Sub1, two domains, two functions: transcription initiation versus elongation

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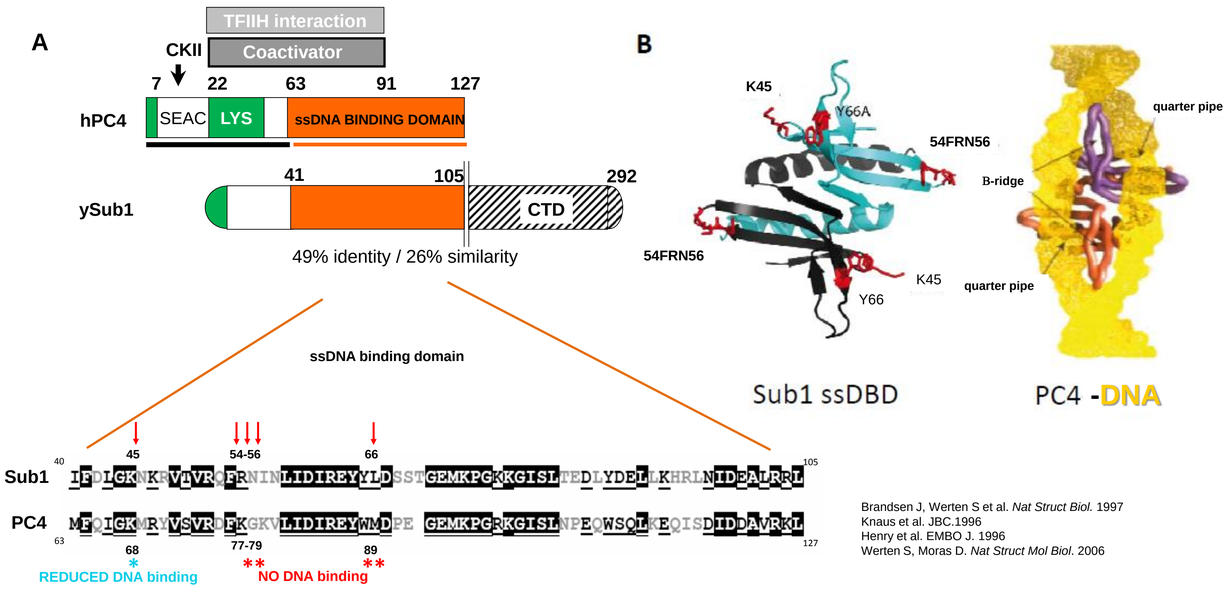
The transcriptional coactivator Sub1 has been implicated in several steps of mRNA metabolism in yeast, such as the activation of transcription, termination and 3'-end formation [1]. Most recently it has been shown that it is a functional component of the preinitiation complex [2]. Additionally, we have shown that Sub1 globally regulates RNA polymerase II phosphorylation [3] and promotes transcription elongation [4]. Sub1 was originally described as a transcriptional stimulatory protein based on its homology to human positive coactivator PC4 and its capacity to bind the general transcription factor TFIIB. Both are ssDNA binding proteins and have been implicated in DNA-dependent processes other than transcription, such as DNA repair and replication [1]. Although Sub1 shows strong similarity to PC4, in particular at the level of their DNA binding domains, Sub1 is much larger. Specifically, it has an extra C-terminal domain with an unknown function, suggesting that Sub1 might have functional differences due to this additional domain.

We have performed a detailed analysis of the ssDNA binding and C-terminal domains and identified the essential residues involved in Sub1 DNA binding capacity, which are not required during transcription elongation. Additionally, our data indicate that the C-terminus domain of Sub1, the function of which was previously unknown, might be important in promoting Sub1 release from the promoter to facilitate transcription elongation. In summary, Sub1 plays a dual function during transcription, being a pre-initiation factor via its DNA binding domain, and a transcription elongation factor through its C-terminus domain.

Sub1 IS A ssDNA BINDING PROTEIN HOMOLOGOUS TO HUMAN PC4



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Knaus et al. JBC.1996
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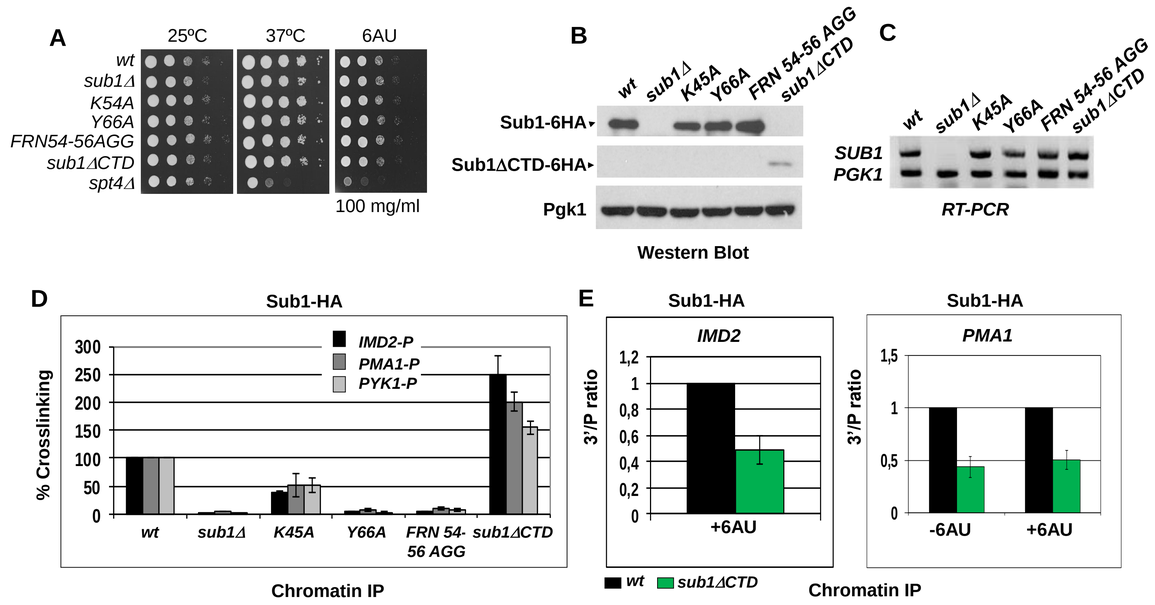
hPC4 and ySub1 share a ssDNA binding domain. A) Yeast Sub1 and human PC4 share high homology at the ssDNA binding domain. Both proteins present also a N-terminal region sensitive to phosphorylation by casein kinase II (CKII). In the figure is detailed the region responsible for PC4 coactivator function, TFIIH interaction and binding to ds- and ssDNA. Much less is known about yeast Sub1, and its phosphorylation. In addition, Sub1 has a long carboxi-teminal domain, not present in PC4. The sequence of the ssDNA binding domain of Sub1 and PC4, the mutated residues in PC4 and their effects on its ability to bind DNA are indicated, as well as the residues of Sub1 mutated in our study. B) Left panel, predicted structural model for Sub1 ssDNA binding domain (ssDBD), showing the three mutated residues. Right panel, published PC4-DNA structure.

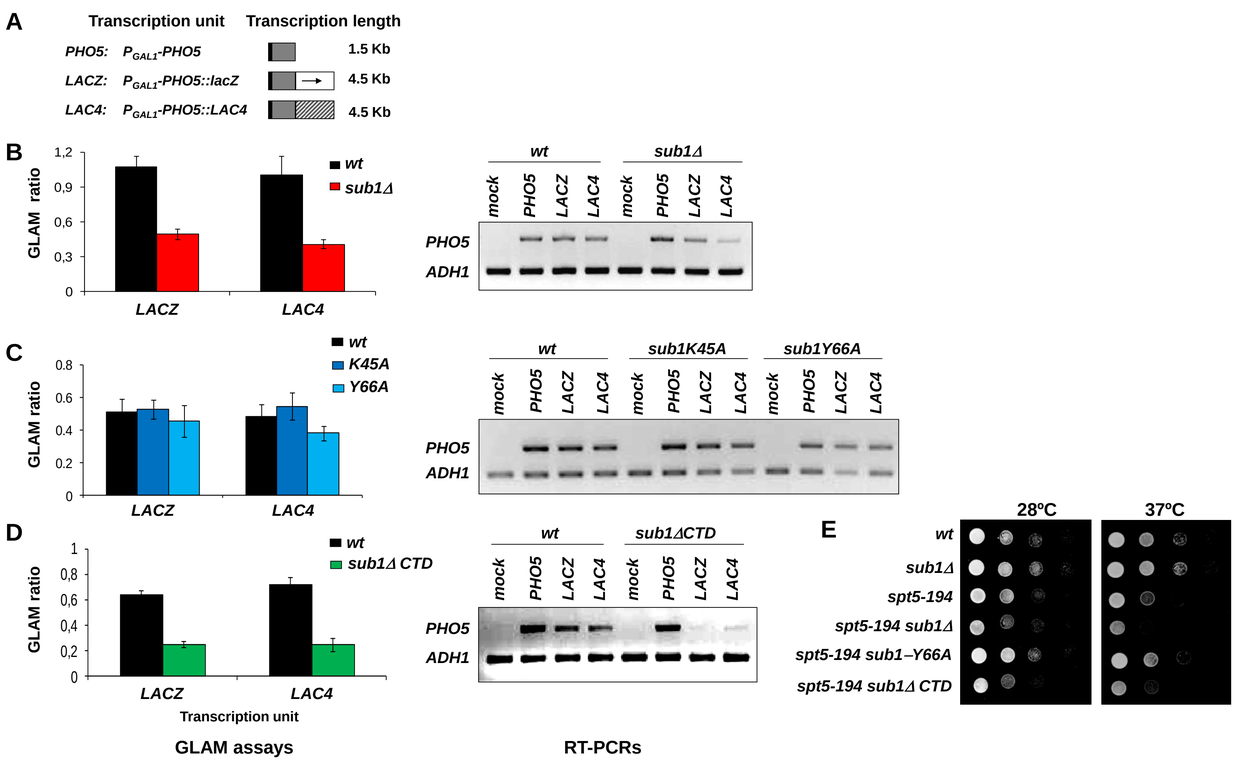
Sub1 RECRUITMENT TO GENE PROMOTERS DEPENDS ON THE ssDNA BINDING DOMAIN



Characterization of three *sub1* ssDNA binding domain mutants (*K45A*, *Y66A* and *FRN54-46AGG*) and a mutant lacking the CT domain, not present in hPC4 (*sub1ΔCTD*). (A) Growth phenotypes. (B) Protein expression levels: *wt* and ssDNA binding mutants present similar levels of Sub1 protein, but a significant decreased of Sub1p is observed in *sub1ΔCTD* cells. This decrease is not due to altered *SUB1* expression, as corroborated by RT-PCR. (D) ChIP assay shows that Sub1 association to three gene promoters is reduced or abolished in the ssDNA binding mutants, whereas is increased in *sub1ΔCTD* cells, indicating that the CT domain could facilitate Sub1 release from the promoter. (E) Sub1 associates with coding regions [4], however this association is reduced in *sub1ΔCTD* mutant, as shown by the 3'/5' ratio of Sub1 occupancy at the *IMD2* gene after 6AU induction, and at the *PMA1* gene before and after 6AU treatment.

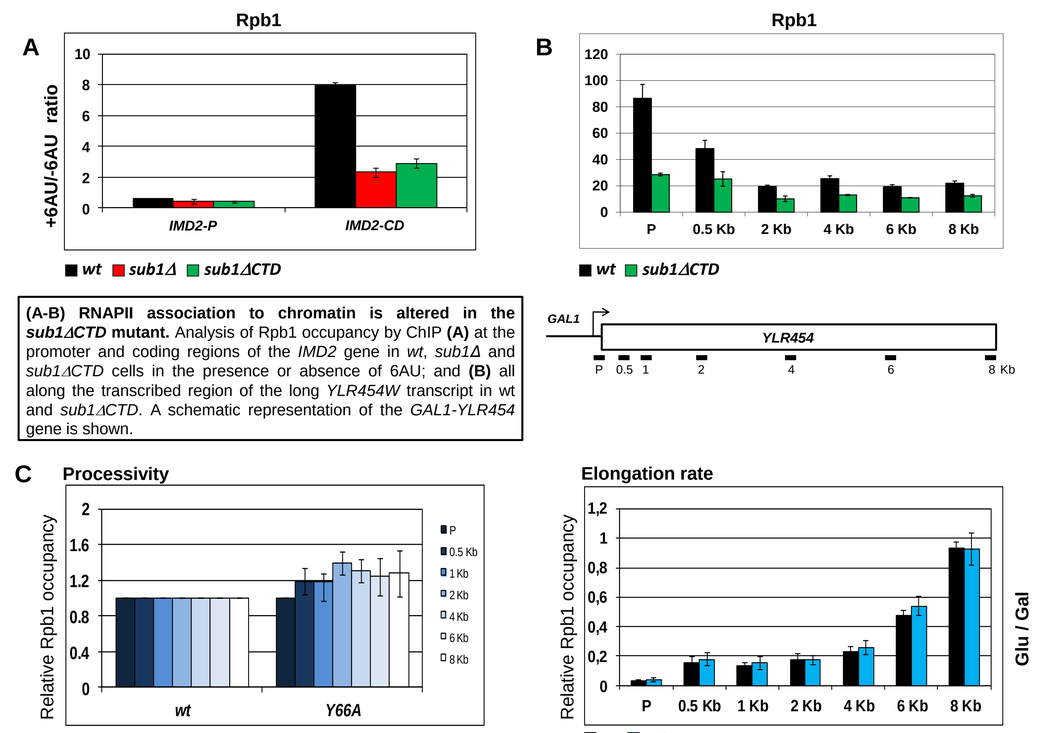
THE CT DOMAIN OF Sub1, BUT NOT THE ssDNA BINDING DOMAIN, IS REQUIRED FOR TRANSCRIPTION ELONGATION

Sub1-CTD influences transcription elongation efficiency



Sub1 CT domain influences transcription elongation efficiency of long transcripts. Our results show that *PHO5* expression decreased in *sub1Δ* and *sub1ΔCTD* strains cells, when expressed as long transcription units containing either the *lacZ* or *LAC4* 3' untranslated sequences, but is not affected when *PHO5* is transcribed from the short transcription unit. On the other side, no effect on elongation is observed in the ssDNA binding domain mutants (*sub1-K45A* and *sub1-Y66A)*. (A) Schematic representation of the three transcription units used in the GLAM assays. The GLAM assay specifically detects defects in elongation and is used to measure the efficiency of **g**ene-**l**ength-dependent **a**ccumulation of **m**RNA [5]. (B-D) The *wt*, *sub1Δ, sub1-K45A*, *sub1-Y66A*, and *sub1ΔCTD* strains were transformed with the plasmid bearing the three transcription units shown in (A). GLAM ratios were calculated as the relative levels of acidic phosphatase activity expressed by the indicated transcription unit (*GAL-PHO5 LacZ* or *GAL-PHO5 LAC4*) with respect to the acid phosphatase activity from the shortest transcription unit (*GAL-PHO5*). The left panel is a representation of the GLAM ratios, and the right panel shows the RT-PCR results of *PHO5* expression from the three transcription units and from the mock strain bearing an empty plasmid. *ADH1* was used as loading control. PCR products were run on an ethidium bromide-stained gel. (E) Allelic genetic interaction between *SUB1ΔCTD* and *SPT5*, coding for the Spt5 elongation factor.

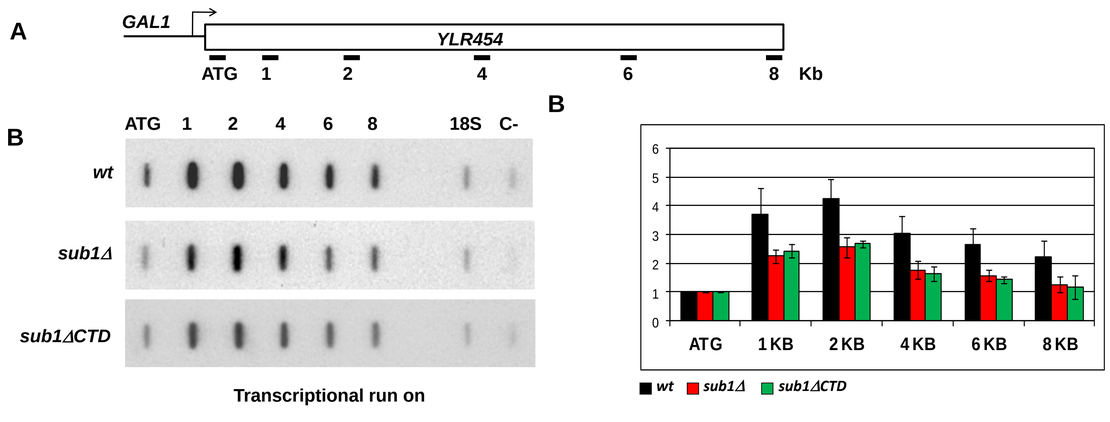
Sub1-CTD influences RNAPII association to chromatin and transcription elongation rate



(A-B) RNAPII association to chromatin is altered in the *sub1ΔCTD* mutant. Analysis of Rpb1 occupancy by ChIP **(A)** at the promoter and coding regions of the *IMD2* gene in *wt*, *sub1Δ* and *sub1ΔCTD* cells in the presence or absence of 6AU; and **(B)** all along the transcribed region of the long *YLR454W* transcript in wt and *sub1ΔCTD*. A schematic representation of the *GAL1-YLR454* gene is shown.

(C-D) The DNA binding domain of Sub1 seems not to participate in transcription elongation; however, deletion of Sub1-CT domain leads to transcription elongation rate defects (C and D, left) Measurement of RNAPII processivity. Relative Rpb1 occupancy at the *GAL1-YLR454* gene in *wt, sub1-Y66A and sub1ΔCTD* cells at the indicated positions after inducing conditions. The qPCR values were first normalized to the values at the promoter (P) and then with respect to the *wt* cells values, taken as 1 for each probe (C and D, right) Measurement of the elongation rate. Relative Rpb1 occupancy of galactose-induced cells in *wt, sub1-Y66A and sub1ΔCTD* cells along the *GAL1-YLR454* gene after 4 min of glucose treatment (glucose/galactose ratio).

Active elongation-competent RNAPII is decreased in *sub1ΔCTD* cells.



Distribution of transcriptionally competent polymerases analyzed by transcriptional run on (TRO) assay shows that active RNAPII is significantly decreased in *sub1Δ* and *sub1ΔCTD* cells when compared to *wt* cells, and the decrease is stronger in the 5' region of the *YLR454* gene. (A) Diagram of the *GAL1-YLR454* gene. Locations of the probes used in the TRO are indicated. (B) TRO of *wt* and *sub1Δ and sub1ΔCTD* cells growing in galactose-containing medium. A representative slot blot filter is shown. (C) Quantitation of blot hybridizations. Hybridization signals were quantitated by PhosphorImager analysis. The bacteria DNA background signal (C-) was subtracted from each probe and, after normalization for 18S rRNA signal, the results were normalized to the ATG probe, which was fixed at 1.

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