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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 *rpb4*Δ mutant [30], the decrease in lifespan in *rpb4*Δ 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 *rpb4*Δ 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

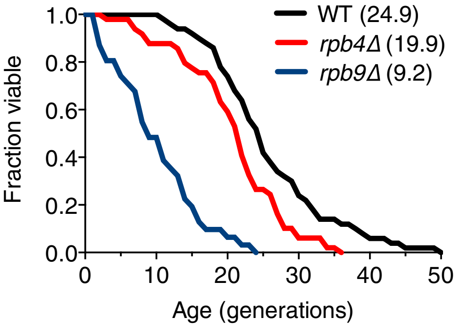

Fig. 1. Loss of Rpb4 subunit results in a shortened lifespan. The average replicative lifespans of BY4741 (WT), *rpb4*Δ, and *rpb9*Δ strains were determined by counting the total number of daughter cells that were produced by each mother cell. The numbers in parentheses denote the mean lifespan of the corresponding strains.