Characterization of chloroplast protein import in *Arabidopsis thaliana* with emphasis on Toc64 and Tic55

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Abstract: Although the chloroplast possesses a genome of its own most genes have been transferred during the evolution to the nucleus to acquire more transcriptional control, and couple the activities of the whole cell to events at the chloroplast. It is estimated that approximately 95 % of all proteins found in the chloroplast are transcribed from nuclear genes. This means that the proteins translated on ribosomes in the cytosol must subsequently enter the chloroplast by some means. The majority of proteins destined to the chloroplast carry an N-terminal address tag known as the transit peptide (TP) that directs the protein to its proper location. This TP is recognized by receptors at the chloroplast outer envelope membrane. These receptors are part of the TOC/TIC (Translocon at the Outer/Inner envelope membrane of Chloroplast) complex mediating chloroplast protein import, which also consists of membrane spanning channels allowing the protein to enter the chloroplast stroma. The process is not spontaneous and requires energy in the form of GTP and ATP. The stepwise translocation process permits a high degree of regulation and control over the translocation process at the inner and outer envelope membrane. Translocation occurs in a concerted manner making sure transport is a unidirectional process. The number of identified TOC/TIC components in the import machinery is currently around 20. In the last year chloroplast protein import has been the subject of much research and the major components and their functions have been characterized. Nevertheless, some of the components still have unclear functions and in some cases the proposed function is supported by a relatively small amount of experimental data.

The Toc64 protein, first discovered in pea (*Pisum sativum*), was proposed to function as a receptor in the chloroplast outer envelope membrane and to interact with the TP of incoming preproteins together with molecular chaperones. In addition it was later proposed that Toc64 recruits other components in the intermembrane space and that the receptor function may only be valid for certain incoming preproteins. In *Arabidopsis thaliana* three homologs (referred to as Toc64-III, Toc64-V and Toc64-I where the roman number indicates chromosome location) for the pea Toc64 exist. We examined a triple *toc64-III/V/I* mutant and compared it to wild-type plants. In all aspects measured the mutant plants were indistinguishable from wild-type plants. Furthermore, import of various preproteins was not affected by the mutation. These findings indicate that Toc64 is not vital for chloroplast protein import in Arabidopsis. Toc64-I did however display a subtle phenotype that could possibly be attributed to altered auxin levels but further analysis of the mutant plants is required.

The Tic55 protein of the inner envelope membrane has been proposed to function as a redox sensing component linking chloroplast protein import to the energetic state of the cell. However this claim is largely based on assumptions and an experiment conducted with diethylpyrocarbonate (DEPC), a chemical suggested to specifically target the Tic55 component. In Arabidopsis the specific effect of DEPC could not be repeated. It can not be ruled out that DEPC acted on multiple targets since an effect was observed that was not mutant specific. We therefore conclude that Tic55 is not vital for chloroplast protein import and that if Tic55 acts as a redox sensor it is probably of minor importance. We also examined the Tic55 homolog Protochlorophyllide (Pchlide)-dependent Translocon Component of 52 kDa (PTC52). PTC52 is believed to constitute a separate translocon that specifically import the NADPH:Pchlide oxidoreductase A (pPORA) protein and in this way form a substrate dependent pathway. Mutating PTC52 did not have an effect on import of various preproteins and more importantly the import of pPORA was not affected. Thus, there is no evidence for a substrate dependent pathway in Arabidopsis. Finally, a proteomic investigation of chloroplasts from the *tic55-II* and *toc64-III/V/I* mutants supported their roles as non-essential components of the chloroplast import machinery since only a handful proteins were significantly up/down-regulated. Further experiments are required to fully elucidate the exact roles of Toc64 and Tic55 in Arabidopsis.

Keywords: *Arabidopsis thaliana*, chloroplast import, non-essential receptor, Toc64, redox control, Tic55, DEPC, substrate dependent pathway, PTC52, proteomics

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This thesis is based on the following papers, which are referred to by their Roman numerals

(I) Aronsson H, Boij P, Patel R, Wardle A, Töpel M, Jarvis P (2007) Toc64/OEP64 is not essential for the efficient import of proteins into chloroplasts in *Arabidopsis thaliana*. Plant J 1: 53-68*

(II) Boij P, Patel R, Garcia C, Jarvis P, Aronsson H (2009) In vivo studies on the roles of Tic55-related proteins in chloroplast protein import in *Arabidopsis thaliana*. Mol Plant doi: 10.1093/mp/ssp079*

(III) Boij P, Björk RG, Aronsson H (2009) The *toc64-1* (*ami1*) mutant line in *Arabidopsis thaliana* shows a root morphology phenotype. (Manuscript)

(IV) Boij P, Aronsson H (2009) Proteomic analysis of chloroplasts from *toc64-III/V/I* and *tic55-II* mutant plants. (Manuscript)

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Abbreviations

AKR2A	ankyrin repeat protein
ceQORH	Quinone Oxidoreductase Homologue
DEPC	diethylpyrocarbonate
DGDG	digalactosyldiacylglycerol
ER	endoplasmatic reticulum
GFP	green fluorescent protein
MGDG	monogalactosyldiacylglycerol
Pchlide	protochlorophyllide
pSS	preprotein of the small subunit of Rubisco
PTC52	Protochlorophyllide-dependent Translocon Component of 52 kDa
SRP	signal recognition particle
TIC	Translocon at the Inner envelope membrane of Chloroplasts
TOC	Translocon at the Outer envelope membrane of Chloroplasts
TP	transit peptide
TPR	tetratricopeptide repeat motif