Rpb4/7 heterodimer regulates RNAPII phosphorylation

Paula Allepuz-Fuster [(1)], Verónica Martínez-Fernández [(2)], Ana I. Garrido-Godino [(2)], Francisco Navarro [(2)] and Olga Calvo [(1)]

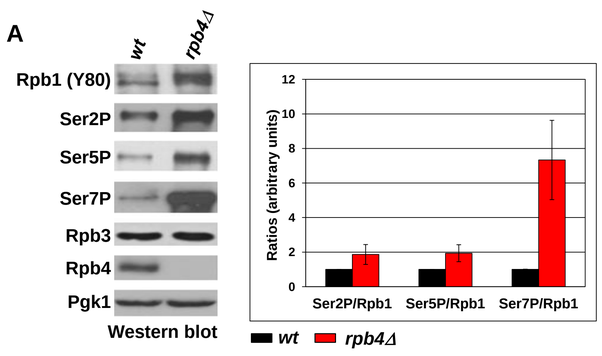
[(1)]*Instituto de Biología Funcional y Genómica. CSIC/USAL. Salamanca. Spain*

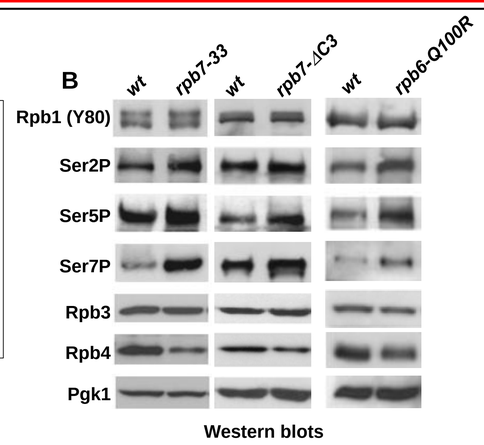
[(2)]*Departamento de Biología Experimental, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, Spain.*

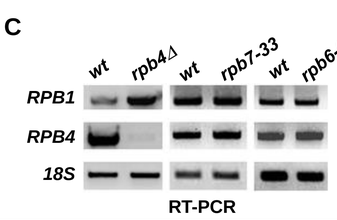
The 12-subunit RNA polymerase II enzyme in eukaryotic cells is essential for transcription of protein-coding genes. The Rpb4 and Rpb7 subunits bind to each other forming a complex in archaebacteria and in eukaryotic cells, and display some unique features that distinguish them from the remaining subunits. In *S. cerevisiae*, the Rpb4/7 heterodimer can dissociate from the rest of the holoenzyme. These subunits are important for transcription initiation, elongation and, in particular, Rpb4 contributes to contranscriptional recruitment of 3'-end processing factors. However, recent evidences suggests that they are also involved in DNA repair, mRNA export and decay, as well as translation [1]. Additionally, several facts suggest that Rpb4/7 may play a role on RNAPII phosphorylation: 1) The close proximity of the Rpb4/7 heterodimer to the CTD of the Rpb1. 2) The *in vitro* concerted interaction of Rpb4 and the CTD phosphatase, Fcp1, in *S. pombe*, suggesting that Rpb4 may play an important role in the assembly of Fcp1 into the RNAPII complex and thus, also in dephosphorylation [2]. 3) The Rpb4-Fcp1 interaction is also conserved in *Drosophila* [3]; and 4) in *Saccharomyces cerevisiae,* Rpb7 genetically interacts with the propyl isomerase Ess1 [4], also involved in CTD phosphorylation regulation. Here, we present genetic and biochemical data showing that effectively Rpb4/7 subcomplex is important for RNAPII dephosphorylation in *S. cerevisiae*, regulating several CTD modifying enzymes.

Results

A FUNCTIONAL Rpb4/7 HETERODIMER IS REQUIRED TO MAINTAIN PROPER RNAPII PHOSPHORYLATION LEVELS

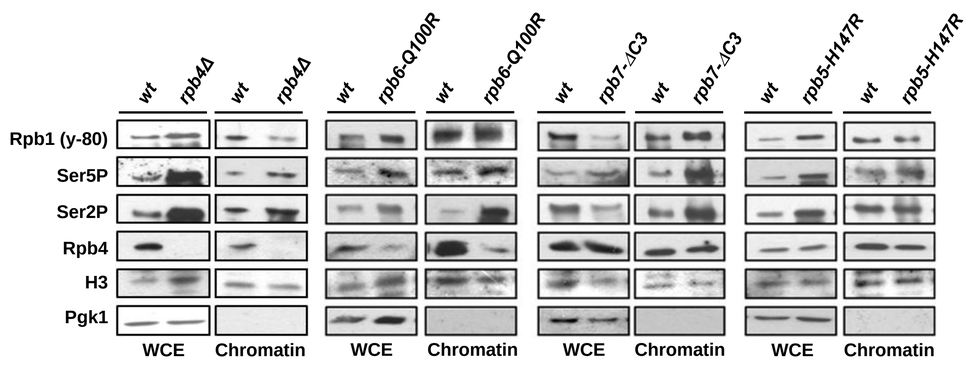


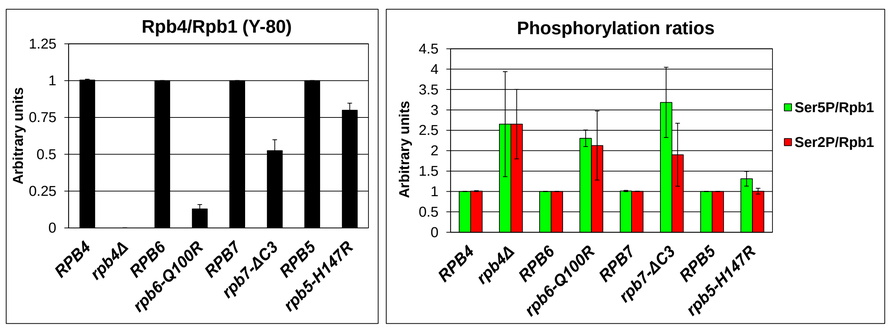




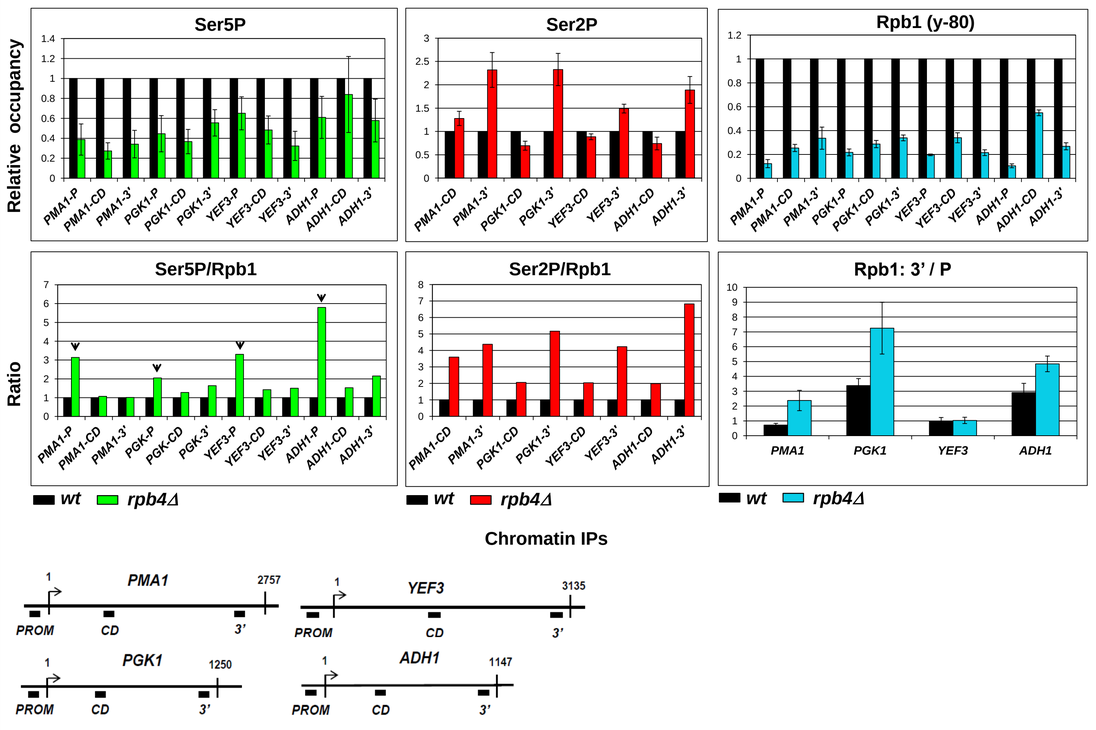
Rpb1-CTD phosphorylation is increased in *rpb4Δ* cells, in *rpb7* mutants and in the *rpb6-Q100R* mutant, in which assembly of Rpb4/7 is altered.

RPB4 deletion or Rpb4 dissociation increase CTD phosphorylation



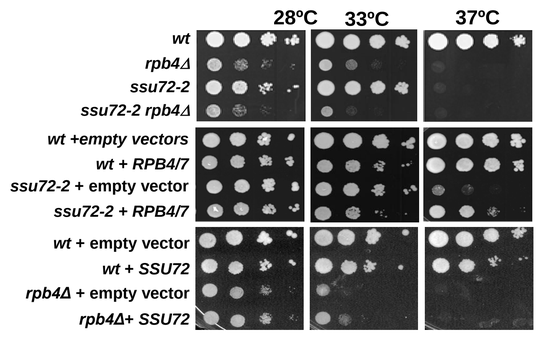


RPB4 deletion increases Rpb1-Ser5P and Rpb1-Ser2P gene occupancy, and3'/P ratio, suggesting a dephosphorylation defect, and therefore altered Rpb1 recycling, consistent with the significant reduction of Rpb1 occupancy at the promoters

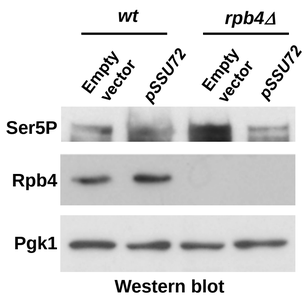


Ssu72 ASSOCIATION TO CHROMATIN AND TO RNAPII IS FACILITATED BY Rpb4/7

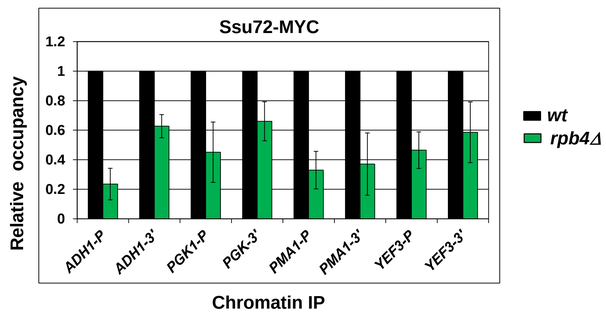
***RPB4/7* and *SSU72* display a strong genetic interaction**



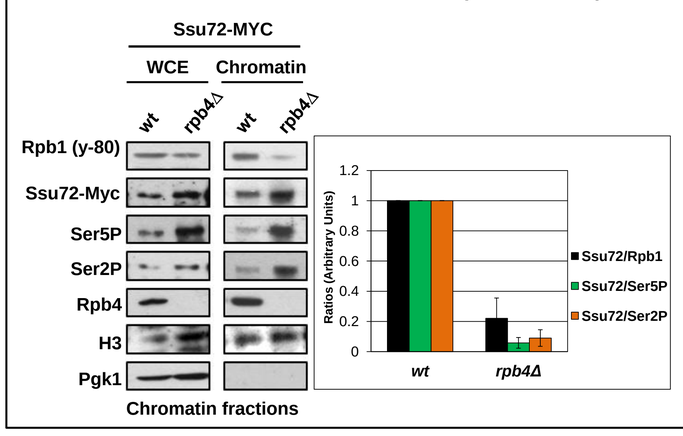
Suppression of *rpb4Δ* growth defect by *SSU72* is dependent on the reduction of Ser5P levels.



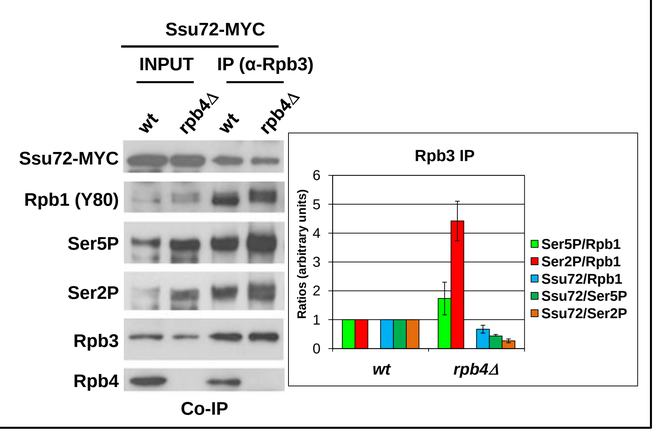
Occupancy of Ssu72 at promoter and 3'-end regions of several genes is significantly decreased in *rpb4Δ* cells



Ssu72 association to chromatin is dramatically reduced in *rpb4Δ*



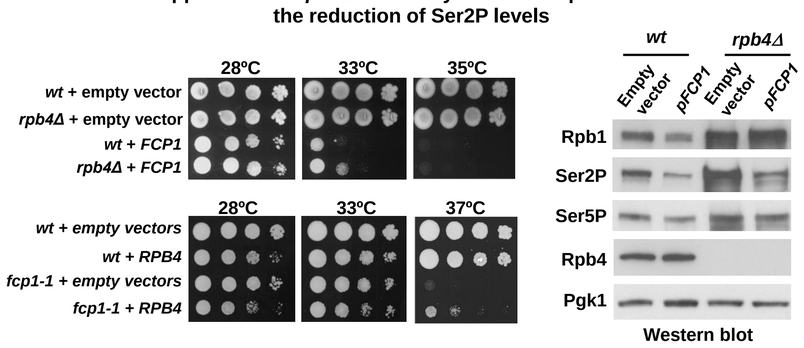
Ssu72 association to RNAPII is weakened when lacking Rpb4



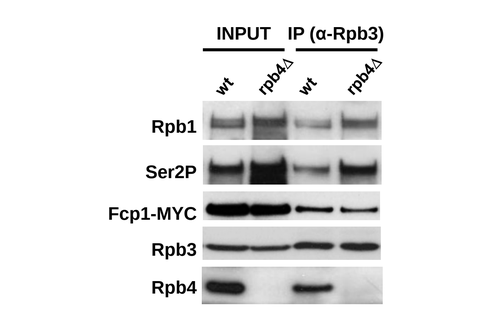
RPB4/7 HETERODIMER IS GENETICALLY LINKED TO *ESS1*

Fcp1 ASSOCIATION TO CHROMATIN AND TO RNAPII DEPENDS ON Rpb4

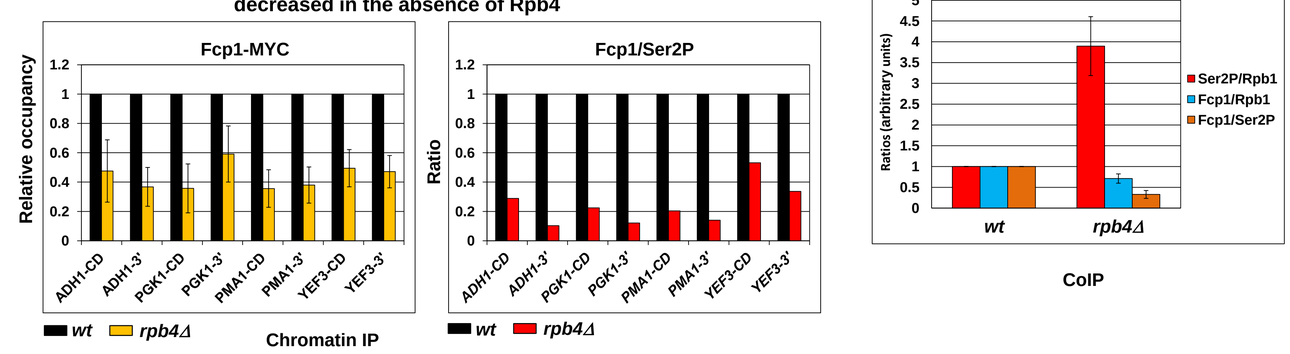
Suppression of *rpb4Δ* defect by *FCP1* is dependent on the reduction of Ser2P levels



Fcp1 association to RNAPII is reduced in *rpb4Δ*



Fcp1 occupancy at coding and 3'-end gene regions is significantly decreased in the absence of Rpb4



Conclusions

Our data clearly demonstrate that Rpb4/7 heterodimer is required to maintain proper Rpb1-CTD phosphorylation. It facilitates Ssu72 and Fcp1 CTD phosphatases association to RNAPII and to chromatin during gene transcription, and is genetically linked to Ess1, which facilitates Ser5P and Ser7P dephosphorylation. Thus, Rpb4/7 may be required for CTD dephosphorylation during transcription termination and, in consequence, RNAPII recycling. Furthermore, Rpb4 has been previously shown to contribute to 3'-end processing factors recruitment [5], and all together these data suggest that possibly the Rpb4/7 could modulate the CTD phosphorylation required for transcription termination, polyadenylation factor recruitment, and RNAPII recycling.

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