

Deciphering the role of specialized ribosomes in plants' translation efficiency

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1 Translational regulation mediated by ribosomal heterogeneity

Translation and its regulation play an important role in plant adaptation. Ribosomes have traditionally been considered passive molecular players regarding which RNA to translate. However, this view is changing due to studies showing that specific and heterogeneous ribosomes can have an active role regulating the translation of different RNA subunits in mammals and bacteria [1]. In plants, the possibilities for specialization are much higher, as each ribosomal family is encoded by two to seven paralogs and there are several hints in the literature pointing towards differential paralog roles. However, whether this heterogeneity provides selective translation of specific mRNAs under particular cell conditions has yet to be demonstrated.

Objectives

To determine whether different paralogs of ribosomal proteins (RPs) are functionally equivalent or not.

Methodology

We have focused on RP family eL24, composed of two paralogs. We are working with T-DNA mutants for each paralog and performing phenotypical characterizations to find situations that may suggest a specific role for each paralog [point 2]. In addition, it has been described that *rpl24b/el24y* is unable to translate uORF-containing transcripts [2]. To determine whether this feature supposes a possible paralog specialization, we have expressed a reporter construct with the 5' leader of the transcription factor *bZIP11* which harbors several uORFs that regulate its translation in the two paralog mutant backgrounds of the eL24 family [point 2]. To figure out whether the paralogs affect in the same way in general translation, polysome profiling technique was performed and differences in gene expression between both paralog mutant was studied doing RNA-seq before and after pelleting polysome in order to refine our analysis [point 3]. Finally, lethality of the double mutant *rpl24a rpl24b* was demonstrated as well as gene dosage [point 4].

Background of the family eL24 in Arabidopsis

The family eL24 is composed of two paralogs ubiquitously expressed throughout the plant with a nearly identical expression pattern. Different phenotypes have already been described for each paralog mutant:

- *rpl24b* is defective in the translation of auxin-related genes [3]
- *rpl24b* shows several phenotypes like slower growth or shorter siliques [3]
- RPL24B bolsters the reinitiation competence of uORF-translating ribosomes [2]

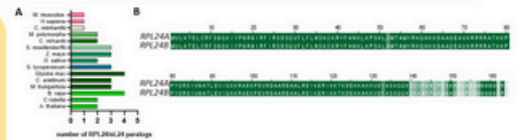


Fig. 1. The presence of several paralogs of the same ribosomal protein family is commonly found in plants. A) Number of paralogs of the family eL24 in different plant families including mammals, algae, and primitive plants. B) Alignment of the two paralogs of the Arabidopsis ribosomal family eL24.

2 Arabidopsis mutants in paralogs of the same riboprotein family exhibit different phenotypes

rpl24b shows growth retardation and auxin-related phenotypes that are not displayed by *rpl24a*

rpl24b seedlings exhibit shorter roots in basal condition, delay in the appearance of the first true leaves, and the germination of both paralog mutants is affected by sucrose in different ways

The eL24 paralogs translate differentially uORF-containing mRNAs

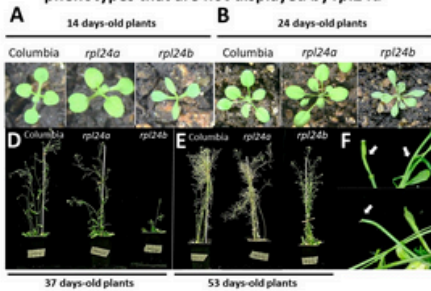


Fig. 2. Photos of *rpl24a* mutants at different growth stages. A) 14 d-old plants. B) 24 d-old plants. C) 37 d-old plants. D) 53 d-old plant. E) Related auxin phenotypes displayed by the *rpl24b* mutant, such as ETIN-like siliques.

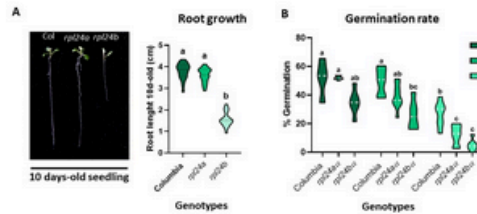


Fig. 3. Although *rpl24b* is always more affected phenotypically, it was also found that the germination of *rpl24a/el24z* is altered under sucrose stress. A) Root length in 10 d-old seedlings, grown in MS 1% sucrose. ANOVA 1-way (Tukey test) with p -value < 0.05. B) Germination percentage after 48h under different sucrose concentrations (0, 1 and 3%). ANOVA 2way (Tukey test), p -value < 0.05. Two independent experiments were analyzed together.

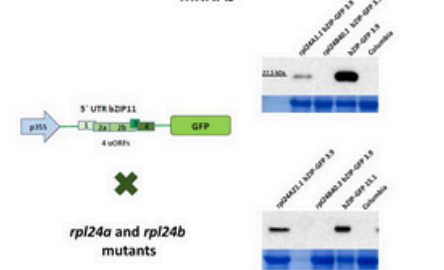


Fig. 4. RPL24B/eL24y is required for the translation of a uORF-containing reporter construct while RPL24A/eL24z seems to be dispensable. A) Schematic of the reporter construct employed that was introduced into the *rpl24a* and *rpl24b* mutants. B) Western blot using anti-GFP from transgenic line 35S-5'UTR bZIP-GFP in *rpl24a* and *rpl24b* background. Two independent experiments were done using different mutant seed batches.

3 Both mutants show altered polysome profiles and RNA-seq reveals the ribosome biosynthesis machinery is affected in *rpl24b* but not in *rpl24a*

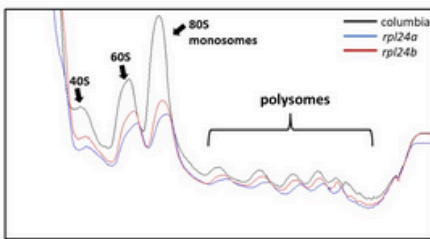


Fig. 5. Both paralog mutants display lower monosome and 60S peak in polysome profiling compared to Columbia. Polysome profiling from 8d-old seedlings of Col-0, *rpl24a* and *rpl24b* grown 3 days in darkness and 5 day in light afterwards.

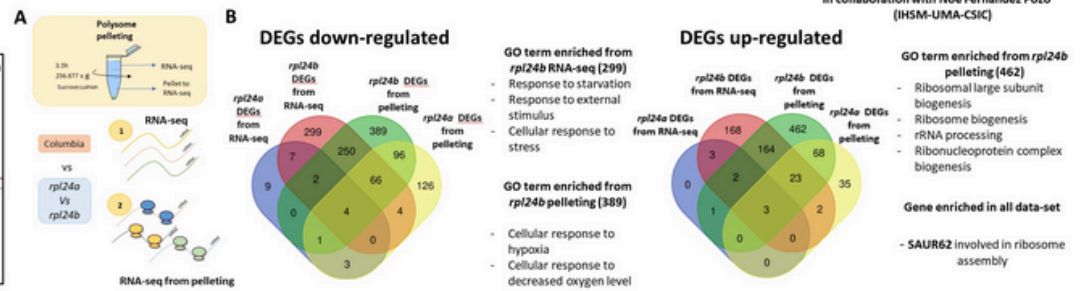


Fig. 6. DEGs obtained through RNA-seq technique of each paralog mutant of family eL24. A) mRNA sequencing was done comparing both the mutants with Col-0 and each paralog mutant with each other. In parallel, polysome pelleting was used to isolate and sequence polysomal RNA. B) Venn diagrams divided in up or down regulated and comparing four list of genes (rpl24a DEGs from RNA-seq, rpl24b DEGs from RNA-seq, DEGs from rpl24a from pelleting-RNA-seq and rpl24b from pelleting-RNA-seq)

4 The double mutant *rpl24a rpl24b* is lethal

How about the double mutant?

A

rpl24a rpl24b Lethal

the obtained percentage of genotypes which included *rpl24b* was lower than expected

Genotype	percentage obtained	Expected percentage
WT	833	828
<i>rpl24a</i> <i>rpl24b</i>	353	123
<i>rpl24a</i> <i>rpl24b</i>	193	828
<i>rpl24a</i> <i>rpl24b</i> <i>rpl24b</i>	26	39
<i>rpl24a</i> <i>rpl24b</i> <i>rpl24a</i>	18	123
<i>rpl24a</i> <i>rpl24b</i>	133	828
<i>rpl24a</i> <i>rpl24b</i>	133	828
<i>rpl24a</i> <i>rpl24b</i>	133	828

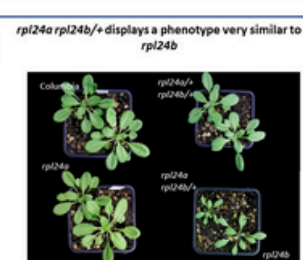
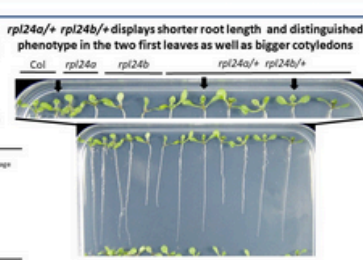


Fig 7. Lethality of double mutant. A) 94 plants were genotyped to obtain F2 population distribution B) Phenotype seen in 10 d-old seedling. C) 35 d-old plants.

5 Conclusions and work in progress

These results suggest that the different paralogs of the riboprotein family eL24 are good candidates to perform specialized functions.

In progress:

We are complementing our mutants and expanding the phenotypical and molecular analysis to determine whether there is specialization in the eL24 family.

Bibliography

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