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CY5 AND CY5-LIKE ARE INVOLVED IN THE DEVELOPMENT OF FUNCTIONAL CHLOROPLASTS IN ARABIDOPSIS

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1. CY5 and CY5-like are members of a small chloroplast-localised protein family with unknown function

The CY5 and CY5-like proteins are nuclear-encoded homologous soluble proteins (42% identity, 57% similarity) predicted to reside in the chloroplast/thylakoid lumen. Their function is unknown and their sequence does not contain any known domains or motifs which could give a hint about their role in the cell. The genes coding for CY5/CY5-like proteins are only found in land plants.

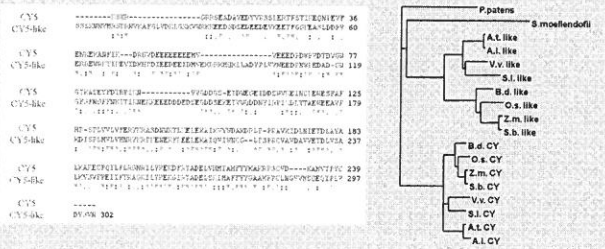


Figure 1. Left: Alignment of the *Arabidopsis* mature CY5 and CY5-like proteins. Signal peptide positions were predicted by ChloroP. **Right:** Phylogenetic tree based on the most conserved part of the CY5 and CY5-like proteins from different plant species.

2. Both CY5 and CY5-like are essential for chloroplast biogenesis

Deficiency in both CY5 and CY5-like leads to a seedling-lethal phenotype. The homozygous mutants can be grown heterotrophically on media supplemented with sucrose (Fig.2). Proteins, despite their homology, are not redundant.

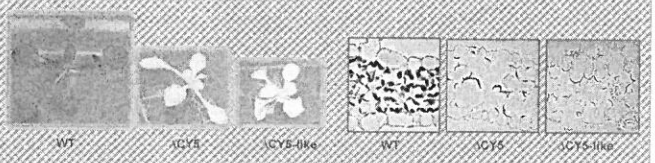


Figure 2. Left: Phenotype of the ΔCY5 and ΔCY5-like T-DNA lines. Plants were grown on ½-MS supplemented with sucrose for 4 weeks. **Right:** Light microscope photograph of a leaf section. The mutant plants clearly lack developed chloroplasts.

3. Amounts of plastid transcripts are greatly reduced whereas the expression of nuclear genes is unaffected

Deficiency in CY5/CY5-like has a very profound impact on plant fitness and many processes in the chloroplasts (and not only) are likely to be affected as a cause of the studied mutations. As a starting point, gene expression levels were analysed by qRT-PCR. Several nuclear- (*Lhcb1, RbcS, PsaD*, tubulin) and plastid-encoded (*psbA, rrn16S*) genes were selected for analysis (Fig.3). Reduced levels of plastid 16S rRNAs in the mutants suggest a severe drop in translation rate.

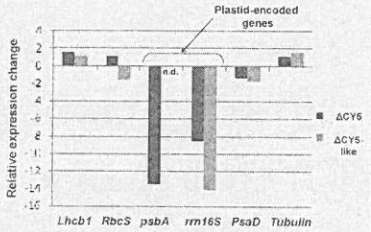


Figure 3. Gene expression in the mutant plants. The transcripts that we have analysed suggest that the gene expression in the plastid is compromised, whereas nuclear expression is not affected by chloroplast malfunction. Typical result of 4 repeats. More transcripts are upon investigation.

4. Thylakoid protein complexes are virtually absent in the mutants

In order to assess protein levels in the plastids of the mutant plants, Western blot analysis was performed (Fig.4). Major plastid protein complexes as well as Rubisco (composed of both plastid- and nuclear-encoded subunits) are barely detectable, whereas nuclear encoded polypeptides such as FNR and the enzymes of the chloroplyll biosynthesis pathway are present in the mutant at least in the wild-type amounts.

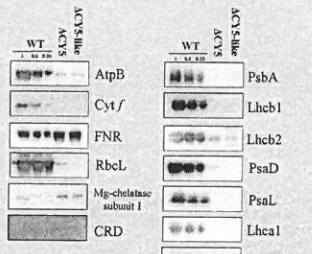
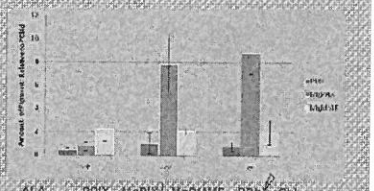


Figure 4. Western blot analysis. Major protein complexes are lacking in the thylakoid membrane of the mutants. A representative blots of three repeats.

5. Flow through the chlorophyll biosynthesis pathway is restricted

To investigate whether the chlorophyll biosynthesis pathway is affected in the studied mutants, the HPLC analysis of the pathway intermediates was performed. It showed that the mutant plants are able to synthesise the chlorophyll precursors, however there is a restriction in the pathway leading to accumulation of MgPPIX.

Figure 5. Analysis of precursors in the chlorophyll biosynthesis pathway. Plants were fed with ALA in order to increase the flow through the pathway. Extracted pigments were analysed by HPLC and normalised to the amount of protochlorophyllide (PChl). Average of three repeats.



6. Conclusions so far

- CY5 and CY5-like are members of a small protein family specific for land plants
- CY5 and CY5-like are essential for development of functional chloroplasts in *Arabidopsis*
- Deletion mutants of *CY5* and *CY5-like* have severely reduced levels of plastid transcripts. The latter is likely to contribute to a profound decrease in major thylakoid protein complexes observed
- Nuclear gene expression is not affected in the mutants suggesting a problem in retrograde signalling. Nuclear-encoded proteins plastid proteins are present in the mutants in at least wild-type level
- Chlorophyll biosynthesis pathway in the mutants is functional, and at least two of the enzymes are present in the plastid. However, the flow through the pathway is restricted at the level of MgPIX methyltransferase

7. On-going experiments

- EM of the chloroplast ultrastructure
- Localisation studies for CY5 and CY5-like proteins (import into isolated chloroplasts)
- Complementation of the mutation, generation of antisense lines
- Interaction partners (co-immunoprecipitation, transient expression of the tagged proteins in tobacco)
- RT-PCR, western blotting, chlorophyll biosynthesis