[Plant Signaling & Behavior 3:4, 231-232; April 2008]; ©2008 Landes Bioscience

Article Addendum

How do plants respond to copper deficiency?

Hiroaki Yamasaki,[1] Marinus Pilon[2] and Toshiharu Shikanai[1,*]

[1]Graduate School of Agriculture; Kyushu University; Fukuoka, Japan; [2]Biology Department and Program in Molecular Plant Biology; Colorado State University; Fort Collins, Colorado USA

Key words: copper, iron, microRNA, superoxide dismutase, plastocyanin, chloroplast

The transition metal copper is essential for all organisms yet excess copper is toxic because of production of free radicals via its free form. Therefore, the levels of copper are precisely regulated in a cell. Under copper depleted conditions, the expression of Cu/Zn superoxide dismutase (SOD) is downregulated and its function is compensated by Fe SOD in chloroplasts of higher plants. We presented evidence that a microRNA, *miR398*, is involved in this downregulation of Cu/Zn SOD genes in *Arabidopsis thaliana* when grown at low copper levels, corresponding to less than 1 μM Cu in tissue culture media. However, a green alga, *Chlamydomonas reinhardtii*, adjusts to copper depletion by modifying the photosynthetic apparatus from copper containing plastocyanin to iron containing cytochrome c_6. During evolution plants modified one of the main strategies to respond to copper deficiency probably to adapt to different metal environments.

Copper is an essential micronutrient and is involved in several metabolic processes.[1] In higher plants, the most abundant copper protein is plastocyanin, which is involved in the photosynthetic electron transport in the thylakoid lumen of chloroplasts. Another major copper protein, Cu/Zn SOD, localizes to the cytoplasm (CSD1), stroma of chloroplasts (CSD2) and also peroxisomes (CSD3), and is involved in the scavenging of reactive oxygen species.[2] Despite its physiological importance, excess copper is toxic for plants because of its potential participation in the Fenton reaction. To minimize the damage by excess copper and also respond to copper deficiency, higher plants have several strategies, including the regulation of copper uptake in root cells,[3] strict copper trafficking via P-type ATPases and copper chaperones[4-9] or regulation of the levels of copper proteins in response to a change in the metal availability.[10,11] In addition plants respond to copper deficiency by expressing the alternative iron proteins which complement the function of copper proteins.[12]

*Correspondence to: Toshiharu Shikanai; Kyushu University; 6-10-1 Hakozaki; Higashiku, Fukuoka 812-8581 Japan; Tel./Fax: +81.92.642.2882; Email: shikanai@agr.kyushu-u.ac.jp

Submitted: 09/25/07; Accepted: 09/27/07

Addendum to: Yamasaki H, Abdel-Ghany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M. Regulation of copper homeostasis by micro-RNA in Arabidopsis. J Biol Chem 2007; 282:16369–78; PMID: 17405879; DOI:10.1074/jbc.M700138200.

Among photosynthetic eukaryotes, the unicellular green alga, *Chlamydomonas reinhardtii*, is best studied with respect to the molecular mechanism of copper homeostasis.[13] Under low copper conditions, Chlamydomonas degrades plastocyanin and its function is replaced by cytchrome c_6 containing iron.[12] A transcriptional factor, Crr1, plays a pivotal role in switching the photosynthetic machinery and probably also in copper sensing.[14] However, higher plants are unlikely to encode the functional homolog of the algal cytochrome c_6.[15] How do higher plants respond to copper deficiency?

In Arabidopsis, the expression of *CSD1* and *CSD2* is downregulated in copper deficient conditions.[5] MicroRNA, *miR398*, is involved in this downregulation of both Cu/ZnSOD genes.[10] While microRNAs extensively regulate a variety of developmental processes,[16] they are also involved in responses to the environmental stresses including deficiency in sulfur[17] and phosphate.[18] *miR398* is expressed only in low copper conditions[10] and directly involved in the degradation of *CSD1* and *CSD2* mRNA.[10,19] Consequently, this regulation allows limited copper to be preferentially transferred to plastocyanin whose expression is independent of the regulation via *miR398*. Even in the absence of the functional homolog of algal cytochrome c_6, higher plants can sustain photosynthesis in the low copper conditions. Meanwhile, the function of CSD2 is replaced by Fe SOD (FSD1). This switching between Cu/Zn SOD and Fe SOD takes place at the range of 0.1–1 μM $CuSO_4$ in agar-solidified medium.[10] Arabidopsis seedlings grown in the soil used in our laboratory express both Cu/Zn and Fe SODs (data not shown), suggesting that the soil contains copper ion equivalent to the range of 0.1–1 μM in the medium. Arabidopsis seedlings can perceive a change in copper concentration in the physiological range for maintaining copper homeostasis. The rice and poplar genomes encode microRNA which is homologous to *miR398*, suggesting a conserved mechanism by which higher plants adapt to copper deficiency.

During evolution from algae to higher plants, one of major strategies to adapt to copper deficiency was altered from the system switching plastocycnin to cytochrome c_6 to that switching Cu/Zn SOD to Fe SOD. We are interested in the strategy in a moss, *Physcomitrella patens*, which is a model plant of the primitive land plants. Based on the genome information (genome.jgi-psf.org//Phypa1_1/Phypa1_1.home.html), the *P. patens* genome encodes at least three Cu/Zn SODs, two chloroplast forms and one cytosolic form. Expression of these CSD genes was analyzed in *P. patens* cultured on the agar medium containing various concentrations of $CuSO_4$ (Yamasaki et al., unpublished data). Both RT-PCR and protein blot analysis indicate that the gene expression is differently regulated across the boundary existing between 0.05 μM and 0.1 μM $CuSO_4$. In contrast, the plastocyanin gene is constitutively expressed at any copper concentrations, as in Arabidopsis. However, a genome wide analysis of microRNA has not identified the microRNA similar to Arabidopsis *miR398* in the *P. patens* genome. It is possible that the expression of *P. patens* CSDs is regulated at transcriptional level. The *P. patens* genome is unlikely to encode a functional homolog of algal cytochrome c_6, and plastocyanin may be essential for photosynthesis even under copper deficient conditions. Taken together all the information, *P. patens* is likely to respond to copper deficiency with the similar strategy of higher plants, rather than that of algae.

Information from three model plants may not be sufficient to conclude something definitively, but we hypothesize that plants acquired the higher plant-type strategy to respond to copper deficiency in the early evolution of land plants (Fig. 1). This is probably related to the evolutionary event that plastocyanin rather than cytochrome c_6 was fixed as an electron carrier for photosynthetic electron transport of land plants. Plastocyanin is also essential for the oceanic diatom *Thalassiosira oceanica*, although other coastal species lack plastocyanin and rely on the function of cytochrome c_6.[20] In the open sea, iron levels are very low and copper is relatively more available. The low availability of iron may have led to a selection for the use of plastocyanin in both the land plants and in *T. oceanica*. In the land plants, which can also express a Cu/Zn SOD this could have lead to the evolution of the regulation of SOD genes. Unlike Chlamydomonas, the land plants do not use a copper-dependent iron uptake system,[21] which is well conserved including animals, and this may allow plants to save copper for photosynthesis. How did the higher plants incorporate the function of microRNA into the regulatory process and how is the regulation via the microRNA related to the other strategies for metal homeostasis? Characterization of three model plants, Chlamydomonas, *P. patens* and Arabidopsis, may lead to answers to these questions.

Acknowledgements

This work was supported by grant-in-aids for Creative Scientific Research (17GS0316) and for Scentific Research on Priority Area (16085206) (to TS) and United States National Science Foundation Grant NSF-IBN-0418993 (to MP).

References

1. Pilon M, Abdel-Ghany SE, Cohu CM, Gogolin KA, Ye H. Copper cofactor delivery in plant cells. Curr Opin Plant Biol 2006; 9:256-63.
2. Bowler C, Van Camp W, Van Montagu M, Inzé D. Superoxide dismutase in plants. Crit Rev Plant Sci 1994; 13:199-18.
3. Sancenon V, Puig S, Mira H, Thiele DJ, Penarrubia L. Identification of a copper transporter family in *Arabidopsis thaliana*. Plant Mol Biol 2003; 51:577-87.
4. Shikanai T, Müller-Moulé P, Munekage Y, Niyogi KK, Pilon M. PAA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. Plant Cell 2003; 15:1333-46.
5. Abdel-Ghany SE, Müller-Moulé P, Niyogi KK, Pilon M, Shikanai T. Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. Plant Cell 2005; 17:1233-51.
6. Seigneurin-Berny D, Gravot A, Auroy P, Mazard C, Kraut A, Finazzi G, Grunwald D, Rappaport F, Vavasseur A, Joyard J, Richaud P, Rolland N. HMA1, a new Cu-ATPase of the chloroplast envelope, is essential for growth under adverse light conditions. J Biol Chem 2006; 281:2882-92.
7. Hirayama T, Kieber JJ, Hirayama N, Kogan M, Guzman P, Nourizadeh S, Alonso JM, Dailey WP, Dancis A, Ecker JR. RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transporter, is required for ethylene signaling in Arabidopsis. Cell 1999; 97:383-93.
8. Himelblau E, Mira H, Lin SJ, Culotta VC, Peñarrubia L, Amasino RM. Identification of a functional homolog of the yeast copper homeostasis gene *ATX1* from Arabidopsis. Plant Physiol 1998; 117:1227-34.

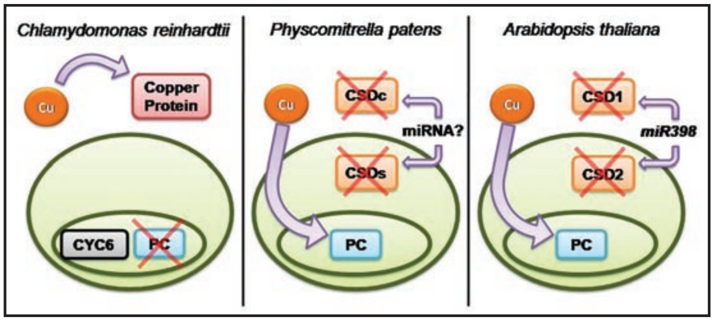

Figure 1. Adaptations to respond to variable copper supply in plants. When copper is limiting, Chlamydmonas degrades plastocyanin (PC) and its function is replaced by cytochrome c_6 (CYC6). In contrast to this algal strategy, Arabidopsis switches from the use of Cu/Zn SOD to iron SOD in response to copper deficiency and this switch involves the function of *miR398*. The strategy of *Physcomitrella patens* is likely to be similar to that of Arabidopsis, although the involvement microRNA is presently unclear.

9. Chu CC, Lee WC, Guo WY, Pan SM, Chen LJ, Li HM, Jinn TL. A copper chaperone for superoxide dismutase that confers three types of copper/zinc superoxide dismutase activity in Arabidopsis. Plant Physiol 2005; 139:425-36.
10. Yamasaki H, Abdel-Ghany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M. Regulation of copper homeostasis by micro-RNA in *Arabidopsis*. J Biol Chem 2007; 282:16369-78.
11. García-Hernández M, Murphy A, Taiz L. Metallothioneins 1 and 2 have distinct but overlapping expression patterns in Arabidopsis. Plant Physiol 1998; 118:387-97.
12. Merchant S, Hill K, Howe G. Dynamic interplay between two copper-titrating components in the transcriptional regulation of cyt c_6. EMBO J 1991; 10:1383-9.
13. Merchant SS, Allen MD, Kropat J, Moseley JL, Long JC, Tottey S, Terauchi AM. Between a rock and a hard place: Trace element nutrition in Chlamydomonas. Biochim Biophy Acta 2006; 1763:578-94.
14. Kropat J, Tottey S, Birkenbihl RP, Depege N, Huijser P, Merchant S. A regulator of nutritional copper signaling in Chlamydomonas is an SBP domain protein that recognizes the GTAC core of copper response element. Proc Natl Acad Sci USA 2005; 102:18730-5.
15. Weigel M, Varotto C, Finazzi G, Rappaport F, Salamini F, Leister D. Plastocyanin is indispensable for photosynthetic electron flow in *Arabidopsis thaliana*. J Biol Chem 2003; 278:31286-9.
16. Zhang B, Pan X, Cobb GP, Anderson TA. Plant microRNA: A small regulatory molecule with big impact. Dev Biol 2006; 289:3-16.
17. Bonnet E, Wuyts J, Rouzé P, Van de Peer Y. Detection of 91 potential conserved plant microRNAs in *Arabidopsis thaliana* and *Oryza sativa* identifies important target genes. Proc Natl Acad Sci USA 2004; 101:11511-6.
18. Chiou TJ, Aung K, Lin SI, Wu CC, Chiang SF, Su CL. Regulation of phosphate homeostasis by MicroRNA in Arabidopsis. Plant Cell 2006; 18:412-21.
19. Sunkar R, Kapoor A, Zhu JK. Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in Arabidopsis is mediated by downregulation of miR398 and important for oxidative stress tolerance. Plant Cell 2006; 18:2051-65.
20. Peers G, Price NM. Copper-containing plastocyanin used for electron transport by an oceanic diatom. Nature 2006; 441:341-4.
21. La Fontaine S, Quinn JM, Nakamoto SS, Page MD, Göhre V, Moseley JL, Kropat J, Merchant S. Copper-dependent iron assimilation pathway in the model photosynthetic eukaryote *Chlamydomonas reinhardtii*. Eukaryot Cell 2002; 1:736-57.