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Title

Arabidopsis Adapts to Copper Deficient Conditions via SPL7, a Master Regulator for Copper Homeostasis

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INTRODUCTION

Plants use copper as a cofactor for a wide variety of physiological processes (Pilon et al 2006). The most abundant copper protein in higher plants is plastocyanin (PC), which localizes the thylakoid lumen of chloroplasts and is involved in the photosynthetic electron transport (Weigel et al 2003). Copper/zinc superoxide dismutase (Cu/Zn SOD), which is involved in the scavenging of reactive oxygen species, is another major copper protein, and localizes the cytoplasm (CSD1), stroma of chloroplasts (CSD2), and also peroxisomes (CSD3) in *Arabidopsis* (Bowler et al 1994). Despite its significance, copper is insolubilized in alkaline soil. To adapt to copper deficient environment, higher plants have some strict strategies to copper deficiency. In copper deficient conditions, higher plants down-regulate the accumulation of CSD1 and CSD2, and their function is compensated by iron superoxide disumutase (FeSOD) specifically expressed in low copper conditions (Abdel-Ghany et al 2005). Previously we demonstrated that an microRNA, *miR398*, was expressed only in copper deficient conditions and was involved in this down-regulation by directing the degradation of *CSD1* and *CSD2* mRNA (Yamasaki et al 2007). Therefore, limited copper is preferentially transported to PC in copper deficient conditions in *Arabidopsis*.

The molecular mechanism of the adaptation for copper deficiency is well understood in green alga, *Chlamydomonas* (Merchant et al 2006). In *Chlamydomonas*, the function of PC is replaced by heme-containing protein, cytochrome c_6 in low copper conditions (Merchant et al 1991). This switching is mediated by a single transcription factor, Crr1 (Kropat et al 2005). Here we present evidence that SPL7, an analogous to Crr1, is a transcription activator for *miR398* and also for several genes involved in copper homeostasis. We propose that SPL7 is a master regulator involved in copper homeostasis in *Arabidopsis*.

RESULTS AND DISCUSSION

In *Arabidopsis*, stability of *CSD1* and *CSD2* mRNAs is down-regulated via *miR398* in copper deficient conditions (Yamasaki et al 2007). To study the molecular mechanism of underlying the copper-dependent transcriptional regulation of *miR398*, we focused on its promoter. Since *miR398c* promoter had 8 GTAC motifs which is an essential motif to respond to copper deficiency in *Chlamydomonas* and is recognized by a transcription factor, Crr1 (Quinn et al 2000; Kropat et al 2005), it was expected that Crr1-like protein might be involved in the regulation of *miR398* in *Arabidopsis*. Crr1 was closely related to SPL (*SQUAMOSA promoter-binding protein-like*) family and showed the highest similarity to SPL7 in *Arabidopsis*. To test the possibility that SPL7 is involved in the regulation of *miR398*, we examined the T-DNA insertion mutant of SPL7 (Figure 1A). Although *miR398* level was up-regulated in low copper conditions in wild-type, the regulation was not observed in *spl7* and *CSD1* and *CSD2* mRNA was stable even in copper deficient conditions, suggesting that SPL7 is a transcription activator for *miR398*. Actually the DNA binding domain of SPL7 was directly bound to the GTAC motif located in the promoter region of *miR398 in vitro* (Yamasaki et al 2009).

In addition to *miR398*, the regulation of several genes which is induced in response to copper deficiency was disturbed in *spl7* (Figure 1A). FeSOD, *FSD1*, was induced in copper deficient conditions in wild-type but not in *spl7*, suggesting that SPL7 is also a transcription activator for FSD1 (Figure 1A). These results indicate that the copper-dependent switching of SOD between Cu/Zn SOD and FeSOD is mediated by single transcription factor, SPL7, in *Arabidopsis*. The up-regulation of some genes involved in the transport of copper across the membrane, *COPT1*, *COPT2*, *ZIP2* and *FRO3*, was also disturbed in *spl7* (Figure 1A). These results indicate that SPL7 is involved in the appropriate copper distribution in low copper conditions. Previously it was reported that three more microRNAs, *miR397*, *miR408* and *miR857* targeted some member of *laccase* family and *plantacyanin* which contains copper, were up-regulated in response to copper deficiency (Abdel-Ghany and Pilon 2008). These microRNAs were not up-regulated in *spl7* (Figure 1A), indicating that SPL7 is also a transcription activator for these microRNAs and is involved in the degradation of copper protein to transfer limited copper to the other essential copper protein like PC in copper deficient conditions. Additionally, many genes were predicted as a target of SPL7 by microarray analysis (Yamasaki et al 2009).

In copper deficient conditions, *spl7* showed severe growth impairment (Figure 1B). This drastic phenotype, however, was recovered by addition of copper to the medium, indicating that the response to copper deficiency via SPL7 is physiologically significant. Taken all results together, we conclude that SPL7 is a master regulator for copper homeostasis in *Arabidopsis*.

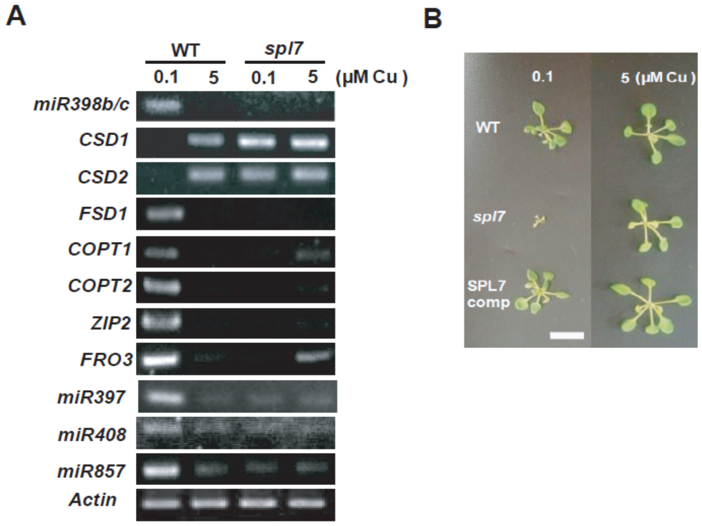

Figure 1. **(A)** RT-PCR analysis of genes responding to copper deficiency. The plants were grown for 3 weeks on MS agar medium containing 0.1 or 5 µM $CuSO_4$. **(B)** Growth phenotypes of the wild type, *spl7*, and SPL7 complement lines (SPL7comp) grown for 3 weeks on MS agar medium containing 0.1 or 5 µM $CuSO_4$. Bar =10 mm.

REFERENCES

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**:**1233-1251.

Abdel-Ghany SE, Pilon M. microRNA-mediated systemic down-regulation of copper protein expression in response to low copper availability in *Arabidopsis*. J. Biol. Chem. 2008;283:15932-15945.

Bowler C, Van Camp W, Van Montagu M, Inzé D. Superoxide dismutase in plants. Crit. Rev. Plant Sci. 1994;13: 199-218.

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. U. S. A. 2005;102: 18730-18735.

Merchant S, 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. Biophys. Acta 2006;1763**:** 578-594.

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: 1383-1389.

Pilon M, Abdel-Ghany SE, Cohu CM, Gogolin KA, Ye H. Copper cofactor delivery in plant cells. Curr. Opin. Plant Biol. 2006;9: 256-263.

Quinn JM, Barraco P, Eriksson M, Merchant S. Coordinate copper- and oxygen-responsive *Cyc6* and *Cpx1* expression in *Chlamydomonas* is mediated by the same element. J. Biol. Chem. 2000;275: 6080-6089.

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: 31286-31289.

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: 16369-16378.

Yamasaki H, Hayashi M, Fukazawa M, Kobayashi Y Shikanai T. *SQUAMOSA* promoter-binding protein–Like 7 is a central regulator for copper homeostasis in *Arabidopsis.* Plant Cell 2009;21:347-361.