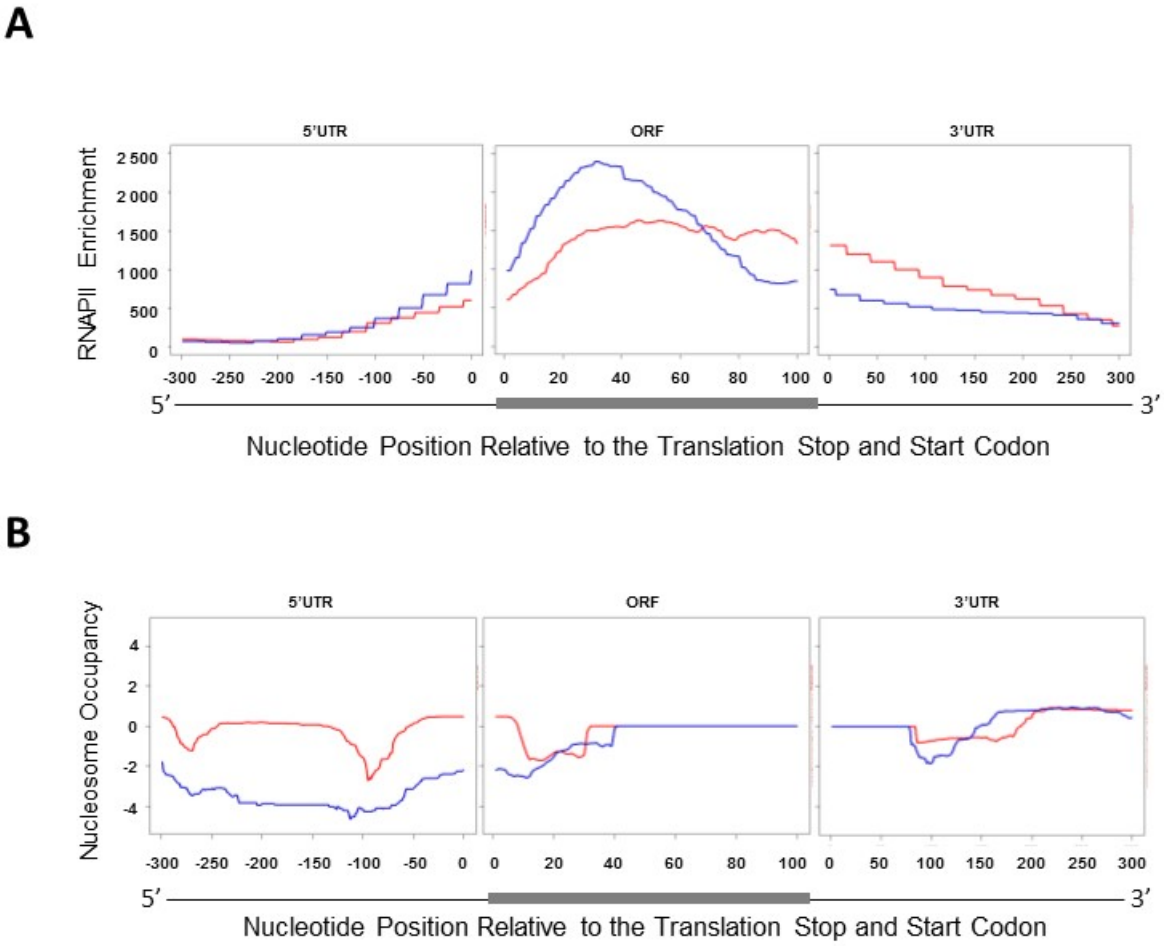
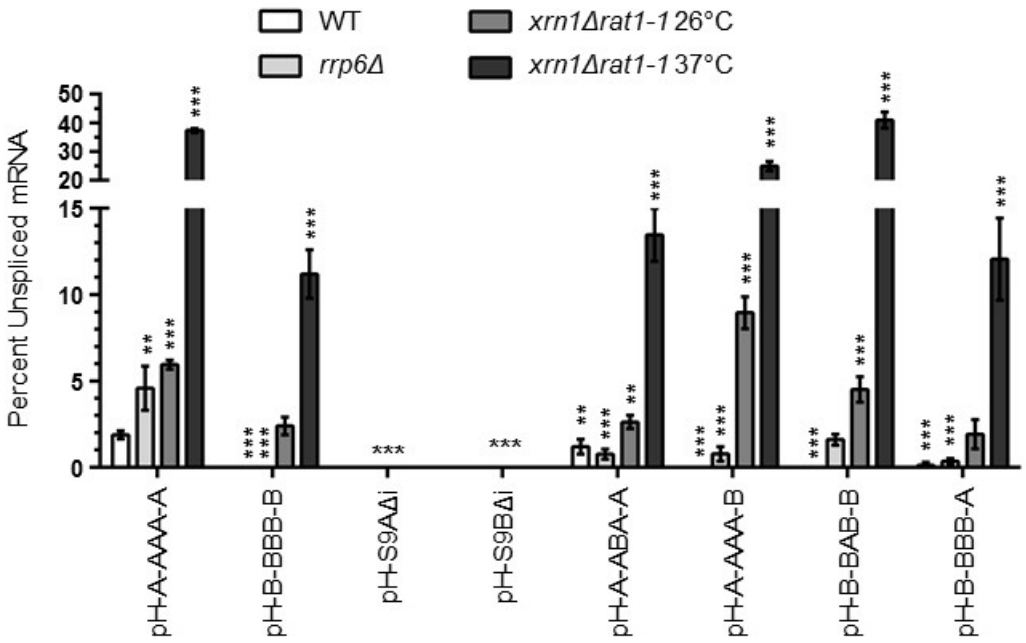
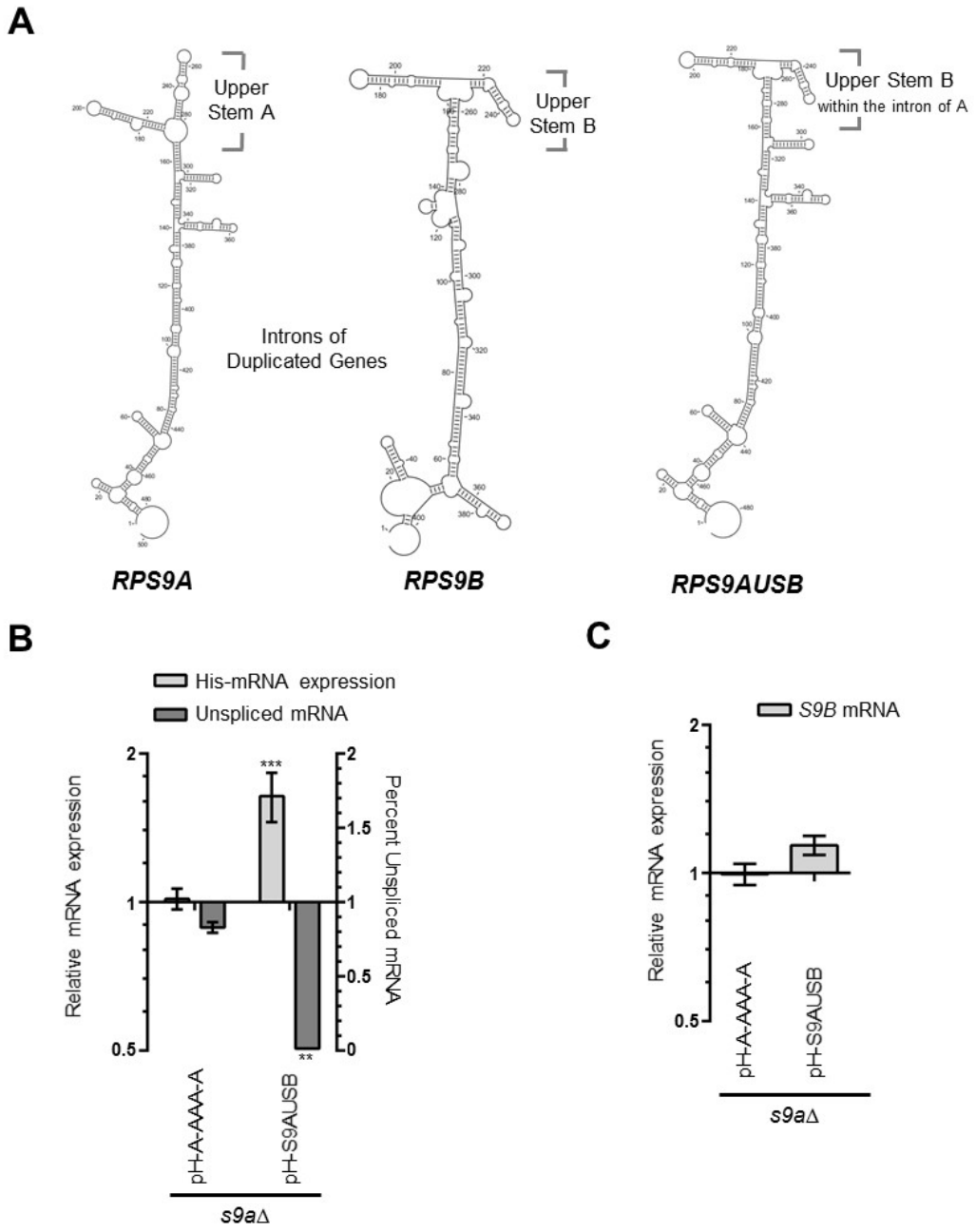
Supplementary Material
Petibon et al., 2016



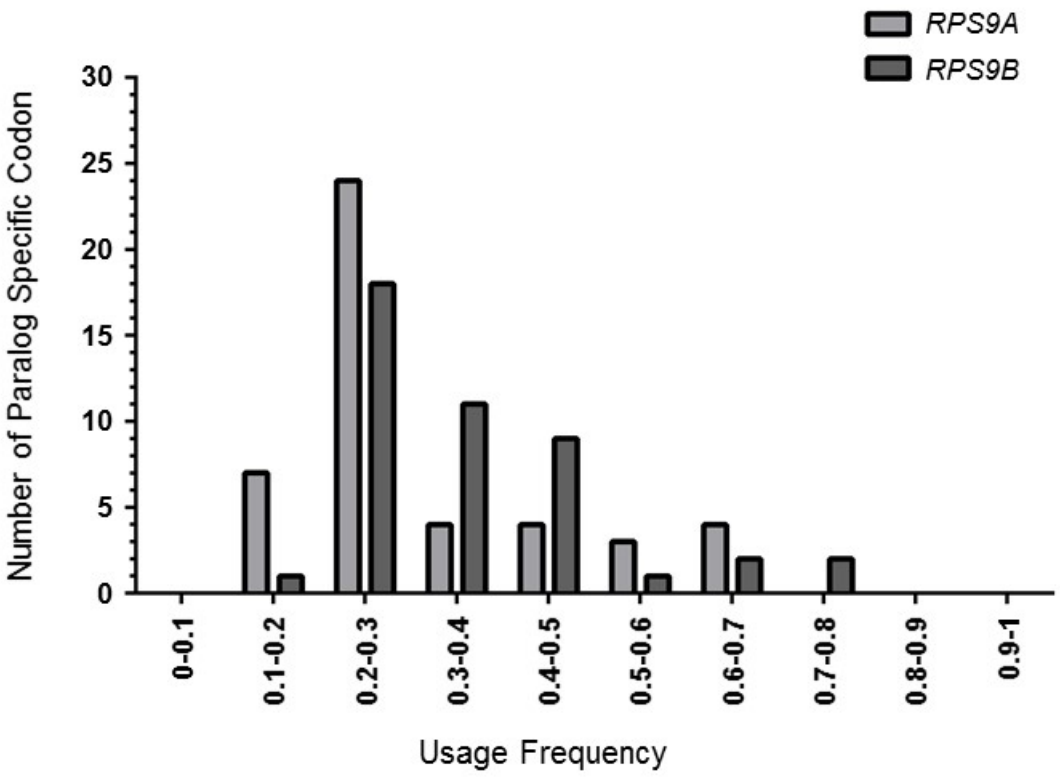
Supplementary Figure S1 (related to Figure 1 and Figure 4). Comparison between *RPS9* paralogs transcription and histones association profiles. **(A)** The transcription profiles of *RPS9A* (blue) and *RPS9B* (red) were obtained by whole genome RNAPII chromatin immunoprecipitation assay (1). **(B)** The nucleosome profiles of *RPS9A* (blue) and *RPS9B* (red) were obtained by whole genome histone ChIP assay (2).



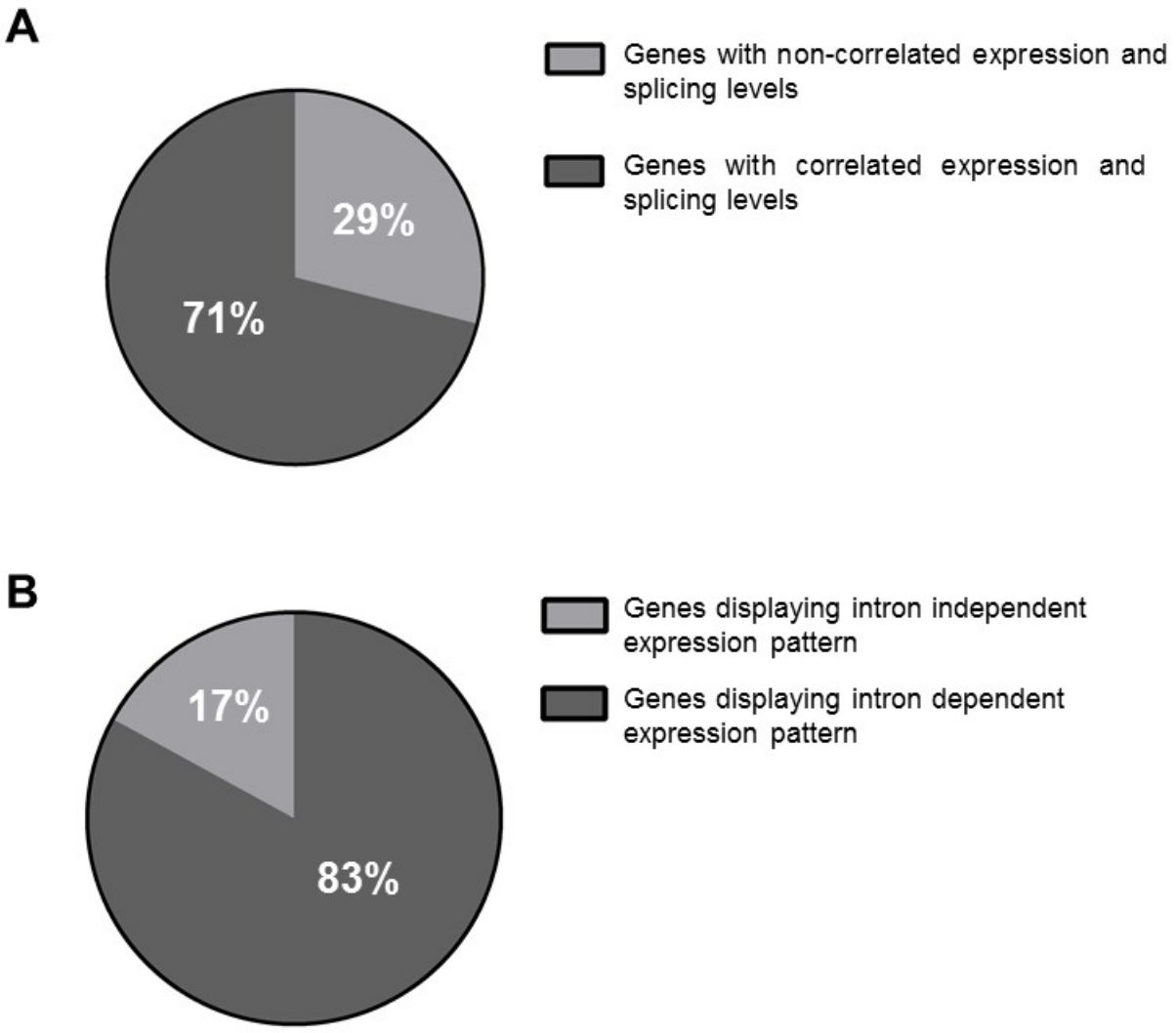
Supplementary Figure S2 (related to Figures 1 and Figure 4). Deletion of nuclear 5'-3' exoribonucleases increases the stability of unspliced *RPS9* unspliced pre-mRNA. Deletion of nuclear exoribonucleases increases the levels of paralog pre-mRNAs. The RNA was extracted from cells expressing tagged versions of *RPS9A* and *RPS9B* and related mutations in wild type strain (WT) yeast and after the deletion of the 3'-5' nuclear exosome component Rrp6p (*rrp6*Δ), the cytoplasmic 5'-3' exoribonuclease Xrn1p (*xrn1*Δ*rat1-1* 26°C) or the inactivation of a temperature sensitive allele of the nuclear 5'-3' exoribonuclease Rat1p in the absence of Xrn1p (*xrn1*Δ*rat1-1* 37°C). The amount of unspliced mRNA was determined using end-point RT-PCR as described in Figure 1F. The experiments were conducted in triplicate and statistically significant differences in splicing efficiency are indicated by asterisks (** p-value < 0.01 and *** < 0.001). Inactivation of the temperature-sensitive allele of *RAT1* (*rat1-1*) (16) was achieved by shifting cells from the permissive (26°C) to the restrictive (37°C) temperature for 4 hours.



Supplementary Figure S3 (related to Figure 2). The *RPS9B* upper stem structure enhances the splicing and expression of *RPS9A*. The intronic upper stem structure of *RPS9A* was replaced with that of *RPS9B* **(A)** and the effect on the splicing and expression **(B and C)** was determined as described in Figure 2. *RPS9A*, *RPS9B* and *RPS9AUSB* indicate wild type *RPS9A*, *RPS9B* and hybrid *RPS9A/B* introns respectively. The position of the sequence replacements is indicated by brackets. The data are the average of 3 independent experiments. The standard deviation is illustrated by error bars. Statistically significant differences are indicated by asterisks (** p-value < 0.01 and *** < 0.001).



Supplementary Figure S4 (related to Figure 4). *RPS9B* has higher usage-frequency codons than *RPS9A*. The codons that vary between *RPS9A* (light grey) and *RPS9B* (dark grey) were identified and plotted relative to their usage frequency. The codon indices were separated in bins of 0.1.



Supplementary Figure S5. Introns inhibit the expression of the majority of the underexpressed dRPG paralog. **(A)** The majority of the under expressed dRPGs (minor copies) contain under spliced introns. The minor and major copies of each dRPG were assigned according to their expression levels as measured by single molecule sequencing mRNA quantification (3) and their relative intron splicing efficiency was determined using a yellow fluorescent protein (YFP)-dependent synthetic splicing reporter (4). Splicing and expression are considered to be correlated when the less expressed copy of a dRPG also contains the under spliced intron of the pair. The results are presented in the form of a pie chart. **(B**) The majority of the under spliced introns found in minor dRPGs inhibit the expression of their host gene. The effect of deleting the under-spliced introns from the minor dRPG copies on the expression of its host genes was determined by quantitative RT-PCR (5) and the percent of deletions with negative and positive effects on gene expression were calculated. Gene expression is considered to be intron-dependent when the intron deletion leads to increased gene expression.

Supplementary References:

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2. Kaplan, N., Moore, I.K., Fondufe-Mittendorf, Y., Gossett, A.J., Tillo, D., Field, Y., LeProust, E.M., Hughes, T.R., Lieb, J.D., Widom, J. *et al*. (2009) The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, **458**, 362-366.
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5. Parenteau, J., Durand, M., Morin, G., Gagnon, J., Lucier, J.F., Wellinger, R.J., Chabot, B. and Elela, S.A. (2011) Introns within ribosomal protein genes regulate the production and function of yeast ribosomes. *Cell*, **147**, 320-331.