

Overexpression of Arabidopsis *ATX1* retards plant growth under severe copper deficiency

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In a previous study, we demonstrated that Arabidopsis *Antioxidant Protein1* (*ATX1*) plays an essential role in copper (Cu) homeostasis, conferring tolerance to both excess and subclinically deficient Cu. The Cu-binding motif MXCXXC was required for the physiological function of *ATX1*. In this study, we found that overexpression of *ATX1* resulted in hypersensitivity to severe Cu deficiency despite enhancing tolerance to subclinical Cu deficiency. However, overexpression of mutated *ATX1*, replacing the Cu-binding motif MXCXXC with MXGXXG, abolished the hypersensitivity, for no differences from the wild type under the same conditions. Thus, the expression of *ATX1* must be cautiously regulated to avoid homeostatic imbalance with the over-chelation of Cu.

Free Cu must be chelated and delivered to its physiological partner proteins by Cu chaperones after Cu uptake from the environment.^{1,2} Arabidopsis has at least three identified Cu chaperones, including the Cu chaperone for superoxide dismutase (SOD; CCS) and two homologs of yeast Antioxidant Protein1 (*ATX1*), Copper Chaperone (*CCH*) and *ATX1*.³⁻⁵ Results from our previous study suggested that *ATX1* and *CCH* have different homeostatic properties and distinct functions *in planta*.⁶ *ATX1* in Arabidopsis contributes to tolerance of both excess and deficient Cu.⁶ In previous study, we also found high Cu accumulation and tolerance in *ATX1* overexpression lines grown in high-Cu-containing soil. These physiological functions of *ATX1* depend on its Cu-binding motif MXCXXC.⁶ *ATX1* possesses excellent Cu-chelating activity and must be strictly regulated.⁷

Here, we found that overexpression of *ATX1* resulted in hypersensitivity to severe Cu deficiency. By contrast, overexpression of mutated *ATX1* did not show the hypersensitivity. Regulation of *ATX1* may have an important role in Cu homeostasis contributing to plant growth and development.

Cu is indispensable for all growth stages of higher plants.⁸ Cu deficiency has been found throughout the world in all climatic zones where crops are grown or animals are kept on farms.^{3,9} Slight Cu deficiencies occur in many crops and cause up to 20% loss in yield without obvious symptoms.⁹ Therefore, optimizing Cu availability in plants to maintain plant growth under Cu deficiency is important.

In a previous study, we demonstrated that overexpression of *ATX1* enhanced tolerance to slight Cu deficiency in Arabidopsis. To investigate whether the overexpression of *ATX1*, with its excellent chelating activity, has any negative effects under Cu deficiency, Arabidopsis transgenic plants overexpressing *ATX1* in

a wild-type and *atx1* mutant background (Wt-*ATX1* and *atx1-ATX1*, respectively) were challenged with severe Cu deficiency. Wt-*ATX1*-1, Wt-*ATX1*-2, *atx1-ATX1*-1 and *atx1-ATX1*-2 were hypersensitive to severe Cu deficiency (Fig. 1A). Fresh weight and root length were lower for Wt-*ATX1*-1, 2 and *atx1-ATX1*-1, 2 than the wild type, *cch*, *atx1* and *cchatx1* mutants under severe Cu deficiency (Fig. 1B and C). The fresh weight for Wt-*ATX1*-1, Wt-*ATX1*-2, *atx1-ATX1*-1 and *atx1-ATX1*-2 was 40%, 39%, 48% and 42%, respectively, that of the wild type. In addition, the root length was about 46%, 50%, 48% and 48%, respectively, that of the wild type. However, the response of *CCH* transgenic lines and the wild type was similar (Fig. 1).

To elucidate whether the MXCXXC Cu-binding motif of *ATX1* plays a role in the hypersensitivity, we examined Arabidopsis transgenic plants overexpressing *ATX1* with MXCXXC Cu-binding motif replaced by MXGXXG in a wild-type and *atx1* mutant background (Wt-CG and *atx1*-CG, respectively) under severe Cu deficiency.⁶ Wt-CG-1, Wt-CG-2, *atx1*-CG-1 and *atx1*-CG-2 transgenic lines were as sensitive as the wild type to severe Cu deficiency, and the biomass and root length were similar for Wt-CG-1, Wt-CG-2, *atx1*-CG-1, *atx1*-CG-2 and the wild type (data not shown). Therefore, *ATX1*-mediated hypersensitivity to severe Cu deficiency depends on the MXCXXC Cu-binding motif, and Cu chelation of *ATX1* is crucial in the hypersensitivity to severe Cu deficiency. Overexpression *ATX1* may create a critical cellular situation with exhausted Cu under severe Cu deficiency that retards plant growth.

Together with the previous report,⁶ our study suggests that optimal levels of *ATX1* protein ensure important functions in the control of Cu availability to affect plant growth and development.

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Submitted: 06/06/12; Revised: 06/17/12; Accepted: 06/18/12
<http://dx.doi.org/10.4161/psb/>

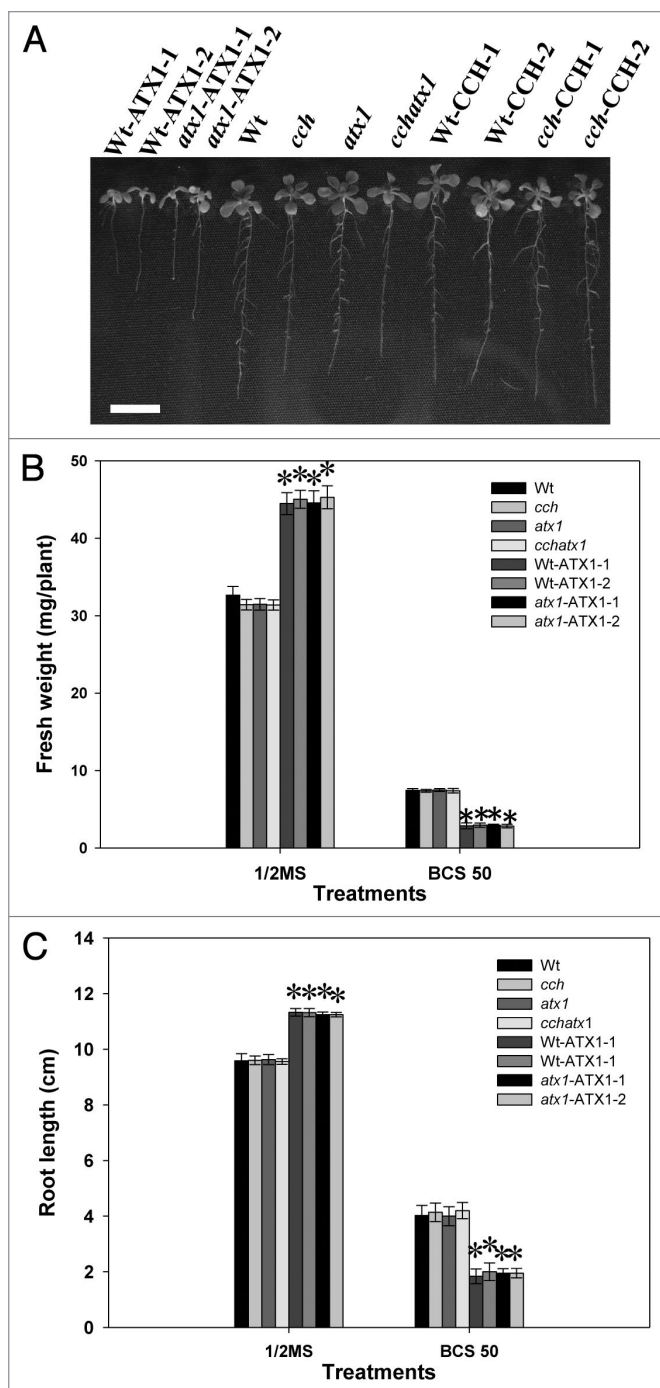


Figure 1. Growth of the wild type (Wt), Cu chaperone mutants (*cch*, *atx1* and *cchatx1*) and transgenic plants with overexpression of *Arabidopsis Antioxidant Protein1 (ATX1)* (Wt-ATX1-1, Wt-ATX1-2, *atx1*-ATX1-1 and *atx1*-ATX1-2) or *Copper Chaperone (CCH)* (Wt-CCH-1, Wt-CCH-2, *cch*-CCH-1 and *cch*-CCH-2) under severe Cu deficiency. A, Seeds of plants were grown vertically on half-strength MS agar plates with 50 μ M Cu chelator bathocuproinedisulfonate (BCS) for 17 d (A). Bar = 1 cm. Plants were grown in half-strength MS medium and treated with BCS 50 μ M for 17 d, and fresh weight (B) and root length (C) were measured. Data are mean \pm SD of 4 replicates with 40 seedlings each. Student's t-test was used for statistical analysis. * $p < 0.01$ compared with the wild type under the same condition. The plant materials and growth conditions were described previously.⁶

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the National Science Council (NSC 97-2311-B-001-008-MY3) and Academia Sinica.

References

- Puig S, Andr s-Col s N, Garc a-Molina A, Pe arrubia L. Copper and iron homeostasis in *Arabidopsis*: responses to metal deficiencies, interactions and biotechnological applications. *Plant Cell Environ* 2007; 30:271-90; PMID:17263774; <http://dx.doi.org/10.1111/j.1365-3040.2007.01642.x>.
- Burkhead JL, Reynolds KA, Abdel-Ghany SE, Cohu CM, Pilon M. Copper homeostasis. *New Phytol* 2009; 182:799-816; PMID:19402880; <http://dx.doi.org/10.1111/j.1469-8137.2009.02846.x>.
- Himmelblau E, Mira H, Lin SJ, Culotta VC, Pe arrubia L, Amasino RM. Identification of a functional homolog of the yeast copper homeostasis gene *ATX1* from *Arabidopsis*. *Plant Physiol* 1998; 117:1227-34; PMID:9701579; <http://dx.doi.org/10.1104/pp.117.4.1227>.
- Chu CC, Lee WC, Guo WY, Pan SM, Chen LJ, Li HM, et al. A copper chaperone for superoxide dismutase that confers three types of copper/zinc superoxide dismutase activity in *Arabidopsis*. *Plant Physiol* 2005; 139:425-36; PMID:16126858; <http://dx.doi.org/10.1104/pp.105.065284>.
- Puig S, Mira H, Dorcey E, Sancen n V, Andr s-Col s N, Garc a-Molina A, et al. Higher plants possess two different types of *ATX1*-like copper chaperones. *Biochem Biophys Res Commun* 2007; 354:385-90; PMID:17223078; <http://dx.doi.org/10.1016/j.bbrc.2006.12.215>.
- Shin LJ, Lo JC, Yeh KC. Copper chaperone antioxidant protein 1 is essential for copper homeostasis. *Plant Physiol* 2012; In press; PMID:22555879; <http://dx.doi.org/10.1104/pp.112.195974>.
- Shoshan MS, Tshuva EY. The MXCXXC class of metallochaperone proteins: model studies. *Chem Soc Rev* 2011; 40:5282-92; PMID:21695339; <http://dx.doi.org/10.1039/c1cs15086c>.
- Marschner H. *Mineral Nutrition of Higher Plants*. London: Academic Press 1995.
- Graham RD, Nambiar EKS. Advances in Research on Copper Deficiency in Cereals. *Aust J Agric Res* 1981; 32:1009-37.