

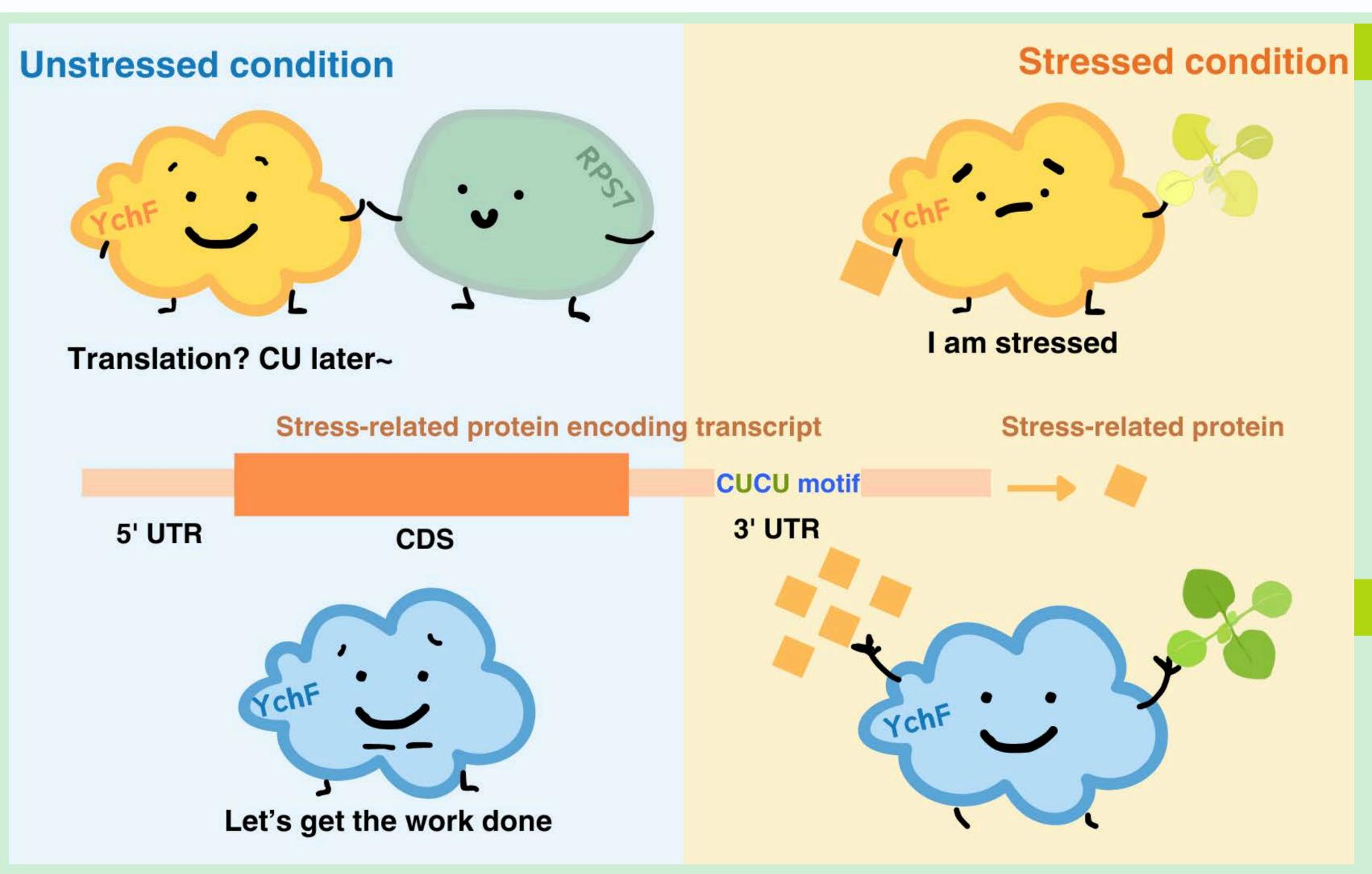
The possible roles of a plant ribosome-associated protein, AtYchF1, on translational regulation in Arabidopsis



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Introduction

A group of unconventional conserved GTPase, YchF, resembles those nucleotide-hydrolyzing proteins from the translation factor (TRAFAC) family that possibly regulate translation. Mammalian and bacterial YchF homologs modulate stress responses by interacting with the translation machinery ²⁻³. Despite the high sequence homology and functional similarities observed among YchF members, detailed mechanisms of how plant YchFs regulate stress responses remain elusive. We hypothesized that plant YchF homologs might influence the translation profile of a specific group of mRNA by ribosomal protein RPS7 interaction, as its bacteria, yeast, and human homologs do.

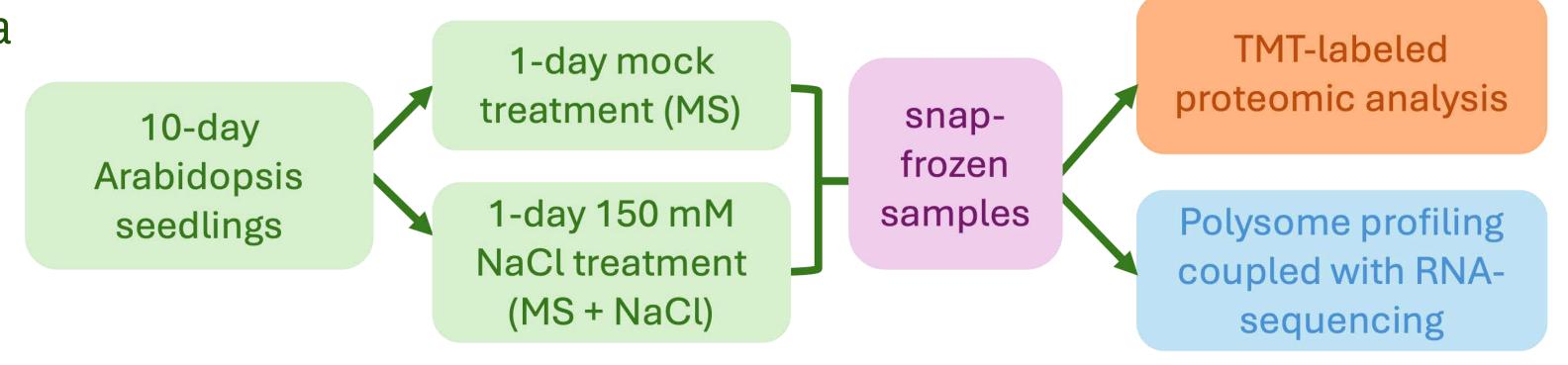
Aims

Our project aimed to reveal the possible role of AtYchF1 in translation by interacting with AtRPS7 in salt-stressed conditions. Our experimental design aimed to investigate a possible stress-regulating mechanism in plants, therefore contributing to providing suitable candidates for climate-smart crop breeding and improvements.

Methodology

To reveal the possible role of AtYchF1 in translation by interacting with AtRPS7 in salt-stressed conditions,

- Transgenic Arabidopsis lines overexpressing *AtYchF1* (the native form) or *AtY34* (a mutant form of AtYchF1 incapable of binding AtRPS7) were constructed.
- 10-day Arabidopsis seedlings were subjected to 150mM NaCl for 1 day.
- Tandem mass tags labeled quantitative proteomics analysis were conducted to estimate the protein profile.
- Polysome sequencing was conducted to estimate the translation profile.



Results

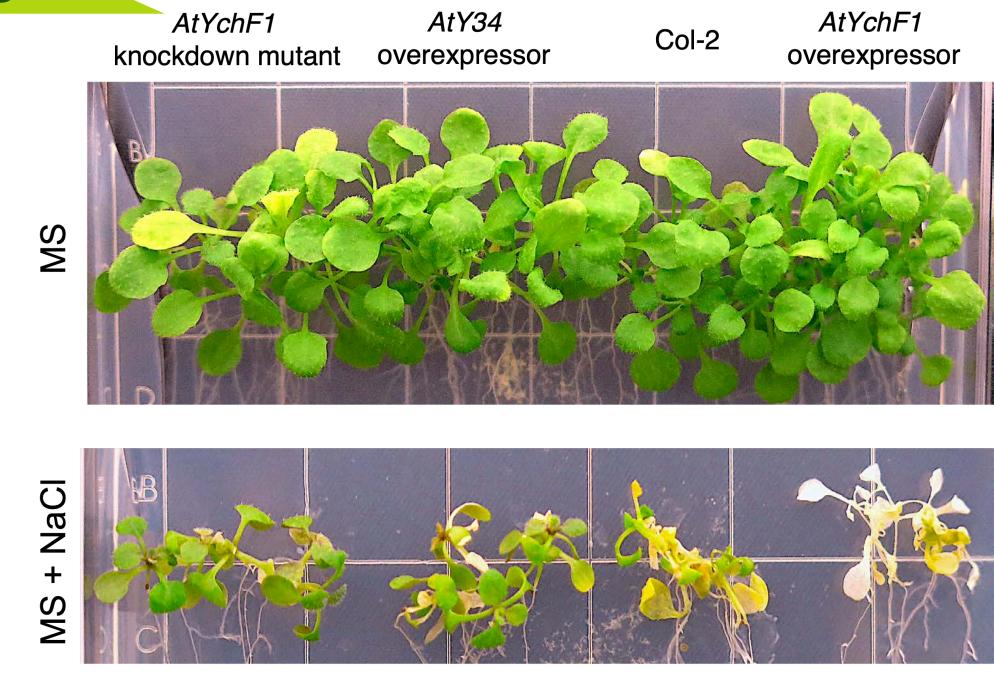


Fig.1 AtYchF1 overexpression enhances salt sensitivity but not AtY34 overexpression. Severe leaf chlorosis was observed in the native AtYchF1-overexpressor under salt treatment, while the AtYchF1-knockdown mutant and the AtY34-overexpressors exhibited minor leaf chlorosis.

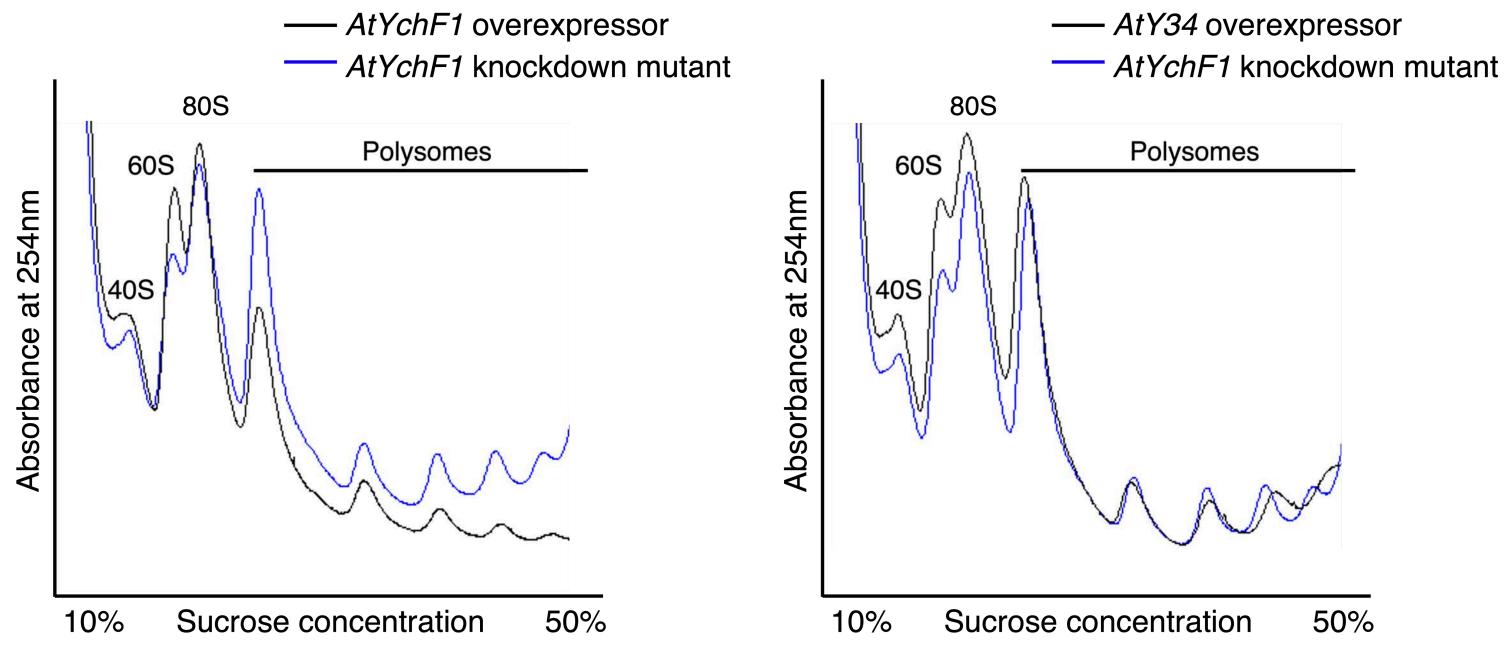


Fig.2 Representative overlaid polysome profiles of *AtYchF1* overexpressor or *AtY34* overexpressor compared to their genetic background, *AtYchF1* knockdown mutant at unstressed conditions. *AtYchF1* overexpressor showed a lowered efficiency in translation compared to *AtY34* overexpressor and its genetic background. In particular, ROS scavenger encoding transcripts showed reduced abundance in the actively translating polysomes of *AtYchF1* overexpressor.

Fig.3 Electrophoretic mobility shift assay showing AtYchF1 and AtY34 recognize and directly bind to the enriched CUCU motifs of the down-regulated ROS scavenger-encoding transcripts. The 3'UTR of a ROS scavenger (AT2G47730) was used as the native RNA probe in the assay. The result suggested that the motif recognition of AtYchF1 is independent of its AtRPS7-binding capability.

Conclusion

Our result showed that AtY34, with the AtRPS7-binding ability, abolished, lowered stress sensitivity. Compared to the *AtY34* overexpressing plants, *AtYchF1* overexpressors had repressed levels of stress-related proteins, along with reduced abundances of the corresponding mRNAs at the actively translating heavy polysome. We proposed AtYchF1 as a translational regulator that influence the translation of stress-related proteins in an AtRPS7-dependent manner. AtYchF1 binds to a specific CUCU-motif on stress-related protein-encoding mRNAs to repress their translation through interacting with AtRPS7. AtYchF1 resulted in the translational repression of the stress-related proteins, therefore promoting salt sensitivity.

References

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