

# PUMPKIN – the sole Plastid <u>UMP Kin</u>ase is involved in Chloroplast RNA Metabolism and Plant Acclimation



Environmental stimuli

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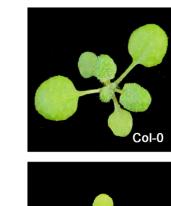
## INTRODUCTION

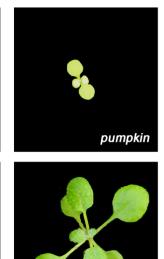
The chloroplast displays a combination of eubacterial and eukaryotic features in terms of gene expression and RNA metabolism. After transcription, precursor transcripts are subjected to multiple processing steps including 5' and 3' end maturation, intercistronic cleavage, splicing and RNA editing. In these processes numerous nuclear-encoded RNA-binding proteins are involved. In this study, we present **PUMPKIN**, a functional **p**lastid **UMP kin**ase which is also involved in **chloroplast RNA metabolism** and its potential role in **plant acclimation**.

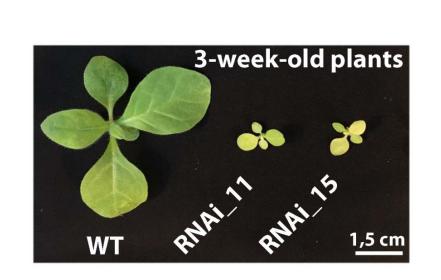
**RNA-binding UMP** kinase activity activity stabilization UMP + ATP trnG In PUMPKIN . ndhA In UDP + ADP petB In \* petD In RNA **Nucleotide** metabolism metabolism

# **RESULTS**

pumpkin knock-out mutants and RNAi lines appear palegreen and are retarded in development



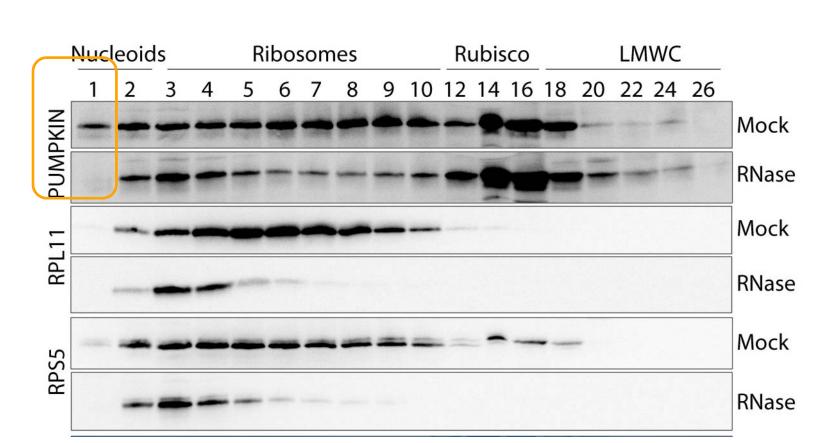






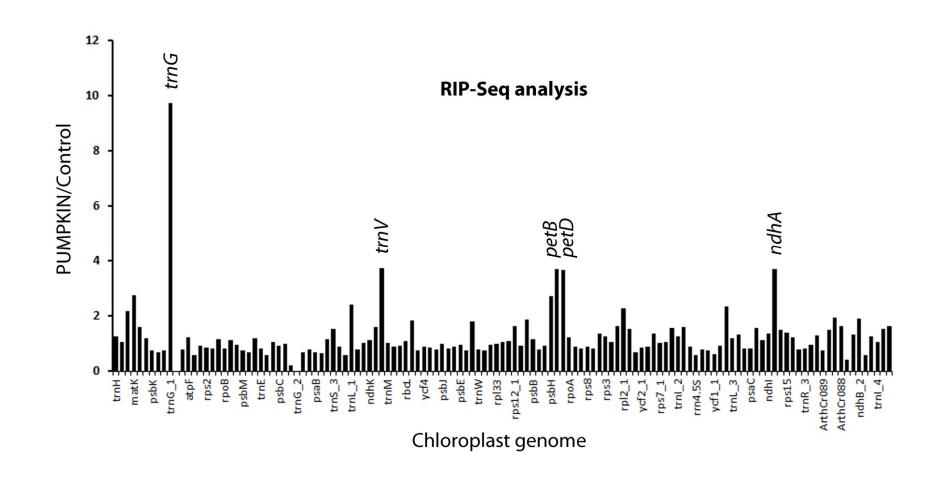
pumpkin = **p**lastid **UMP kin**ase

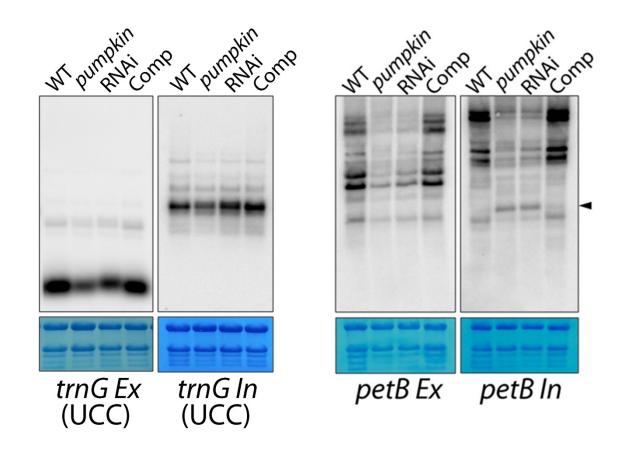
**PUMPKIN** is localized in chloroplasts where it is associated with RNA in high molecular weight complexes



Native size-exclusion chromatography of soluble WT chloroplast extracts

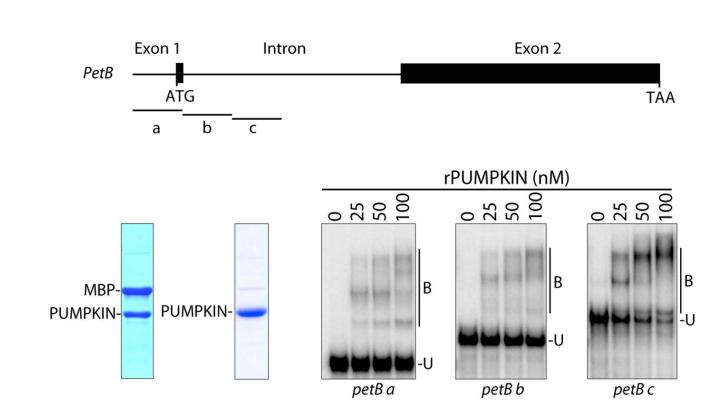
PUMPKIN associates with the intron regions of its RNA targets and affects their stabilization





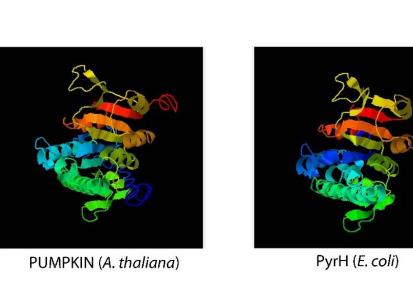
RNA-gel blot analyses of the two targets trnG and petB

#### PUMPKIN binds to its target RNA with high affinity and specificity

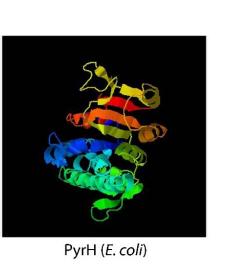


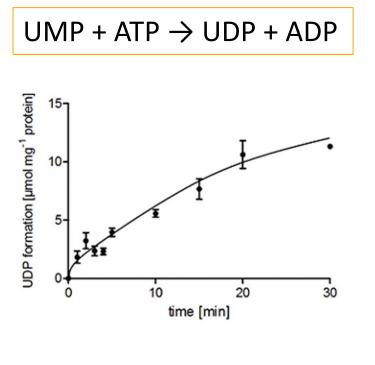
EMSA with recombinant PUMPKIN and *petB* RNA-probes

**PUMPKIN** is a functional UMP kinase and forms a homomultimer – similar to eubacterial UMPKs



3D structure prediction

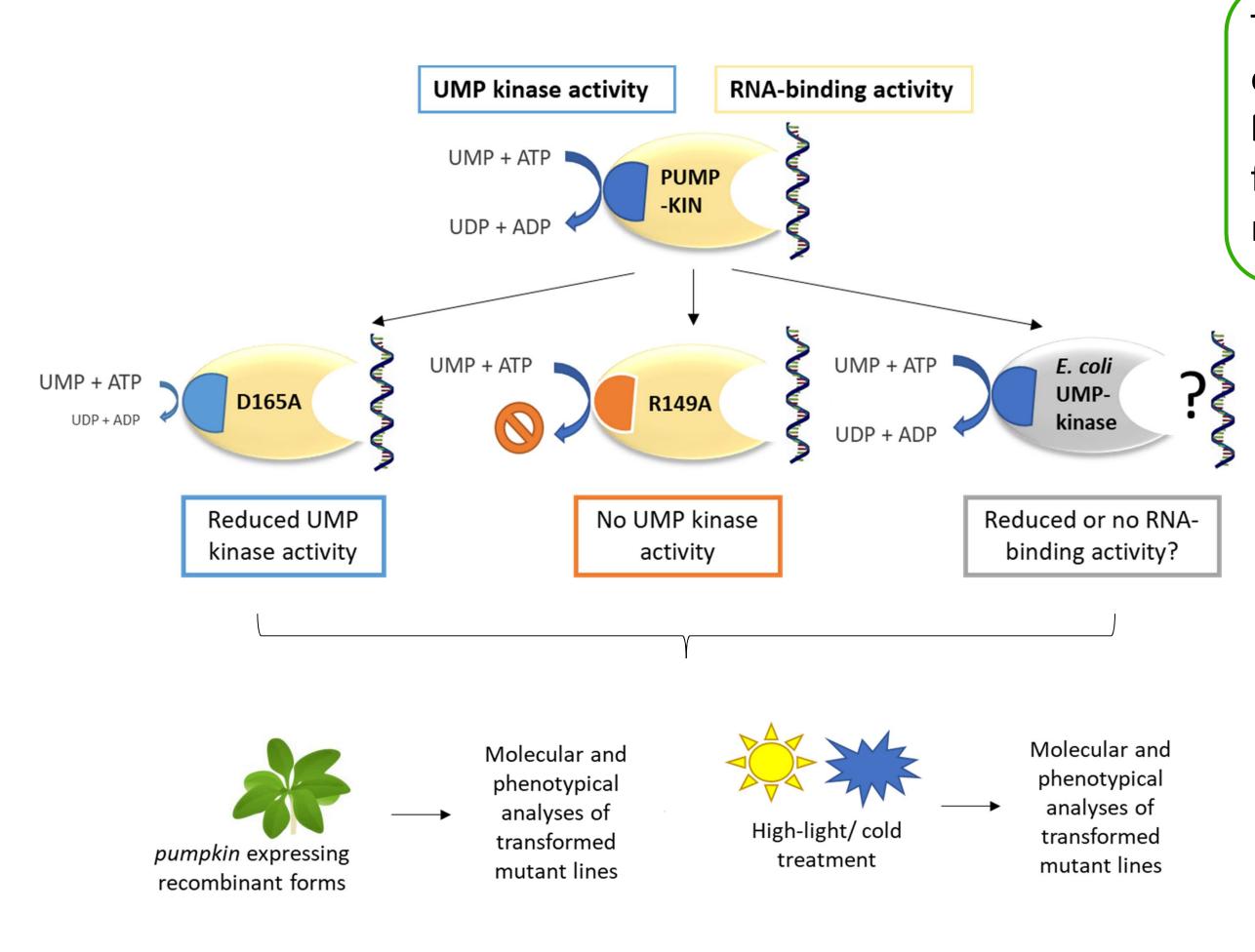


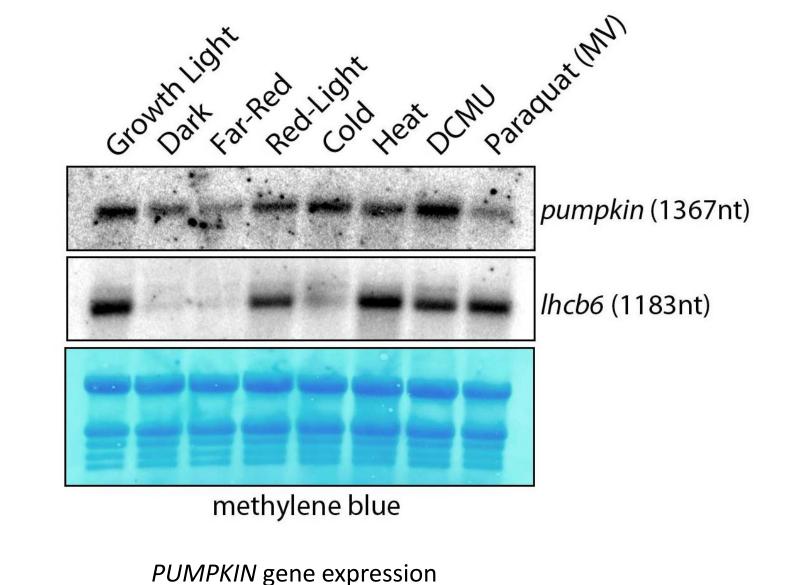


Enzymatic assay with recombinant PUMPKIN

## **PERSPECTIVES**

The expression of pumpkin is highly responsive to different stimuli, pointing to a potential role of PUMPKIN in adopting the plants' needs to various conditions. Using mutated versions of PUMPKIN (D165A and R149A) with reduced or no UMPK activity as well as the E. coli homolog for complementation we want to decipher to which extent the two functions contribute to the resulting mutant phenotype and play a role under various acclimation conditions.





### REFERENCES

Schmid L-M, Ohler L, Möhlmann T, Brachmann A, Muiño JM, Leister D, Meurer J, Manavski N (2019) PUMPKIN, the sole Plastid UMP Kinase, Associates with Group II Introns and Alters Their Metabolism. Plant Physiology, 179: 248-264.

Manavski N, Schmid, L-M, Meurer, J (2018) RNA-stabilization factors in chloroplasts of vascular plants. Essays in Biochemistry, 62: 51–64.

Nagel R (2019) Moonlighting Enzymes: How Often Are We Missing Secondary Functions? Plant Physiology 179: 15-15