IN BRIEF

Global Analysis of Copper Responsiveness in *Chlamydomonas*

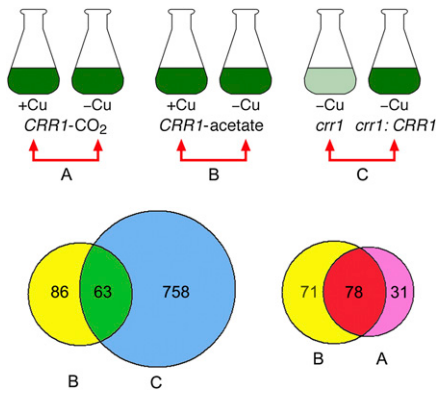
Aerobic organisms require copper due to its key roles in oxygen-requiring chemical reactions. Copper ions aid electron transfer to molecular oxygen during aerobic respiration, act as cofactors in superoxide dismutases, and are an essential component of plastocyanin, an electron carrier in oxygenic photosynthesis. Previous work by Eriksson et al. (2004) identified COPPER RESPONSE REGULATOR1 (CRR1) as a key regulator of copper homeostasis in *Chlamydomonas*. In copper-deficient conditions, CRR1 activates copper sparing pathways to conserve copper. For example, it downregulates the copper-containing plastocyanin protein and upregulates the iron-containing cytochrome c_6 protein that can serve as its replacement.

Castruita et al. (pages ■■■) used high-sensitivity global transcriptomic, proteomic, and lipidomic methods to identify *Chlamydomonas* genes regulated by copper, test whether mRNA abundance correlates with protein abundance, and examine the role of copper nutrition in the modification of thylakoid membrane lipids. By identifying differentially expressed transcripts in pairwise combinations of conditions (see figure) they defined a nutritional copper regulon and distinguished between primary responses to copper limitation versus secondary responses due to reduced growth rate as a result of limiting copper.

Using digital gene expression tag profiling ('t Hoen et al., 2008) and RNA sequencing (Nagalakshmi et al., 2008), they reproducibly identified distinct sets of differentially regulated genes with expression levels as low as 1 mRNA per cell and showed that any variance was independent of the method used. Using an iterative alignment protocol, over 90% of expressed sequences were aligned uniquely to the *Chlamydomonas* genome. Real-time PCR conducted in parallel gave good correlations (0.88 and 0.97) with these results.

Among differentially expressed CRR1 targets, redox proteins were overrepresented. For phototrophic cells, copper-responsive expression was seen in copper transport proteins and in several proteins involved in iron homeostasis, highlighting the role of copper in iron assimilation. RNA and protein abundance for fatty acid desaturases also increased in copper limiting conditions, and lipid analysis showed an increase in polyunsaturated fatty acids, especially in digalactosyldiacylglyceride, a major thylakoid lipid. The authors then used elevated liquid chromatography mass spectrometry (LC-MS^E; Silva et al., 2006) to identify and quantify over 1000 proteins. For 17 of 18 proteins corresponding to differentially expressed transcripts, the change in protein abundance paralleled the change in mRNA abundance.

The authors discuss the technical and biological advantages of these methods, validate their methods and analysis pipeline with simulation studies, and present a comprehensive model for copper allocation under copper limiting conditions. This work not only advances our understanding of copper biology in *Chlamydomonas* but also provides a general strategy to analyze the global response to essential nutrients and strategies for survival under nutrient-limiting conditions.



Top: Pairwise comparisons used to identify differentially expressed *Chlamydomonas* transcripts: presence versus absence of copper during growth of wild-type cells either autrophically **(A)** or heterotrophically **(B)** and presence versus absence of CRR1 in copper-deficient conditions using the complemented *crr1* mutant **(C)**. Bottom: The numbers of unique and shared transcripts in these comparisons. (*Adapted from Figure 2 of Castruita et al. [2011].*)

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