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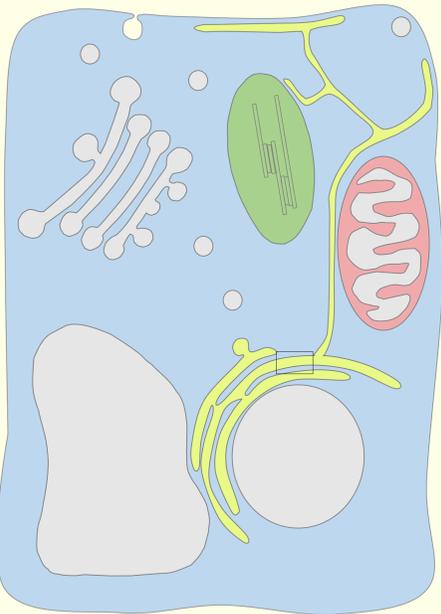
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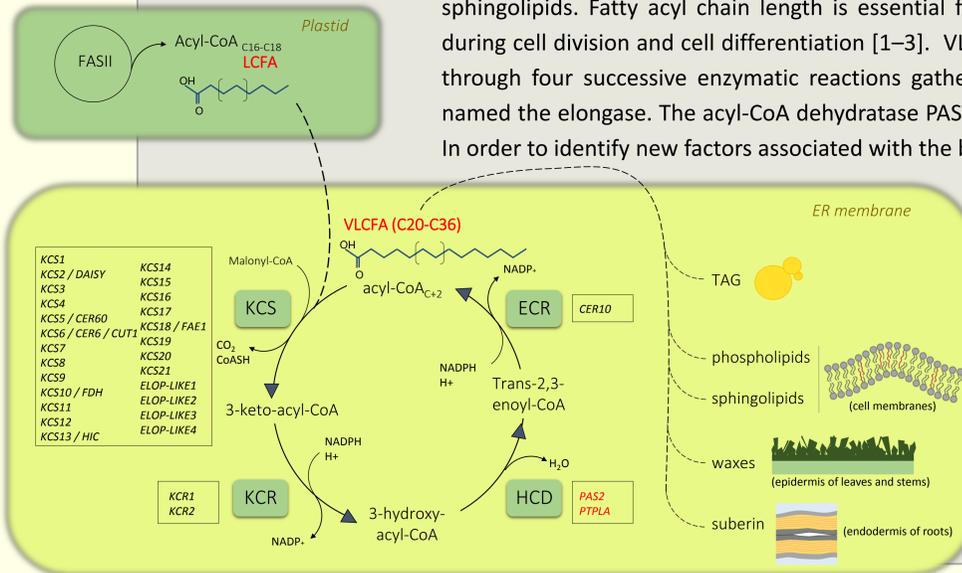
Dual Fatty Acid Elongase Complex Interactions In Arabidopsis

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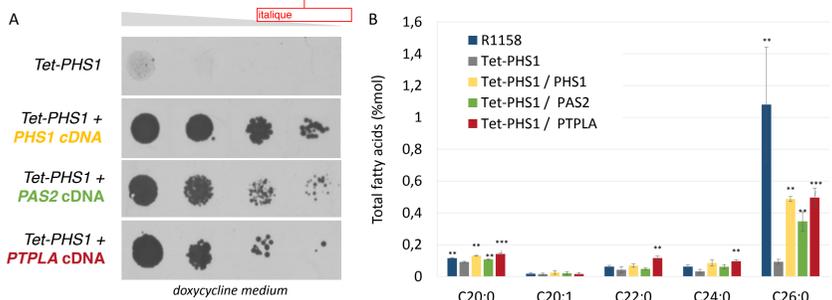
INTRODUCTION



Very long chain fatty acids (VLCFAs) are required for the synthesis of triacylglycerols, waxes, phospholipids and sphingolipids. Fatty acyl chain length is essential for plant development in particular for membrane trafficking during cell division and cell differentiation [1–3]. VLCFAs are elongated by the sequential addition of two carbons through four successive enzymatic reactions gathered in the endoplasmic reticulum within a protein complex named the elongase. The acyl-CoA dehydratase PASTICINO2 (PAS2) is involved in the third step of elongation [3]. In order to identify new factors associated with the biosynthesis of VLCFAs, a yeast multicopy suppressor screen

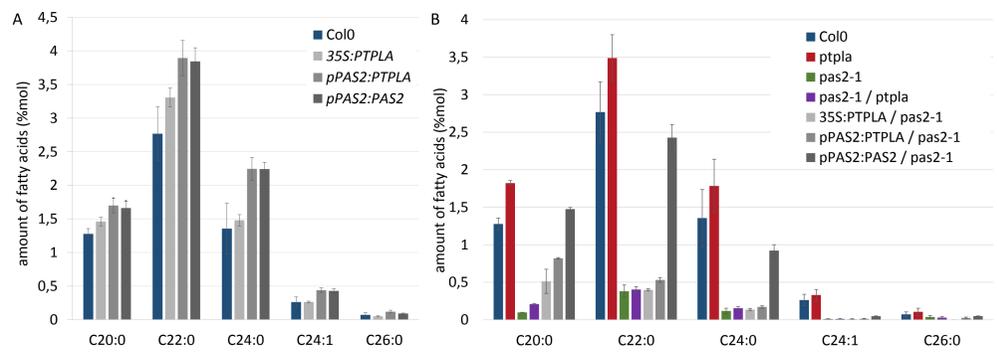
with an *A. thaliana* cDNA library was carried out in *phs1* strain. Loss of function of *PHS1*, the yeast *PAS2* ortholog, prevents growth and induces cytokinesis defects. The screen identified the *PTPLA* as a dehydratase-like gene involved in VLCFA elongation in both yeast and plant. Functional analysis of *PTPLA* in Arabidopsis demonstrated the existence of a new fatty acid elongase complex activity independent of *PAS2*-based complex but also uncovered unsuspected regulatory interactions between the two complexes.

1) Arabidopsis thaliana PTPLA restores VLCFA elongation in yeast *Tet-PHS1* strain



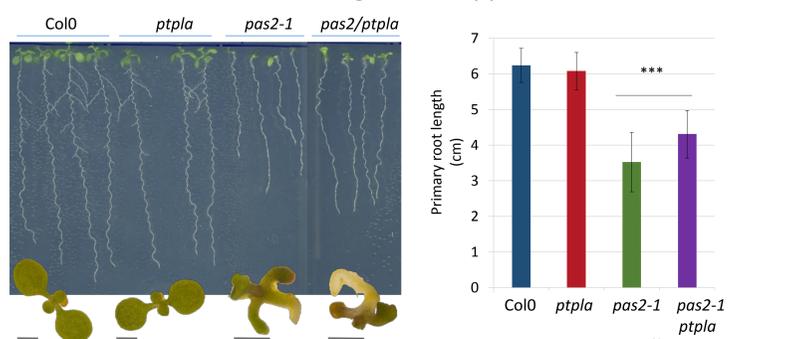
(A) *PTPLA* expression restores growth of *Tet-PHS1* in presence of doxycycline (DOX). *Tet-PHS1* yeast strain was transformed with yeast expression vector pFL61 alone or with yeast *PHS1*, Arabidopsis *PAS2* or *PTPLA*. R1158 is the wild type control strain. (B) *PTPLA* expression induces fatty acid elongation in yeast *Tet-PHS1* in presence of DOX.

2) *PTPLA* expression enhances VLCFA contents in Arabidopsis thaliana



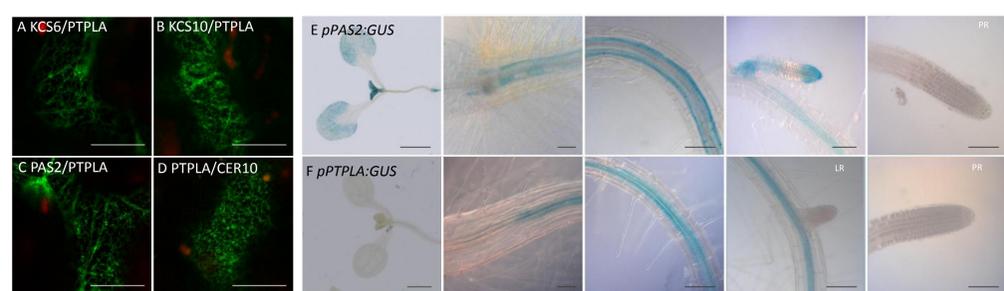
Relative fatty acid content of wild-type Col0 (A) and *pas2-1* and *ptpla* mutants (B) lines expressing 35S:*PTPLA* and *pPAS2:PTPLA* constructs. FAMES analysis were performed on roots of 14 days-old seedling.

3) Absence of *PTPLA* increases root length in leaky *pas2-1* mutant



The *pas2* mutants show strong developmental defects such as tumorous-like phenotype and reduction of primary root length [3,4]. The figures show the effect of *ptpla* mutation of primary root length. Vertically grown 10 day-old seedling (top) and the corresponding primary root length

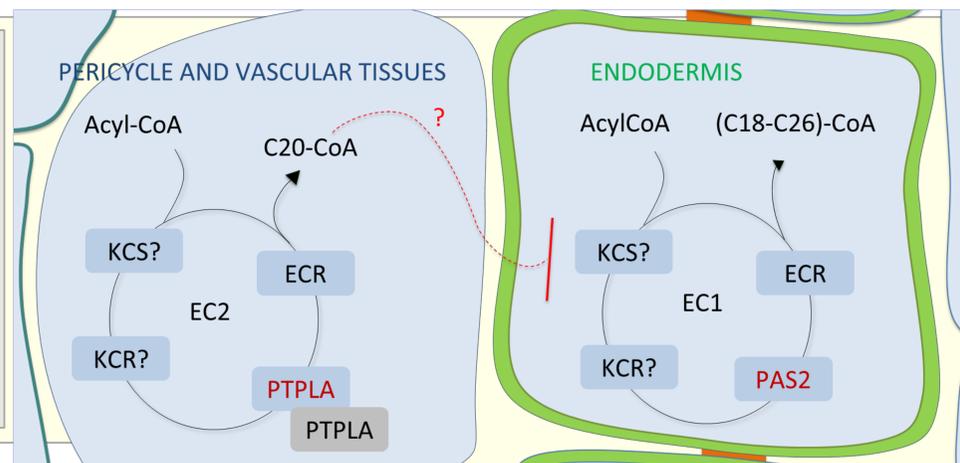
4) *PTPLA* interacts with EC members in the ER and is expressed in root.



(A-D) BiFC interactions in tobacco leaves. (E-F) *pPAS2:GUS* is present in the epidermis of leaves and cotyledon, endodermis and secondary roots whereas *pPTPLA:GUS* is restricted to inner tissues of the pericycle and/or vascular tissues.

CONCLUSION AND MODEL

PTPLA was able to restore yeast *phs1* growth and VLCFA elongation but not the plant *pas2* defects. *PTPLA* interacted with elongase members in the ER and its absence induced the accumulation of 3-hydroxyacyl-CoA (non shown) as expected from a dehydratase involved in fatty acid (FA) elongation. However, loss of *PTPLA* function led to increased VLCFA levels, effect that was dependent of the presence of *PAS2* indicating that *PTPLA* activity repressed FA elongation. The two dehydratases have specific expression profiles in the root with *PAS2*, mostly restricted in the endodermis, while *PTPLA* was confined in the vascular tissue and pericycle cells. Comparative ectopic expression of *PTPLA* and *PAS2* in their respective domains confirmed the existence of two independent elongase complexes comprising *PAS2* or *PTPLA* that were functionally interacting in a non-cell autonomous manner.



References

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Acknowledgements

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