

Supplementary Material

# Non-Coding, RNAPII-Dependent Transcription at the Promoters of rRNA Genes Regulates Their Chromatin State in *S. cerevisiae*

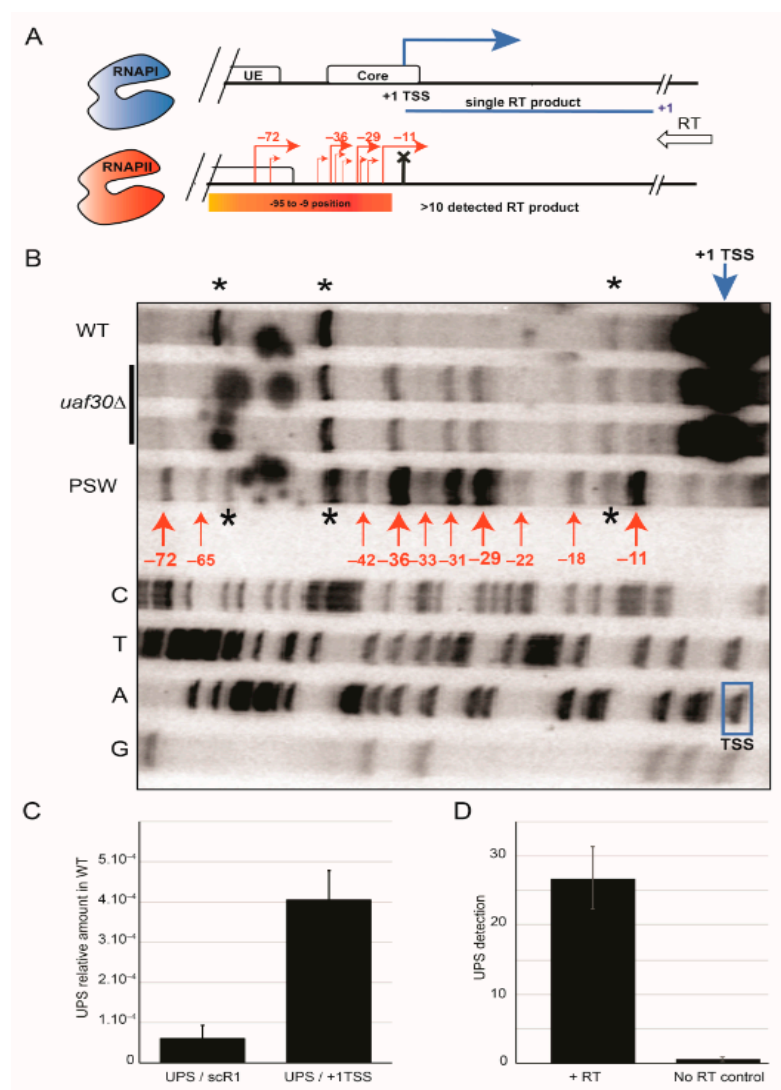
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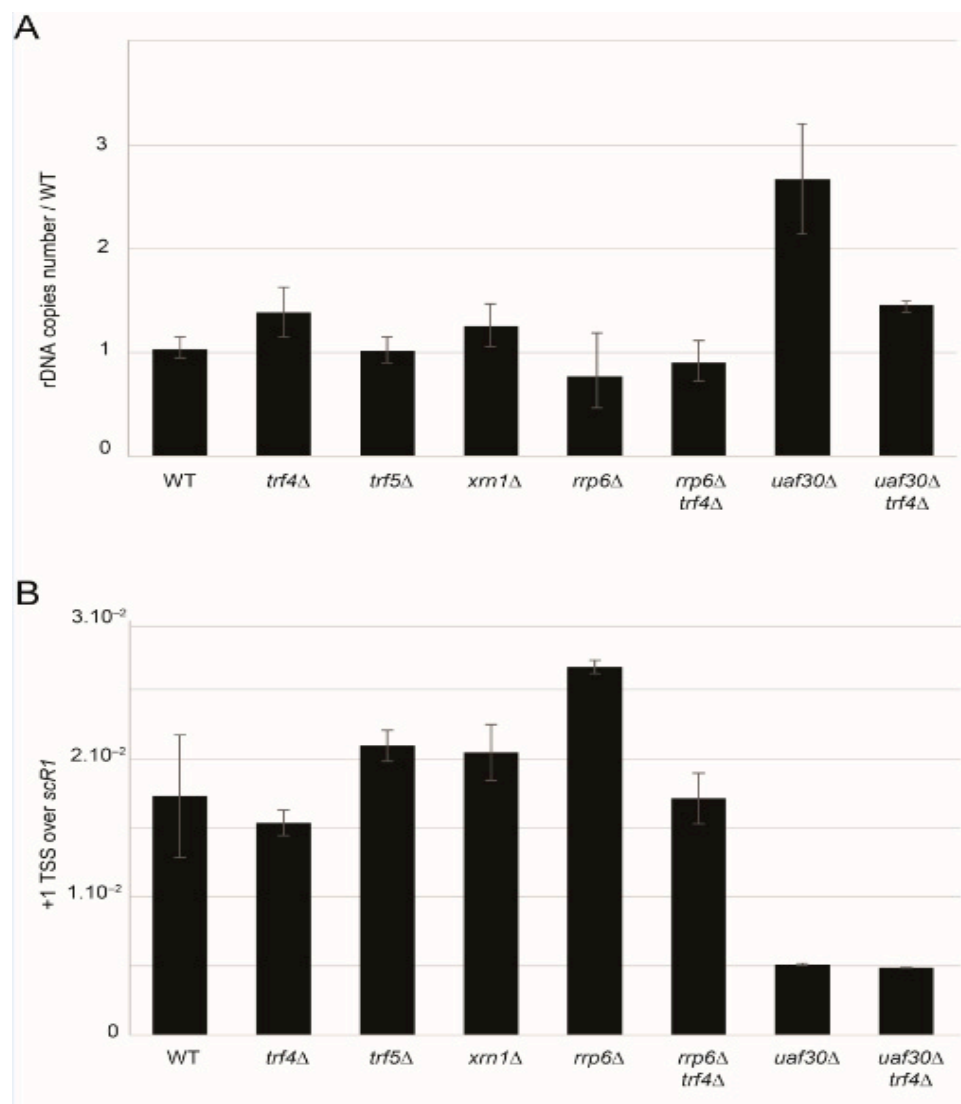
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**Figure S1.** UPS lncRNAs are transcribed from the IGS2 region in rDNA. **A.** Schematic representation of the 35S promoter region composed of UE (Upstream Element) and Core promoter. RT forward

primer was used for reverse transcription. Blue arrows represent +1 TSS. Red arrows represent position of 5' of UPS rRNA. **B.** Primer extension reaction with radiolabelled RT primer was resolved in 10% denaturing acrylamide gel. +1 TSS is marked by blue arrows and UPS by red ones. Star (\*) represents a non-specific RT product. Sequencing reaction was used as molecular weight C. Quantification of UPS in wild type strains. UPS were quantified using RT primer for reverse transcription (1  $\mu$ g of total RNA) relative to *scR1* and +1 TSS. Error bars correspond to the standard deviation of three independent cultures. **D.** 1  $\mu$ g of total RNA was used with or without reverse transcription (no RT), showing that UPS can be detected about 50-fold over background.



**Figure S2.** rDNA copy number and +1 TSS rRNA accumulation in various mutants. **A.** Quantification of rDNA copy number was performed using qPCR and are shown relative to WT control (BY4741). **B.** Quantification of +1 TSS in various mutants relative to *scR1* accumulation. Error bars correspond to the standard deviation of three independent cultures.

**Table S1.** Strains used in this study.

Name	Genotype	Source / reference
WT BY4741	<i>MATa, his3Δ, leu2Δ0, met15Δ0, ura3Δ0</i>	Euroscarf
WT BY4742	<i>MATα, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0</i>	Euroscarf
WT BY4743	<i>MATa/α, his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0</i>	Euroscarf
<i>rrp6Δ</i>	<i>MATα, lys2, ura3, his3, leu2, rrp6::KAN-MX4</i>	Euroscarf
<i>trf4Δ</i>	<i>MATα, ura3, his3, leu2, trf4::KAN-MX4</i>	Euroscarf
<i>trf5Δ</i>	<i>MATα, lys2, ura3, his3, leu2, trf5::KAN-MX4</i>	Euroscarf
<i>rrp6Δ trf4Δ</i>	<i>MATα, met15, lys2, rrp6::NAT-MX, trf4::KAN-MX4</i>	This study
<i>uaf30Δ</i>	<i>MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, met15Δ0, uaf30::KAN-MX4</i>	This study
<i>xrn1Δ</i>	<i>MATa, his3Δ1, leu2Δ1, ura3Δ0 met15Δ0, xrn1::KAN-MX4</i>	Euroscarf
<i>rat1-1</i>	<i>MATa, his3Δ1, leu2Δ1, ura3Δ0 met15Δ0, rat1-1::NAT-MX</i>	[1]
<i>rpb4Δ</i>	<i>MATa, his3Δ1, leu2Δ1, ura3Δ0 met15Δ0, rpb4::KAN-MX4</i>	Euroscarf
<i>uaf30Δ trf4Δ</i>	<i>MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, met15Δ0, uaf30::KAN-MX4 trf4::NAT-MX</i>	This study
<i>rpa49Δ</i>	<i>MATα, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0, lys2Δ0 rpa49::alpha NAT-MX</i>	This study
<i>pMET3-RAT1</i>	<i>MATa, ade2-1 (ochre), can1-100 (ochre), his3-11,15, leu2-3,112, trp1-1 (ambre), ura3-1 HIS3::pMET3-RAT1</i>	[2]
<i>pMET3-RAT1 rrp6Δ</i>	<i>MATa, ade2-1 (ochre), can1-100 (ochre), his3-11,15, leu2-3,112, trp1-1 (ambre), ura3-1 HIS3::pMET3-RAT1, rrp6::KAN-MX4</i>	This study
WT YPH499	<i>MATa, ura3-52, lys2-801_amber, ade2-101_ochre, trp1-Δ63, his3-Δ200, leu2-Δ1</i>	[3]
<i>rpb1-1</i>	<i>MATa, leu2-Δ1, ura3-52, trp1-Δ63, lys2-801, his3-Δ200, rpb1-1 (rpb1-G1437D)</i>	[4]
<i>rDNA 190C</i>	<i>MATa, leu 2-3, 112 ura3-1, his3-11, trp1-1, ade2-1, can1-100, fob1::HIS3 rDNA copy number 190</i>	[5]
<i>rDNA 25C</i>	<i>MATa, leu 2-3, 112 ura3-1, his3-11, trp1-1, ade2-1, can1-100, fob1::HIS3 rDNA copy number 25</i>	[5]
PSW	<i>MATa, rpa135::LEU2, rrn9::HIS3, ade2-1, ura3-1, trp1-1, leu2-3, 112, his3-11, can1-100</i>	[6]

**Table S2.** Primers used in this study.

Name	Sequence 5'-3'
RT (35S rRNA) - R	ACACGCTGTATAGAGACTAGGC
A (35S rRNA) - R	GACTCTCTCCACCGTTTGACG
B (35S rRNA) - F	AAAGCAGTTGAAGACAAGTTCGAA
C (UPS) - R	ACGACAAGCCTACTCGAATTCGT
D (UPS) - F	GAGGAAAAGTAGTTGGGAGGTAC
sCR1 - F	GGCTGTAATGGCTTTCTGG
sCR1 - R	GTGCGGAATAGAGAACTATCC
pGAL7 - F	CGAACAGTAGCTGATCTCAG
pGAL7 - R	ATTTAACCAAATGGTGAAGGAGGACCTCGC
PMA1 - F	CCTCTTCATCATCCTCTTCAGC
PMA1 - R	AGCGTCATCGTAAGTCTTAGCAG
PCR rDNA - F	CTTGTCTCAAAGATTAAGCCATGC
PCR rDNA - R	ACCACAGTTATACCATGTAGTAAAGGAACT

## References

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