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Involvement of *S. cerevisiae* Rpb4 in subset of pathways related to transcription elongation

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ABSTRACT

Yeast Rpb4, a subunit of RNA pol II is not essential for viability but is involved in multiple cellular phenotypes such as temperature sensitivity, enhanced pseudohyphal morphology, and decreased sporulation. Both *in vivo* and *in vitro* studies strongly support involvement of Rpb4 in transcription initiation, while its role in transcription elongation is not entirely consistent. Here we show that Rpb4 is not required for recruitment of RNA pol II on the coding region of *YLR454w*, a representative long gene. Yet we find strong genetic interaction of *rpb4*Δ with mutants in many transcription elongation factors such as Paf1, Spt4, Dst1, Elp3 and Rpb9. We demonstrate that, Rpb4 interacts functionally with Paf1 to affect the transcription elongation of the *FKS1* gene. Our results suggest that while Rpb4 is not required for general transcription elongation, it could support transcription elongation for specific of class of genes by interaction with other elongation factors.

1. Introduction

The eukaryotic RNA pol II consists of twelve different subunits named Rpb1–Rpb12. The Rpb4 subunit of RNA pol II is non-essential for viability in budding yeast and the null cells display several phenotypes. These included cellular phenotypes such as decreased sporulation and enhanced pseudohyphal morphology of diploids. The molecular defects associated with gene expression in *rpb4*Δ cells are failure of activated transcription, RNA 3′ end processing, mRNA export and decay, translation and DNA repair (Goler-Baron et al., 2008; Harel-Sharvit et al., 2010; Kumari and Sharma, 2013; Pillai et al., 2001; Runner et al., 2008; Sampath and Sadhale, 2005; Verma-Gaur et al., 2008). Early *in vitro* experiments have shown that Rpb4 is required for promoter-directed transcription initiation but not for subsequent progression of RNA pol II on the template human histone H3.3 gene (Edwards et al., 1991). However, the subsequent *in vivo* studies have indicated functions for Rpb4/Rpb7 subcomplex in transcription elongation as well. A genome-wide study showed that degree of association of Rpb4 and Rpb7 at coding regions of genes is similar to that of Rpb3, which was taken to represent the core RNA pol II (Jasiak et al., 2008; Verma-Gaur et al., 2008). However, a recent report contradicts the predicted *in vivo* role of Rpb4/Rpb7 in transcription elongation (Mosley et al., 2013). This report found that Rpb4/Rpb7 subcomplex was not associated with the core RNA pol II that was immunoprecipitated with some of the known elongation factors (Rtr1, Set2). Thus, the role of Rpb4/Rpb7 in transcription elongation *in vivo* is still debated.

Here, we investigated the influence of RNA polymerase II lacking Rpb4 on transcription elongation. We examined the recruitment of pol II at promoter and on different coding segments of the long gene *YLR454w*. We found that the association of RNA pol II on these regions is not affected in the absence of Rpb4. To study genetic interactions of *rpb4*Δ with transcription elongation factors, double mutants were generated and cell growth was examined. Synthetic lethality of *rpb4*Δ with mutants of transcription elongation factors *PAF1*, *SPT4*, *DST1*, and *RPB9* and synthetic sickness with mutants in histone acetyltransferase genes *ELP3* and *GCN5* revealed new interactions between Rpb4 and the subcomplexes involved in transcription elongation. Our data also implicates that Rpb4 supports Paf1 mediated transcription elongation of the *FKS1* gene in response to active PKC (Protein Kinase C) signaling pathway. Overall, these results indicate that Rpb4 functions in transcription elongation, relevant to specific pathways.

2. Materials and methods

2.1. Yeast strains

The following *Saccharomyces cerevisiae* strains were used in the study.

Wt BY4742 (S288c, MATα, *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

Abbreviations: Δ, deletion; ::, novel junction (fusion or insertion); μ, micron; μg, microgram; °C, degrees Celsius; 6AU, 6-azauracil; 5-FOA, 5-fluoro orotic acid; kb, kilobase(s) or 1000 bp; LacZ, β-galactosidase; M, molar; OD, optical density; ORF, open reading frame; p, plasmid; P, promoter; P_{GAL1}, promoter of *GAL1*; pol, polymerase; SD, synthetic drop-out medium; SDS, sodium dodecyl sulfate; Ura⁻, absence of uracil; wt, wild type; *ACT1*, actin.

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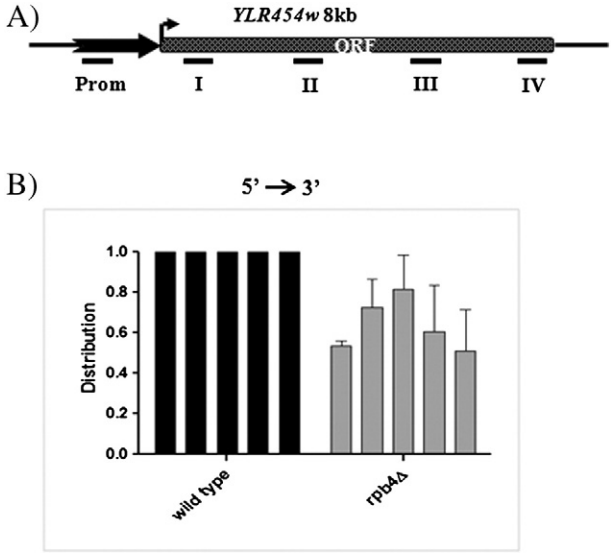

Fig. 1. Occupancy of RNA pol II is not affected in *rpb4*Δ cells on coding region of *YLR454w*. A) Schematic representation of primer positions on *YLR454w* locus used to determine RNA pol II occupancy. B) Chromatin IP followed by real-time PCR analysis of Rpb3 (RNA pol II) relative occupancy at the five indicated positions on *YLR454w* genomic region in wild type and *rpb4*Δ with values in the wild-type strain normalized to 1.0 at each position. Chromosome V intergenic region was used as negative control. The error bar shows the SD in three independent experiments, for occupancy observed in the mutant strain.

rpb4Δ::KANMX4 (*S288c*, (*MAT**a*** *his3Δ1 leu2Δ 0 lys2Δ 0 ura3Δ 0 YJL140w::kanMX4*)).

rpb4Δ::HIS3 (*MATa*, *his3 Δ-200*, *ura3–52*, *leu2–3*, *112*, *lys2*, *rpb4Δ::HIS3*).

Wt Rpb3-TAP (MATa; *ade2*; *arg4*; *leu2–3*, *112*; *trp1–289*; *ura3–52*; *YIL021w::TAP-K.l.URA3*).

*rpb4*Δ Rpb3-TAP (MATa, *his3Δ1*, *leu2–3*, *112*, *ura3–52*, *RPB3::TAP-K.l.URA3*).

*paf1*Δ (BY4742, S288c MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

*spt4*Δ (BY4742, S288c background MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

*dst1*Δ (BY4742, S288c MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

*rpb9*Δ (BY4742, S288c MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

*elp3*Δ (BY4742, S288c MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

*gcn5*Δ (BY4742, S288c MATα; *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*).

2.2. Culture and growth conditions

All strains were grown in YPD (yeast extract 1%, peptone 2%, dextrose 2%) and synthetic complete (2% dextrose) media as per the standard procedure.

5-Fluoroorotic acid (Sigma-Aldrich) was added to the final concentration of 0.1% w/v in synthetic complete medium containing uracil (20 μg/ml final concentration). Similarly, 6-azauracil (Sigma-Aldrich, solvent DMSO) was added as 75 μg/ml final concentration into synthetic complete medium lacking uracil. To test the effect of cell wall damaging agents, final concentrations of sodium dodecyl sulfate 0.005%, Congo red 250 μg/ml (solvent absolute ethanol) and NaCl 1 M were added to the synthetic complete media.

2.3. 5-FOA assay

All the cells were grown in YPD medium for 24 h and diluted to OD_{600} of 1. Ten-fold serial dilutions were spotted on synthetic complete (SC) and SC with 0.1% 5-FOA plates. The plates were incubated in the dark for 2 days at 28 °C before taking photograph.

2.4. 6-Azauracil assay

For 6AU sensitivity assay, all the strains were grown in YPD medium for 24 h and diluted to OD_{600} to 1.0, and these were further serially diluted (1:10 dilution steps) and spotted on plates of SD Ura^- or SD Ura^- with 75 μg of 6AU per ml. Plates were incubated at 28 °C for 3–6 days before taking photographs.

2.5. Chromatin immunoprecipitation and real-time PCR

Chromatin IP was carried out as described previously (Kuo and Allis, 1999). Anti-protein A antibody (3 μg, Sigma-Aldrich P3775) was used for immunoprecipitation.

Real-time PCR was carried out as per manufacturer's instructions (KAPA Biosystems).

3. Results and discussion

3.1. Rpb4 is not required for recruitment of RNA pol II on coding region of YLR454w

The 8 kb long gene *YLR454w* in yeast has been used previously to characterize the transcription elongation in cells with mutants in

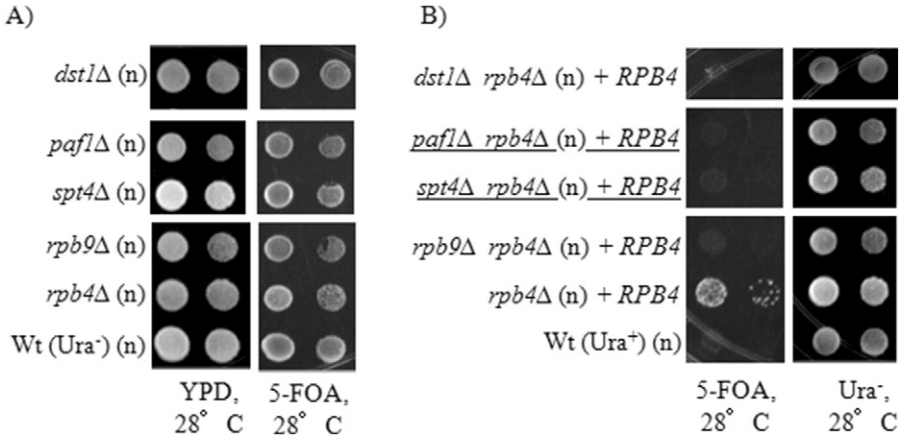

Fig. 2. Growth phenotype of elongation mutants before and after eviction of *RBP4* plasmid. Ten-fold serial dilutions of OD_{600}1 cells are spotted. A) Growth of haploid single deletion mutants on YPD and 5-FOA (+uracil) plates at 28 °C for 2 days. B) Growth of haploid double deletion mutants carrying *RPB4* on *URA3* marked plasmid on 5-FOA (+uracil) and synthetic complete (−uracil) plates at 28 °C for 2 days. Wild type cells with chromosomal integration of *URA3* marker were used as positive control in (B).