Copper Chaperones in Arabidopsis thaliana: Intra Cellular Copper Trafficking: Uptake, Delivery and Regulation

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Introduction: Cu and chloroplasts

The chloroplast is the site of photosynthesis. Copper is an essential element to chloroplast function as a co-factor of superoxide dismutase (SOD) and plastocyanin, which function in photosynthesis. Chloroplasts have a complex structure due to the presence of three membranes, so how is Copper delivered throughout the complex internal structure of plant chloroplasts? In plant chloroplasts, membrane transporters have been identified that transport Copper across these membranes. In microalgae, a family of small cytoplasmic proteins called "metallochaperones" or Copper Chaperones carry out the delivery of copper from transporters to targets. These chaperones bind to copper and insert the copper ions into an active site of a specific partner, a copper dependent enzyme or another transporter. In the genome of Arabidopsis thaliana possible genes encoding for copper chaperones have been identified based on the similarity of sequences with microbial copper chaperones. We named these, CpCCS (Chloroplastic Copper Chaperone for SOD), A.TX2 (similar to yeast Antioxidant protein) and CpCopZ. The three A. complementation knockout mutants have been obtained and analyzed by some experimental data.

Fig 1: Metal ions in photosynthesis

Copper is a co-factor of plastocyanin and required for photosynthetic electron transport.

Fig 3: Arabidopsis thaliana our model plant species

Three possible Cu-chaperones were identified. How do these function?

Search for T-DNA insertions in ATX2, CpCopZ and CpCCS

Fig 6: T-DNA KO searches for ATX2, CpCopZ and CpCCS. T-DNA lines were obtained from the SALK collection. The chromosomal DNA is indicated for each gene with arrows. The triangles represent the insertion of T-DNA by Agrobacterium. The primers to test by PCR if the insert is present are indicated with arrows. The inserted pictures show PCR products visualized on ethidium bromide stained agarose gels.

Two approaches are taken:

1) Complementation of yeast mutants,
2) Analysis of plant T-DNA insertion mutants in which each gene is inactivated.

Fig 7: Native gel assay for SOD activity in yeast. In the WT yeast the Ccs protein delivers Cu to SOD, which becomes active. The ccs yeast mutant (Δccs) with the empty vector does not have the SOD activity. The ccs yeast mutant with the vector and the YNCCS insert shows SOD activity, indicating Cu delivery to yeast SOD by the plant Ccs.

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Fig 2: Working model for copper transport in a plant cell. Copper routing is indicated by arrows.

Proteins that require Cu are indicated as grey boxes and membrane transporters of known function are indicated as blue circles. Copper enters the cell by a Cpt transporter. In the cytoplasm Atx2 may collect Cu and deliver it to the Paa1 transporter to deliver Cu to the chloroplast stroma. We hypothesize that CpcCs can take Cu from Paa1 to Csd2 (superoxide dismutase) to relieve oxidative stress in chloroplast. CpCpZ may be a chaperone to deliver Cu to Paa2 and plastocyanin.

Fig 4: Complementation of a yeast ccs/lys7 mutant with A.rCcCS

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