

## Metallothioneins, a Diverse Protein Family

Metal ions are essential for growth, but in excess, these compounds can become highly toxic. Both plants and animals have adapted numerous ways to maintain metal homeostasis while mitigating detrimental effects of excess metals ions, including the metal-chelating proteins metallothionein (MT) and phytochelatin. Although phytochelatins have been shown to play an important role in the detoxification of certain heavy metals in both plants and animals, the role of MTs in this process has not been conclusively shown in plants.

### BACKGROUND

Metallothioneins and phytochelatins are Cys-rich metal chelators that represent the two principal groups of metal-binding proteins found across most taxonomic groups. Although MTs were originally identified in animals by their ability to protect against cadmium toxicity, their role in plant systems is still being determined. MTs have been reported to play a role in other cellular processes, including the regulation of cell growth and proliferation, DNA damage repair, and scavenging of reactive oxygen species (ROS; Cherian and Kang, 2006). A zinc-donating role has also been found for several metalloproteins (Cherian and Kang, 2006). Thus, although the importance of MTs throughout the life cycle of a plant has been demonstrated, how they fulfill these roles is not clearly understood (Blindauer and Schmid, 2010).

MTs are able to bind a variety of metals by the formation of mercaptide bonds between the numerous Cys residues present in the proteins and the metal, and it is the arrangement of these residues that in part determines the metal-binding properties of the MT proteins (Cobbett and Goldsbrough, 2002; Blindauer and Leszczyszyn, 2010). Based on the Cys arrangement, plant MTs have been grouped into four types, types 1 through 4. Although MTs are expressed throughout the plant, some have been found to be expressed in a tissue-specific manner.

### WHAT WAS SHOWN

One question about MTs is whether all four types are capable of functioning as metal chelators or if some have been coopted into other functions. To address this question, Guo et al. (2008) expressed six *Arabidopsis* (*Arabidopsis thaliana*) MTs representing each of the four types in both the copper-sensitive yeast mutant  $\Delta cup1$  and the double mutant  $\Delta zrc1$

$\Delta cot1$ , a zinc-sensitive yeast mutant lacking two zinc vacuolar transporters. Expression of the *Arabidopsis* MTs in the  $\Delta cup1$  mutant returned copper tolerance and accumulation to wild-type levels, indicating that all of the MTs tested are able to function as copper chelators. Although all four MT types provided some increased tolerance in the zinc-sensitive mutants, the type 4 MTs, typically expressed in seeds, provided greater zinc tolerance and increased accumulation than the other MTs. Together, these yeast complementation assays suggest that all four types of MTs are capable of functioning as metal chelators, leaving open the question, what is their function?

Phenotype analysis of plants lacking the phloem-expressed MT1a or MT2b as single mutants, double mutants, or RNA interference (RNAi) transgenics indicates that neither of these two genes is essential for growth and development under normal growth conditions (soil or hydroponic), as no phenotypic difference between the mutants and the wild type was observed. After exposure to excess copper, a phenotypic difference became apparent in the roots of three of the mutants, where a significant decrease in copper accumulation in the roots of the *mt1a* mutants, *MT1a-RNAi*, and *mt1a-mt2b* double mutant, but not *mt2b*, relative to the wild type was observed. No difference in copper accumulation was observed in the shoot of any of the mutants, nor was there an accumulative effect in the double mutant, suggesting that MT1a, but not MT2b, plays an important role in copper accumulation in roots. This study strongly suggests that in *Arabidopsis*, MT-1a, -2a, -2b, and -3 are likely to function as copper-binding MTs, whereas MT-4a and -4b are more likely to be zinc binding.

### THE IMPACT

Although phenotype analysis of MT2b mutants suggests that it does not play a significant role in the sequestration of excess copper in roots (Guo et al., 2008), recent work has implicated MT2 in ROS tolerance. Zhu et al. (2009) demonstrated that two known inducers of ROS, cold exposure and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) stress, increased the expression of *MT2a* in *Arabidopsis*. An *mt2a* T-DNA insertion mutant proved to be sensitive to cold stress during germination, with higher production of H<sub>2</sub>O<sub>2</sub> relative to the wild type. *MT2a* expression is increased after cold exposure in the catalase mutant *cat2*, which has increased levels of H<sub>2</sub>O<sub>2</sub> when grown under ambient conditions (Queval et al., 2007). Reciprocally, the *mt2a* mutant has increased *CAT2* expression, leading the authors to hypothesize that these two proteins might be complementary to one another in antioxidant processes.

## CONCLUSION

Although many recent studies have started to reveal the roles of MTs in plants, there is still much more information needed. The large diversity in the metal-binding regions of plant MTs suggests that they have the ability to bind a greater range of metals than their animal counterparts and, consequently, a greater range of function. An important area of MT research currently is the elucidation of the structure of the plant MTs and their metal-thiolate clusters (for review, see Freisinger, 2010). This knowledge will aid in the determination of the function of this diverse group of proteins.

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