Rapid isolation of plant mitochondria from *Arabidopsis thaliana* for metabolite analyses and proteomics

Franziska Kuhnert[1] and Andreas P. M. Weber[1]

1. Plant Biochemistry, Heinrich-Heine University, Duesseldorf, Germany

Studying mitochondrial metabolism is a key factor in understanding many processes within the plant. However, isolation of a sufficient amount of intact mitochondria is still a rather challenging process. A variety of protocols have been established to address this matter. Most of them are based on differential centrifugation and density gradients. Generally, such protocols yield a sufficient amount of isolated mitochondria, but they are rather time-consuming as the procedure takes several hours and requires a large quantity of biological material.

Recently, Chen and colleagues reported a rapid isolation of intact mitochondria from HeLa cells using co-immunoprecipitaion of a transgenic 3xHA-eGFP-OMP25 fusion protein.

To adapt and optimize this method for tissues of *Arabidopsis thaliana*, I fused the outer mitochondrial membrane protein TOM5 N-terminally with GFP and a 3xHA-tag followed by stable transformation of *Arabidopsis* plants. Transgenic lines that show stable expression of the 3xHA-GFP-TOM5 construct in mitochondria could be isolated and were used for co-immunoprecipitation experiments showing a significant enrichment of intact mitochondria compared to wild-type plants.

This adapted method allows to specifically enrich intact mitochondria from different *Arabidopsis thaliana* tissues in less than 25 minutes. Additionally, we could reduce the amount of starting material to less than 3 g. Isolated mitochondria show activities for different mitochondrial enzymes, such as malate dehydrogenase, aspartate aminotransferase and GABA transaminase, and contain metabolites such as malate.

The described 3xHA-GFP-TOM5 transgenic plants will serve as basis for further proteomic and metabolite analyses regarding mitochondrial metabolism in plants.