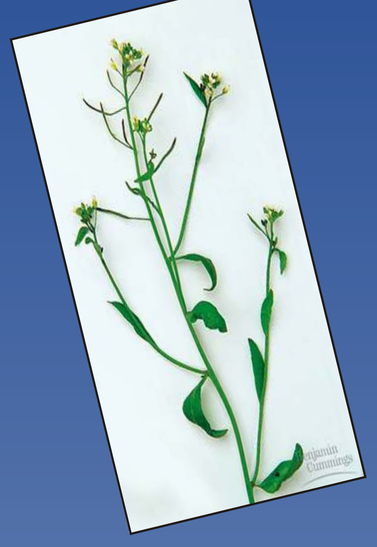

Protein import into chloroplasts- Redoxregulation at the TIC-Complex

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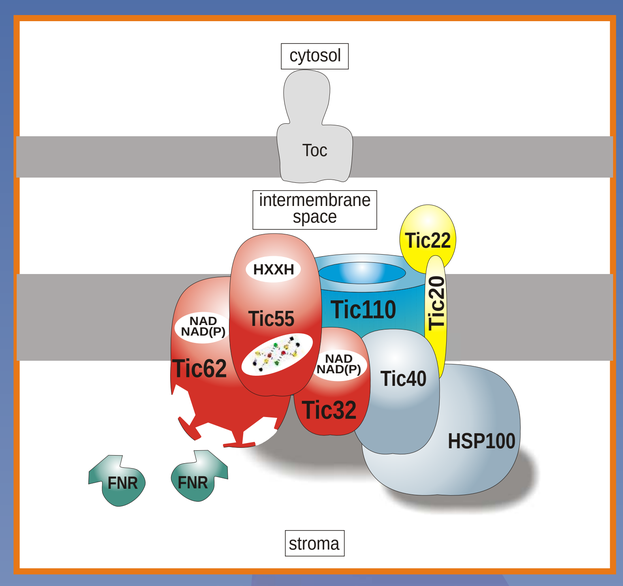
Introduction

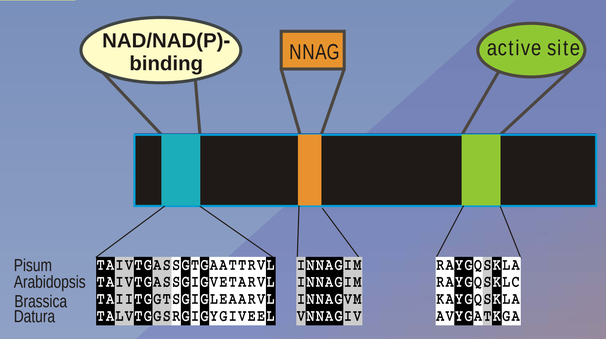
The vast majority of chloroplast proteins is translated in the cytosol and posttranslationally imported into the organelle.
It could be shown that the import process is regulated on different levels via different means: e.g. phosphorylation of precursor proteins in the cytosol, nucleotide (GTP, ATP) dependent control of precursor binding and translocation at the outer envelope translocon (Toc-complex) and finally redox control at the inner envelope translocon (Tic-complex).
With Tic55, a protein containing a Rieske iron-sulfur cluster and an iron-binding site, and a new component Tic62, containing a NAD(P)+-binding site and a FNR binding site, we found two candidates that implicate regulation of import over the inner envelope in a redox dependent manner.
Recently we found a 32 kDa protein at the inner envelope that shows high homologies to SDH (short chain dehydrogenases) with the typical NAD(P)+-binding site, the catalytical site and a domain with so far unknown function. It strongly interacts with Tic110 and it could also be co-immunoprecipitated with other Tic-components. Therefore we called it Tic32. We are now studying the role of Tic32 during protein import and since Tic 32 seems to be another dehydrogenase, we want to find out whether it is involved in regulation of protein import together with Tic55 and Tic62.



Model of the Tic complex

Tic32

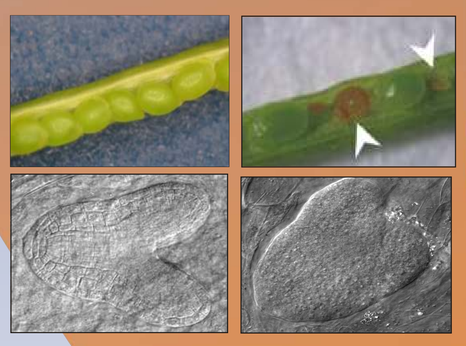
Motif



Tic32 was identified by affinity chromatography as the sole interaction partner of the N-terminus of Tic110.
Tic32 belongs to a family of short chain dehydrogenases (SDR). The family of SDR's is highly divergent, but most of them contain the N-terminal (NAD(P) coenzyme-binding pattern GXXXGXG, the highly conserved NNAG sequence of unknown function, and the C-terminal enzyme active site YXXXK.

Phenotype

Arabidopsis thaliana Tic32 knock-out plants are embryo-lethal

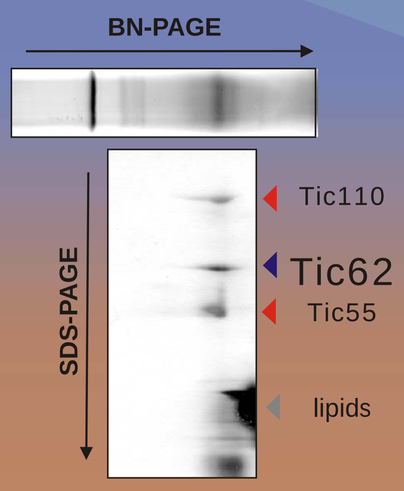


WT ΔTic32

The embryo-lethal phenotype is associated with Tic32. An immature silique from a wild-type plant showed only green seeds, whereas heterozygous siliques from Tic32 plants segregated green and white seeds, the latter turning purplish/brown in older siliques. To characterize the nature of seed lethality associated with Tic32, wild type mutant seeds were cleared in Hoyer's solution and observed with Normarski optics. Mutants show aberrant cell division and no later stage than the heart stage was found.

Tic62

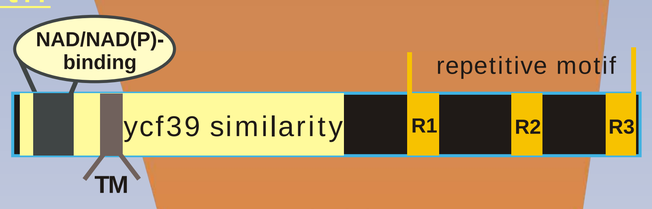
Identification



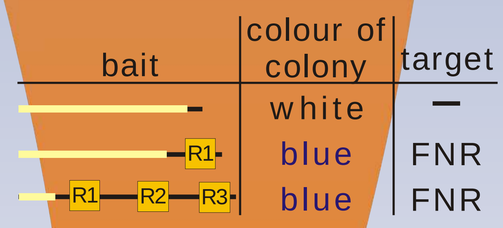
A core Tic complex

The molecular composition of a core translocation complex of the inner envelope membrane was examined by Blue-Native gelelectrophoresis . The composition of the isolated complex was further verified by SDS-PAGE). The purified core translocation complex consists only of Tic110, Tic55 and an unknown 60kDa-protein (Tic62). Tic 40, Tic22 and Tic20 can not be detected as subunits of this core complex.

Motif



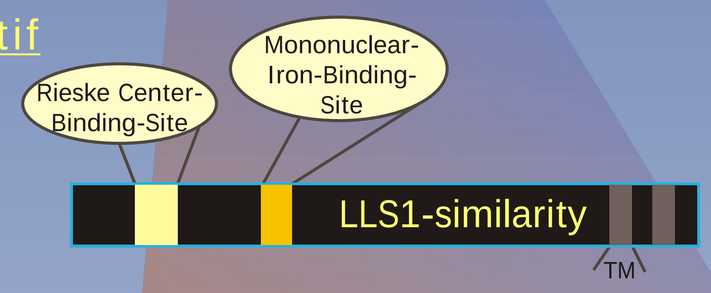
Two Hybrid-Screen



The C-terminal repeats mediate interaction of Tic62 with a stromal Ferredoxin-NAD(P) Oxidoreductase (FNR) as shown by the yeast Two-Hybrid system and affinity chromatography.

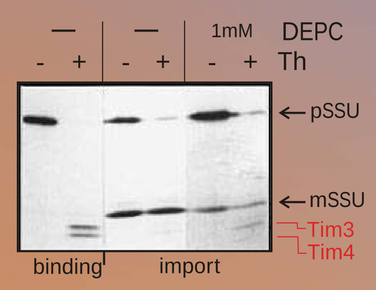
Tic55

Motif



Tic55 has been shown to be localized in the inner envelope of chloroplasts. It contains two putative membrane spanning a-helices, a Rieske-type iron-sulfur-cluster and a mononuclear-iron-binding-site. The primary structure of Tic55 shows highest homology to LLS1 from maize.

Import



Import of pSSU is inhibited by DEPC

DEPC modifies histidine residues of Rieske iron-sulfur clusters and thereby abolishes electron transfer.
Intact chloroplasts were treated with DEPC after binding of precursor protein to the outer envelope membrane. pSSU binding was permitted at low temperature in the presence of 3mM ATP. Under these conditions pSSU partially enters the translocation machinery as demonstrated by the appearance of Tim3 and Tim4 upon thermolysin treatment. After increasing the temperature pSSU is completely translocated into the organelle. There, it is processed to the mature form and becomes resistant to thermolysin. Much less pSSU could be chased into the mature form if the chloroplasts were treated with DEPC.
The appearance of Tim3/4 indicates that pSSU translocation is inhibited at the stage of TIC. These results suggest that DEPC exerts its function at the inner envelope, namely the Rieske-sulfur protein Tic55.

Working hypothesis for regulation of plastid protein import

Conclusion

The Tic Complex contains three proteins which exhibit features indicative of redox regulation: The Rieske-type protein Tic55 and the two dehydrogenases Tic32 and Tic 62.

Accordingly, we believe that the translocation process across the chloroplast inner envelope membrane is regulated via redox mediated processes.