CY5 and CY5-like proteins are important at an early stage of chloroplast biogenesis

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Plant chloroplasts evolved from a cyanobacterial ancestor after a single endosymbiotic event, which was followed by an extensive reduction of the plastid genome size. This has caused a limiting coding capacity of the plastid genome and most of the chloroplast proteins that have a structural or regulatory function are encoded in the nucleus and synthesized in the cytosol as precursors.

The CY5 and CY5-like proteins are nuclear-encoded homologous soluble proteins predicted to reside in the thylakoid lumen. Deficiency in either CY5 or CY5-like proteins leads to a seedling-lethal phenotype and homozygous knockouts of these proteins result in mutants with a pigment deficient phenotype that can only be grown on media supplemented with sucrose.

HPLC analysis of the chlorophyll biosynthesis pathway intermediates showed that the mutant plants are able to synthesise all the chlorophyll precursors. However, there is a restriction in the accumulation of Mg Protoporphyrin monomethyl ester (MgPME). This can be explained by the decreased level of the enzyme – MgProtoporhyrin IX methyltransferase, which is a membrane associated enzyme that catalyse the conversion of Mg Protoporphyrin IX to its methylester.

Ultrastructure analysis showed that mutant plants do not have fully differenciated chloroplasts. In the young CY5 and CY5-like mutant plants the initial thylakoid membrane formation is observed. However, further thylakoid formation is arrested at an early stage.
Expression level of investigated nuclear genes, coding for chloroplast proteins, is not affected. In contrast, both mutants have severely reduced levels of plastid encoded polymerase (PEP) dependent plastid transcripts. In contrast, nuclear encoded polymerase (NEP) dependent plastid transcripts are not negatively affected.

Additionally, analysis of protein levels in the mutant plants was performed. Nuclear encoded components of the photosynthetic complexes are barely detectable whereas nuclear encoded proteins with functions not related to photosynthesis are present in amounts comparable to wildtype. The fact, that some chloroplast encoded proteins are detected, suggets that plastidic translation machinery is present and functional.

To conclude, deficiency in CY5/CY5-like has a very profound impact on plant fitness and many processes in the chloroplasts suggesting that both proteins play an important role in early stages of chloroplast biogenesis, probably at a specific step in thylakoid biogenesis. Most likely, lack of CY5/CY5-like proteins affects the activity of the plastid encoded polymerase (PEP) and thereby preventing normal thylakoid formation.

Poster