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The plant CDF family member TgMTP1 from the Ni/Zn hyperaccumulator *Thlaspi goesingense* acts to enhance efflux of Zn at the plasma membrane when expressed in *Saccharomyces cerevisiae*

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Objectives

We have characterized a *Thlaspi* gene that confers Zn tolerance to yeast

Materials and Methods

1. Material

Yeast-*Saccharomyces cerevisiae*,
Plant-*Arabidopsis thaliana* plants

2. Methods

Thlaspi genes that can functionally complement Zn-sensitive phenotype of cation diffusion facilitator (CDF) family deficient yeast mutant were characterized.

Results and Discussion

To avoid metal toxicity, organisms have evolved mechanisms including efflux of metal ions from cells and sequestration into internal cellular compartments. Members of the ubiquitous cation diffusion facilitator (CDF) family are known to play an important role in these processes. Overexpression of the plant CDF family member metal tolerance protein 1 (MTP1) from the Ni/Zn hyperaccumulator *Thlaspi goesingense* (TgMTP1), in the *Saccharomyces cerevisiae* Delta zinc resistance conferring (*zrc*)1 Delta cobalt transporter (*cot*)1 double mutant, suppressed the Zn sensitivity of this strain. *T. goesingense* was found to contain several allelic variants of TgMTP1, all of which confer similar resistance to Zn in *Delta zrc*1 *Delta cot*1. Similarly, MTP1 from various hyperaccumulator and non-accumulator species also confer similar resistance to Zn. *Delta zrc*1 *Delta cot*1 lacks the ability to accumulate Zn in the vacuole and has lower accumulation of Zn after either long- or short-term Zn exposure. Expression of TgMTP1 in *Delta zrc*1 *Delta cot*1 leads to further lowering of Zn accumulation and an increase in Zn efflux from the cells. Expression of TgMTP1 in a V-type ATPase-deficient *S. cerevisiae* strain also confers increased Zn resistance. In vivo and in vitro immunological staining of hemagglutinin (HA)-tagged TgMTP1::HA reveals the protein to be localized in both the *S. cerevisiae* vacuolar and plasma membranes. Taken together, these data are consistent with MTP1 functioning to enhance plasma membrane Zn efflux, acting to confer Zn resistance independent of the vacuole in *S. cerevisiae*. Transient expression in *Arabidopsis thaliana* protoplasts also reveals that TgMTP1::green fluorescent protein (GFP) is localized at the plasma membrane, suggesting that TgMTP1 may also enhance Zn efflux in plants.