Ribosomal protein Rpl22 regulates the splicing of its own transcripts in *Saccharomyces cerevisiae*

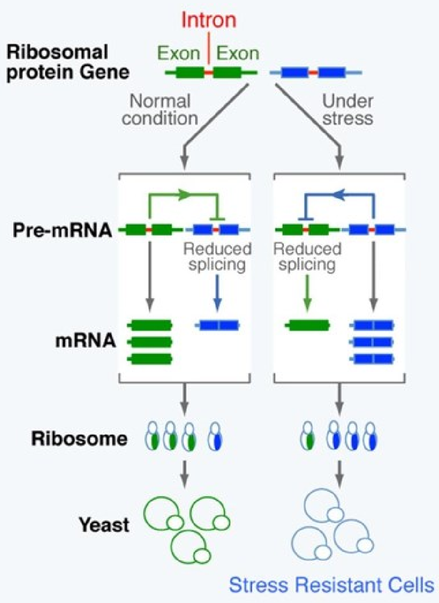
Filip Nemčko (supervised by Kateřina Abrhámová)

Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

INTRODUCTION

Ribosomal protein (RP) genes in *S. cerevisiae*

- **major group of retained duplicated genes after whole-genome duplication (~11%)**[1]
- **overrepresented among intron-containing genes (~74%)** [1]
- **massively expressed in growing yeast cells**[2]
  - ~50% of RNA polymerase II transcription
  - ~90% of pre-mRNA splicing
- **tightly regulated and coordinated on different levels of expression**
- **documented a widespread intron-dependent intergenic regulation controlling the ratio of RP paralogs**[3]
  - intron deletion in one paralog affects the expression of the paralog *in trans*
  - capable to change the relative concentration of paralogs -> impact on the composition of ribosomes (functionally „specialized“ ribosomes)



modified[3]

CONCLUSIONS

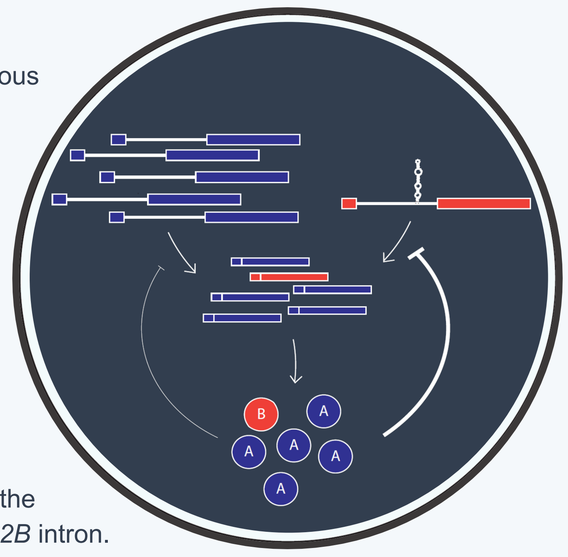
1 Introns modulate the expression of paralogous *RPL22* and *RPL2* genes.

2 Intergenic regulation of *RPL22* paralogs is mediated by introns.

3 Expression of *RPL22* paralogs is regulated by asymmetric splicing.

4.1 Rpl22 interacts with a specific region of *RPL22B* intron.

4.2 Some orthologous Rpl22 proteins retained the ability to interact with the I2 region of *RPL22B* intron.



AIMS OF THE PROJECT

I. Identify candidate paralogous ribosomal protein genes to study intron-dependent intergenic regulation.

II. Bring a mechanistic insight into the intron-dependent intergenic regulation of *RPL22* paralogs.

1) Which step of the Rpl22 expression is regulted?
2) What is the molecular mechanism of regulation?
3) Which part of the intron is responsible for the regulation?

FUTURE PLANS

I. Localize the step in spliceosome assembly, which is blocked by Rpl22 protein by using chromatin immunoprecipitation (ChIP) of splicing factors in following strains
- *RPL22AΔ* (non-inhibited)
- wt (intermediary inhibited)
- *RPL22AΔi* (inhibited)

II. Identify a regulatory element inside the *RPL22* intron responsible for the Rpl22 regulation in yeast *Kluyveromyces lactis*, which did not undergo the whole genome duplication, but retained the regulation.

RESULTS

1

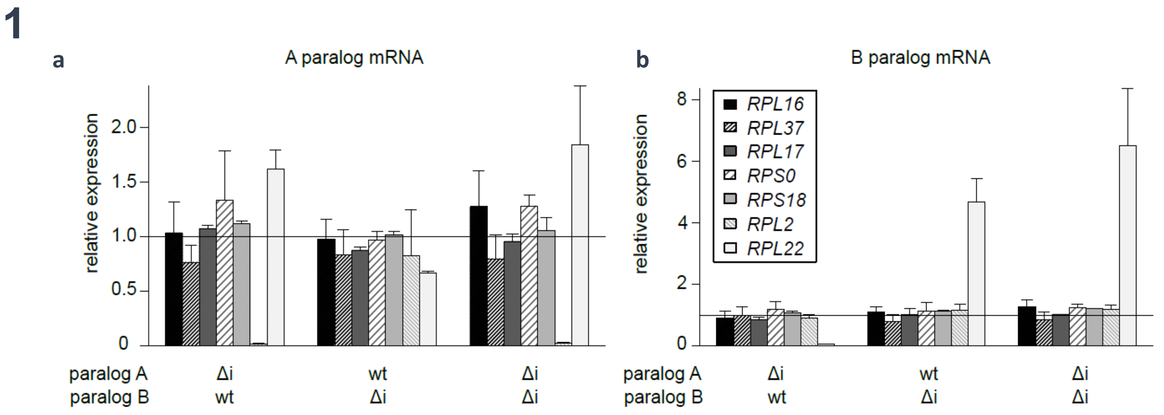


Fig 1. Impact of intron deletions on the expression of duplicated ribosomal protein genes. We selected 7 paralogous RP genes with the most noticeable intron-dependent intergenic regulation (as published in [3]) and re-tested the impact of intron deletion on the expression of both paralogs using the same strains and primers as in [3]. We only identified *RPL22* pair with evident intergenic regulation. We also found a strong intron-dependent intragenic regulation of *RPL2A* gene. Plots show mean fold changes of “A” (a) and “B” (b) paralog mRNA in mutants with intron deletion (Δi) in “A”, “B” or both paralogs as determined by RT-qPCR relative to WT. Data were normalized to *SPT15* expression and to the RNA level in WT cells. Error bars represent s.d. from two (*RPL17*, *RPS0*, *RPS18*) or three (*RPL2*, *RPL16*, *RPL37, RPL22*) biological replicates.

3

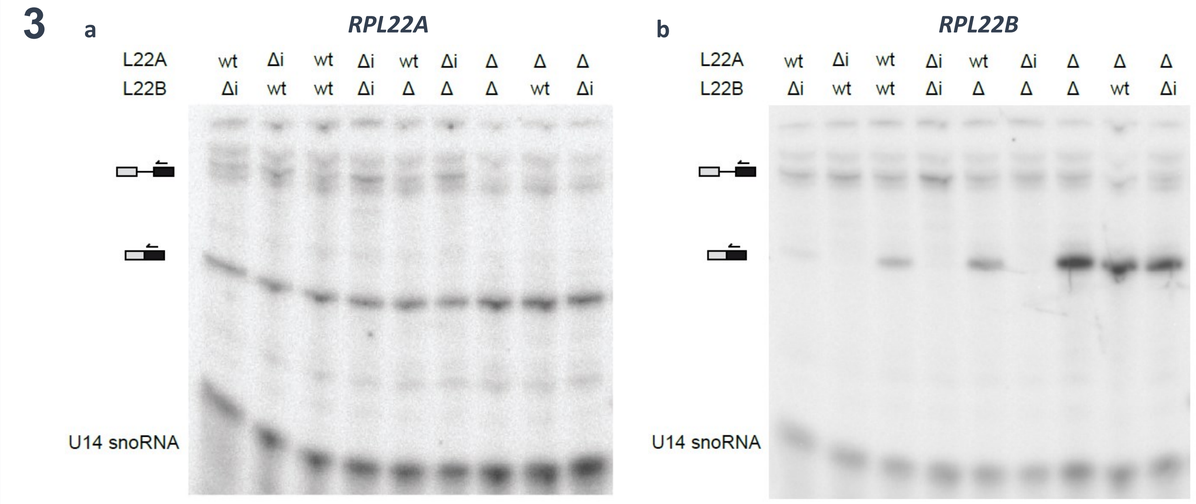


Fig 3. Splicing of *RPL22B* reporter is highly sensitive to the presence of Rpl22. Gels show radioactively labeled primer extension products from cells bearing variants of endogenous *RPL22* paralogs and expressing *RPL22A-CUP1* (a) or *RPL22B-CUP1* (b) splicing reporter. U14 snoRNA was used as a loading control. Each gel is representative of three independent experiments.

2

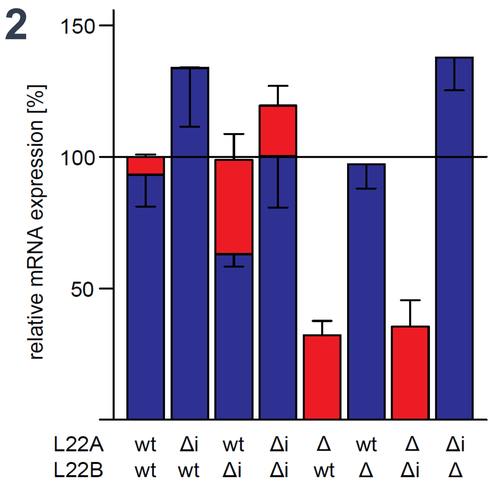
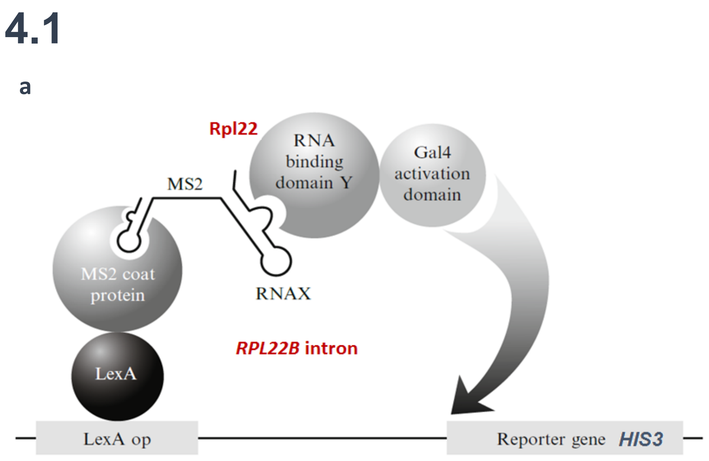


Fig 2. Introns mediate intergenic regulation of *RPL22* paralogs. Plot shows mean fold changes of mRNA levels of *RPL22A* and *RPL22B* relative to WT as determined by RT-qPCR. Data were normalized to the *SPT15* expression and to the RNA level in WT cells. Error bars represent s.d. from three biological replicates. In the case of *RPL22AΔ* and *RPL22BΔ*, two biological replicates were measured by K. Abrhámová.

4.1



4.2

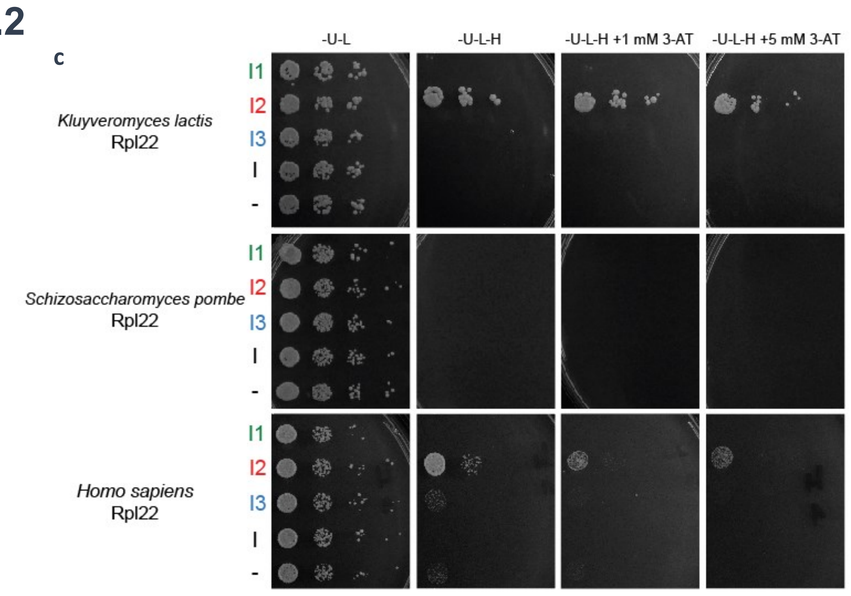


Fig 4. Interactions of Rpl22 proteins with *RPL22B* intron. (a) Diagram depicting the general principle of three-hybrid system adapted from[4]. (b) Rpl22A, Rpl22B, or their RNA-biding mutants, or (c) Rpl22 protein from *Kluyveromyces lactis, Schizosaccharomyces pombe* and *Homo sapiens* in combination with different parts of *RPL22B* intron were assayed for expression of *HIS3* reporter gene. 10-fold serial dilutions of cells were spotted on plates with increasing concentrations of 3-AT. “-U”, “-L” and “-H” denote the lack of uracil, leucine and histidine in the medium. IRE (iron response element) and IRP (iron regulating protein) served as a positive control. Rpl22 protein from *S. pombe* lost the ability to interact with *RPL22B* fragment. (d) RNAfold structure prediction of *RPL22B* intron (“I”). Regions tested in three-hybrid system are shown in color. I1 region (green) represents nucleotides 11 to 123 of *RPL22B* intron, I2 (red) - nt 165 to 236 and I3 (blue) - nt 256 to 321. (d)

ACKNOWLEDGEMENTS

This work was supported by the Grant Agency of the Czech Republic (14-19002S). I would like to thank my supervisor K. Abrhámová, as well as other members of the Laboratory of Regulation of Gene Expression, especially P. Folk, J. Libus, F. Půta, M. Oplová and A. Dědková for their help and productive discussion.

REFERENCES

[1] Planta, R. J. & Mager, W. H. The list of cytoplasmic ribosomal proteins of *Saccharomyces cerevisiae*. *Yeast* (1998).
[2] Warner, J. R. The economics of ribosome biosynthesis in yeast. *Trends Biochem. Sci.* (1999).
[3] Parenteau, J. *et al.* Introns within ribosomal protein genes regulate the production and function of yeast ribosomes. *Cell* (2011).
[4] SenGupta, D. J. *et al.* A three-hybrid system to detect RNA-protein interactions in vivo. *Proc. Natl. Acad. Sci. U S A.* (1996)