Looking for specialized ribosomes in plants. Characterization of the riboprotein families L10 and L24

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1 Translational regulation mediated by ribosomal heterogenity

Translation and its regulation play an important role in plant adaptation and ribosomes have traditionally been considered "passive" machines in this process. This view is changing as studies showing evidences for their active role in translational regulation in mammals and bacteria are appearing [1]. The likelihood of ribosomal specialization is particularly high in plants, with up to seven paralogs per family of ribosomal proteins in Arabidopsis.

2 Objetives

To determine whether different paralogs of ribosomal proteins (RPs) are functionally equivalent.

3 Methodology

We have focused on two RP families, **L10 and L24.** Each of these families is composed of three paralogs, and differential phenotypes have been described in mutants of each paralog [2,3,4] (**point 4**). These phenotypical differences could suggest a possible paralog specialization. We are working with T-DNA mutants for each paralog and performing phenotypical characterizations to find situations that may suggest a specific role for a paralog (**point 5**). Then, the dynamics of the different RPs paralogs will be studied employing polysome profiles using tagged versions of the RPs (**point 6**). These constructs will also be used to perform complementation analyses in which each paralog mutant will be transformed with each tagged paralog of the same family to determine whether the different paralogs are interchangeable.

In addition, rpl24b is unable to translate uORF-containing transcripts [4]. To determine whether this feature supposes a possible paralog specialization, we have used a reporter construct with the 5´leader of the transcription factor bZIP11 which harbors several uORFs that regulate its translation [5] (**point 7**).

4 Background of the families RPL10s and RP24s

The three paralogs of RPL10 and RPL24 are ubiquitously expressed throughout the plant with a nearly identical expression pattern but their paralog mutants show differential phenotypes, which make them suitable to study their possible paralog-specialization in ribosomes.

Different phenotypes have already been described for each paralog mutant:

RPL10

- ❑ *rpl10a* is lethal and not UV-regulated [2]
- ❑ *rpl10b* shows abnormal growth and it is down-regulated by UV [2]
- ❑ *rpl10c* do not exhibit any visible phenotype and it is up-regulated by UV [2]

RPL24

- ❑ *rplr24b* 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 [4]

5 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*.**

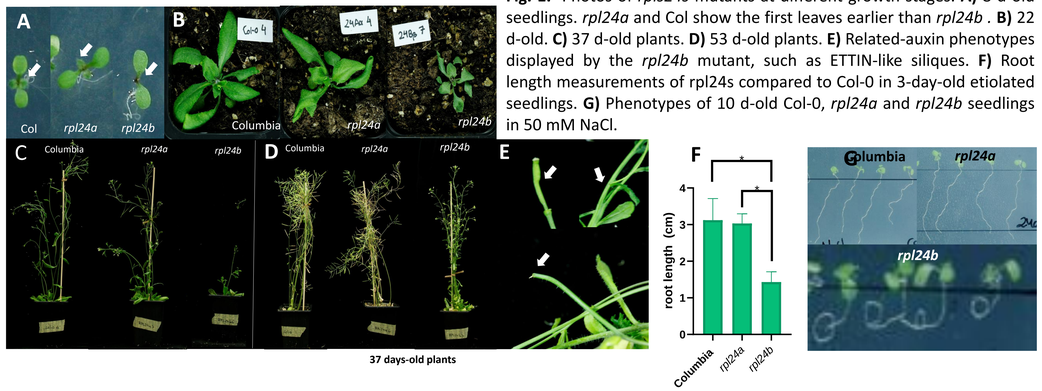

Fig. 1. Photos of *rpls24s* mutants at different growth stages. **A)** 8 d-old seedlings. *rpl24a* and Col show the first leaves earlier than *rpl24b* . **B)** 22 d-old. **C)** 37 d-old plants. **D)** 53 d-old plants. **E)** Related-auxin phenotypes displayed by the *rpl24b* mutant, such as ETTIN-like siliques. **F)** Root length measurements of rpl24s compared to Col-0 in 3-day-old etiolated seedlings. **G)** Phenotypes of 10 d-old Col-0, *rpl24a* and *rpl24b* seedlings in 50 mM NaCl.

***rpl10a* shows dramatic growth retardation compared to *rpl10b* and *rpl10c*. *rpl10b* and *rpl10c* have a lower seed set than Col -0.**

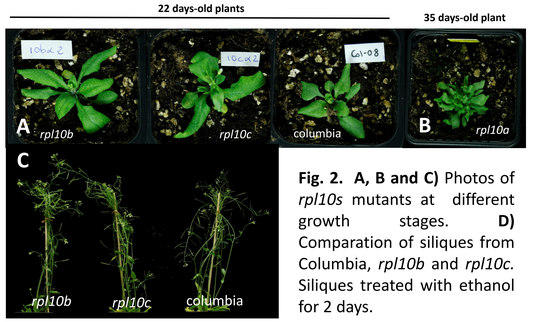

Fig. 2. A, B and C) Photos of *rpl10s* mutants at different growth stages. **D)** Comparation of siliques from Columbia, *rpl10b* and *rpl10c*. Siliques treated with ethanol for 2 days.

6 Flag-tagged RPL10 paralogs are succesfully incorporated into translating ribosomes

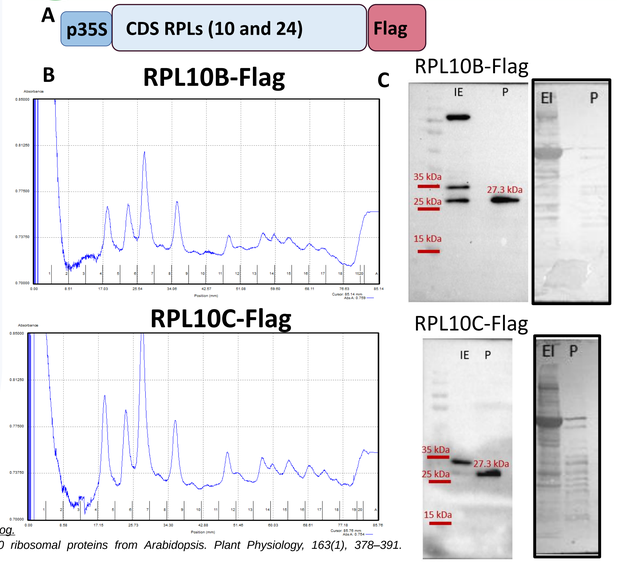

Fig. 3. Flag-tagged RPLs are successfully incorporated into translating ribosomes A) Schematic of the constructs. **B)** Polisome profiles of Nicotiana leaves that transiently express Flag-tagged versions of RPL10B and 10C; and **C)** Western blot of the crude extract (IE) and polysomal fractions (P) using anti-Flag. IE: crude extract P: polysomal pellet . Construct size: 27.3 kDa.

7 The RPL24 paralogs translate differentially uORF-containing mRNAs

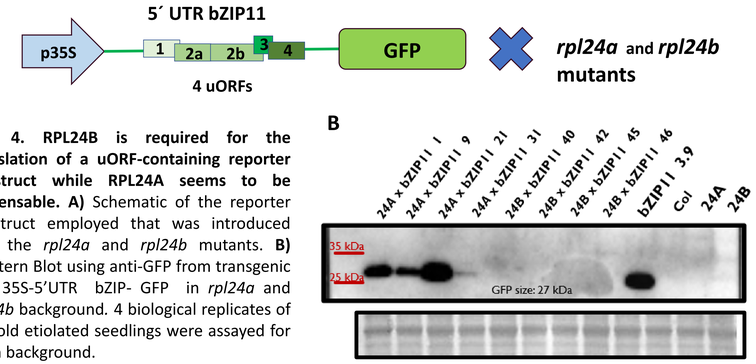

Fig. 4. RPL24B is required for the translation of a uORF-containing reporter construct while RPL24A seems to be dispensable. A) Schematic of the reporter construct employed that was introduced into the *rpl24a* and *rpl24b* mutants. **B)** Western Blot using anti-GFP from transgenic line 35S-5'UTR bZIP- GFP in *rpl24a* and *rpl24b* background. 4 biological replicates of 3 d-old etiolated seedlings were assayed for each background.

8 Conclusions and work in progress

There results show that the different paralogs of the riboprotein families L10 and L24 are good candidates to perform specialized functions.

In progress: we are generating and checking the viability of our transgenic lines in order to complement our mutants and monitor the translation dynamics of the different paralogs under different environmental conditions.

References

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