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

Jared Bodecker, Salah Abdel-Ghany, Jason Burkhead and Marinus Pilon

Biology department, Colorado State University, Fort Collins, CO 80523, USA;

Introduction: Cu and chloroplasts

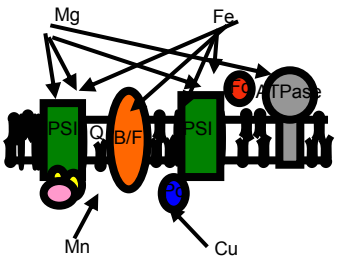


Fig 1: Metal ions in photosynthesis Copper is a co-factor of plastocyanin and required for photosynthetic electron transport

Fig 3 *Arabidopsis thaliana* our model plant species.
Three possible Cu-chaperones were identified. How do these function?

Two approaches are taken:
1) Complementation of yeast mutants

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

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 then 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 microbes, a family of small cytoplasmic proteins called "metallochaperones" or Copper Chaperones carries 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 chaperones. We named these, *CpCCS* (Chloroplastic Copper Chaperone for SOD1), *ATX2* (similar to yeast Antioxidant protein) and *CpCopZ*. The three *A. thaliana* Cu Chaperone proteins may be required for Cu transport to the internal compartments of the chloroplast. The location of these proteins has been determined by *GFP*-fusions.To test the function of the putative copper chaperones in copper delivery two approaches are taken: 1) complementation of yeast mutants, deficient in copper chaperones and 2) analysis of the function and need for these proteins in plants with insertions (KO-mutants) in each gene.

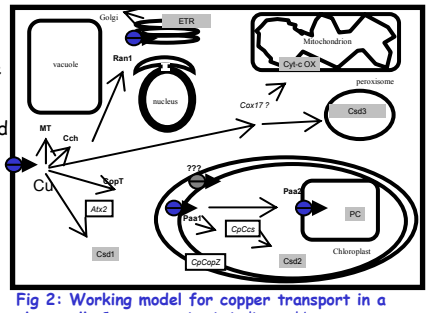


Fig 2: Working model for copper transport in a plant cell. Copper routing is indicated by arrows. Proteins that require Cu are indicated as grey boxes and membrane transporters of known function are indicated as blue circles. Copper enters the cell by a CopT transporter. In the cytoplasm Atx2 may collect 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 relief oxidative stress in chloroplast. CpCopZ may be a chaperone to deliver Cu to Paa2 and plastocyanin.

Overview of Gene insertion into a plasmid vector (pFL61) for yeast complementation

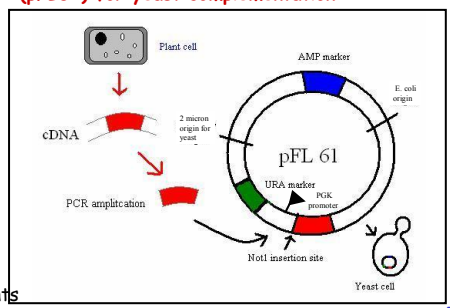
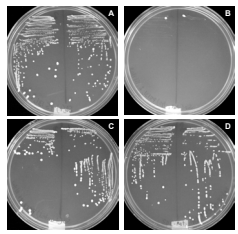


Fig 3 plasmid construction for complementation. We inserted the plant Cu chaperone genes into the pFL61 vector and transformed a yeast mutant (KO of Cu chaperone gene in yeast) with the plasmid. (Some results of this process are in the next pictures to the right.)

Complementation of a yeast *ccs/lys7* mutant

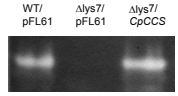
Complementation of PS131 (△lys7) mutant with *AtCpCCS*



A) PS131(△lys7)/pFL61 on minimal medium
B) PS131(△lys7)/pFL61 on –Met, -Lys medium
C) PS131(△lys7)/CpCCS(19) on minimal medium
D) PS131(△lys7)/CpCCS (19) on –Met, -Lys medium

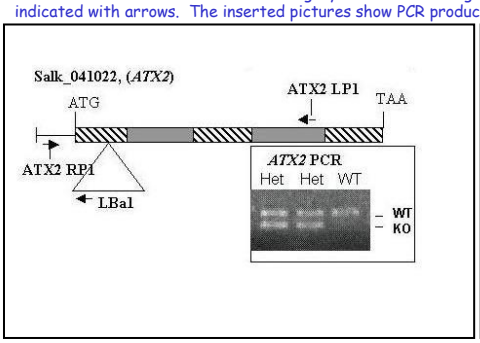
Fig 5: Native gel assay for SOD activity in yeast. In the WT yeast the Ccs protein delivers Cu to SOD, which becomes active. The *ccs* yeast mutant *(△lys7)* with the empty vector does not have the SOD activity The *ccs* yeast mutant with the vector and the *CpCCS* insert, shows SOD activity, indicating Cu delivery to yeast SOD by the plant Ccs.

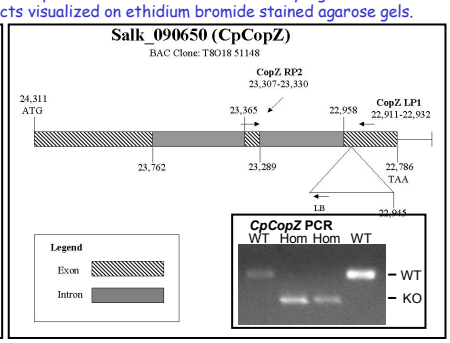
Fig 4: Complementation. The PS131(*△lys7/ccs*) yeast mutant is deficient in Cu delivery to SOD, a reactive oxygen species scavenging enzyme. This makes cells hyper-sensitive to oxidative stress and auxotrophic for lysine and methionine, because enzymes in the lysine and methionine biosynthesis pathways are highly sensitive to oxygen radicals. Plant CCS rescues the growth on minus lys/met media, empty pFL61 does not.

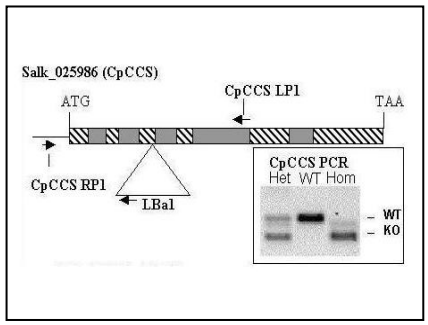


Search for T-DNA insertions in *ATX2*, *CpCopZ* and *CpCCS*

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 exons as hatched boxes and introns as grey boxes. 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.







Summary and Discussion

We have identified 3 putative Cu chaperones and started a functional analysis. By the transformation of yeast mutants with plant Cu chaperones genes, we can analyze the Cu transporting activity of each single protein. CpCcs functions as a Cu chaperone for SOD proteins. This is only a first step towards the overall goal of knowing the exact processes of how Cu enters the plastid, and how it is delivered and regulated by these Cu chaperones and other proteins involved in Cu homeostasis.

With the analysis of different plant lines supplied by the SALK Institute, we were able to find both heterozygous and homozygous KO's for the three Cu Chaperones genes that may involved in Cu homeostasis in plant chloroplasts. Since the homozygous KO lines are viable, the genes are not essential under the tested growth conditions (well fertilized soil, high Cu). Similar results were found previously for *paa1* and *paa2* null mutants. With these KO's we can analyze models of Cu delivery and homeostasis by comparing photosynthesis, the activity of Cu proteins, and growth between wild type and single/double mutants. By altering the Cu supply, we can analyze the physiological and biochemical responses of the plastid regulation of Cu. With the full understanding of how metal ions are transported and regulated, we maybe able to increase plant productivity, and nutritional value and improve metal tolerance and/or accumulation properties of plants.

Acknowledgement:This work was supported by NSF grant # MCB-0091163 to MP