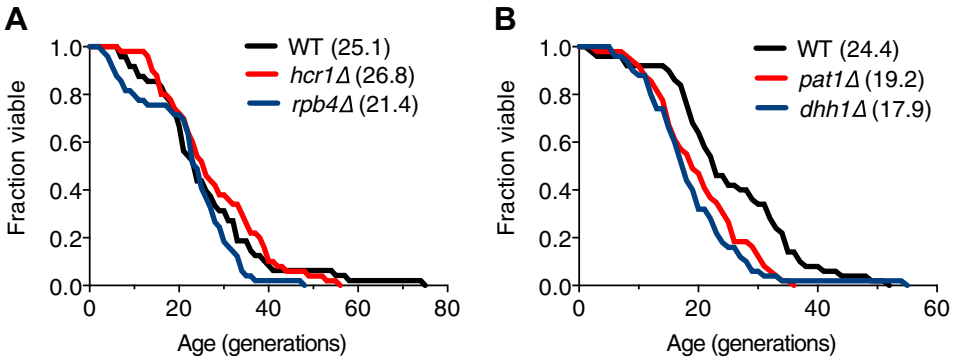


Fig. 4. Replicative lifespan was impaired by loss of mRNA decay factors. (A) Replicative lifespan analyses of the BY4741 (WT), *hcr1*Δ, and *rpb4*Δ strains. (B) Replicative lifespan analyses of the BY4741 (WT), *pat1*Δ, and *dhh1Δ* strains. The mean lifespans are shown in parentheses as in Fig. 1.

volved in these processes play a role in the regulation of cellular lifespan, we performed lifespan analyses using cells deficient in Hcr1, Pat1, or Dhh1. Hcr1 is an eIF3 component, is non-essential for cell viability, and exhibits a reduced growth phenotype [39]. It has also been reported that Hcr1 physically associates with Rpb4/7 [5], and its loss has been shown to result in synthetic sickness with an *rpb4*Δ strain during the stationary phase of growth [5]. However, our results shows that the loss of Hcr1 had no effect on cellular aging (Fig. 4A), which suggests that the shortened lifespan in the *rpb4*Δ strain is most likely not due to a defect in translation initiation. In contrast, the loss of the decapping activators Pat1 and Dhh1 resulted in a reduced lifespan that was comparable to that observed in the *rpb4*Δ mutant (Fig. 4B). These results argue that the shortened lifespan that is observed in the *rpb4Δ* mutant and in the *pat1*Δ and *dhh1Δ* mutant cells was a result of a defect in mRNA decay or P-body formation. Although the two mutually interacting proteins Pat1 and Dhh1 play independent roles in mRNA degradation [3], our results indicate that mRNA decay regulators are required for normal lifespan.

4. Discussion

Transcription by RNApII is one of the most important steps in gene expression by transmitting genetic information from DNA into mRNA. The RNApII complex is composed of 12 subunits, which are designated Rpb1 to Rpb12 based on decreasing order of the subunit molecular weights [40]. A large number of studies have uncovered multiple transcription-related cellular functions for these subunits, including roles in stress responses and DNA repair [11,41]. Recently, Choder and co-workers demonstrated an additional indispensable cellular function for RNApII, which integrates multiple stages of gene expression [4,5]. They proposed that the Rpb4/7 heterodimeric subunits are responsible for coupling transcriptional and post-transcriptional processes, which are dependent on the recruitment of Rpb4/7 to RNApII [4,5,16,17]. In the present study, we found that the Rpb4/7 subcomplex also regulates cellular lifespan through an mRNA decay process. We observed that the Rpb4 subunit is required for normal lifespan and that Rpb7 physically interacts with proteins involved in mRNA decay pathways, such as Tpk2 and Pat1. Furthermore, loss of Pat1 resulted in a reduced lifespan similar to that observed in *rpb4*Δ cells. Thus, our results strongly suggest that regulation of mRNA decay by Rpb4/7 subunits is required for normal lifespan.

Replicative lifespan is defined as the number of mitotic cycles and takes advantage of the asymmetrical division that occurs during the yeast proliferative process [18]. Connections between replicative lifespan and RNApII subunits can be implied from an interesting study in fission yeast that suggested that *S. pombe* Rpb4 plays an important role in cell separation by regulating the transcription of genes that are involved in cell division [42]. In fission yeast, Rpb4 is an essential protein and is not required for responses to environmental stress [42], whereas in budding yeast, it is a non-essential protein but is indispensable for the response to temperature stress [29]. It therefore remains unclear whether the role of *S. pombe* Rpb4 in cell division could account for the deficiency in maintaining normal numbers of cell divisions that are observed in the *S. cerevisiae rpb4*Δ mutant strain (Fig. 1).

We found that ejection of Rpb4/7 from RNApII by Asr1 via ubiquitylation on Rpb1 and Rpb2, which serves as a primary step for Rpb4/7 shuttling between the nucleus and cytoplasm [13], has no effect on lifespan (Fig. 2). Similar results were obtained with the *cul3Δ* and *san1Δ* cells, which further excludes the possibility that the ubiquitylation and concurrent degradation of the RNApII subunit Rpb1 or aberrant nuclear proteins contributes to lifespan (Fig. 2). As a consequence of Rpb4/7 dissociation by Asr1, RNApII is inactivated [13], which suggests that polymerase activation is not critical for the regulation of lifespan. Moreover, the influence of Rpb4/7-mediated translation initiation on lifespan is likely ruled out as well because the deletion of Hcr1, which is an eIF3 subunit that interacts with Rpb4/7 physically and genetically [5], does not alter lifespan (Fig. 4A). Of the multiple stages of gene expression that are coordinated by RNApII subunits after mRNA transcription, the mRNA decay step appears to be one of the processes that regulate replicative lifespan. Pat1, an mRNA decay activator that is strongly associated with the Rpb4/7 heterodimer [3,16,17] (see also Fig. 3), showed a positive role in yeast replicative lifespan (Fig. 4B). Consistent with these results, the Dhh1 deletion mutant showed a reduced lifespan that was comparable to the *pat1Δ* mutant (Fig. 4B) even though Dhh1 participates in transcription repression and mRNA decay in a Pat1-independent fashion [3]. Our observations therefore provide a potential mechanism for an mRNA decay process that is required for normal lifespan most likely through coupling with the RNApII subcomplex Rpb4/7. Future studies that address the underlying mechanisms between RNApII and mRNA decay on the regulation of cellular replicative lifespan will be interesting and promising.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.10.079.

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