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Manganese matters: Feeding manganese into the secretory system for cell wall synthesis

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Main text

Manganese (Mn) is an essential element for plants, playing major roles as a co-factor of enzymes involved in photosynthesis and oxygen species detoxification (Schmidt & Husted, 2019). Mn acts in various plant cell compartments and need to be transported in chloroplast and mitochondria (He *et al.*, 2021). The Mn/Ca efflux transporters of the UPF0016/GTD family (Demaegd *et al.*, 2014), PAM71 and CMT1 have been shown to allow Mn transport at the thylakoid and inner membranes of the chloroplast, respectively (Fig. 1) (Schmidt *et al.*, 2020). In this issue of New Phytologist, Yang *et al.* (pp. 000-000) investigated the function of another member of the UPF0016/GTD transporter family, PML3, in *Arabidopsis thaliana*. Using a functional GPF tagged protein, they showed that PML3 localizes in the cis-Golgi compartment as previously proposed by Hoecker *et al.* (2020)(Fig. 1). In agreement with the role of UPF0016/GTD in Mn transport, the growth of *pml3* mutant is impaired under Mn limitation. The authors further determined that the *pml3* phenotype is mainly caused by a defect in protein glycosylation and cell wall biosynthesis. Although it was known that Mn acts as a co-factor for glycosyl transferases, this publication provides the first genetic evidence that Mn loading in the Golgi is essential to sustain this process and that PML3 plays a dominant role in Mn loading into this cell compartment when Mn availability is limited. These results support a highly conserved role of PML3 and its homologues in distant organisms in Mn influx into the Golgi for protein glycosylation (Potelle *et al.*, 2016).

Before this study, it was assumed that the main effect of Mn deficiency on growth was the consequence of decreased PSII efficiency. However, photosynthesis does not appear to be affected in the *pml3* mutant. Furthermore, even with ample Mn supply, the *plm3* mutant is still strongly affected in reproductive functions, probably as a direct consequence of a defect in pectin deposition during the fast elongation of the pollen tube (Zhang *et al.*, 2021). This result provides a cellular basis for the essential role of Mn in fertility.

The study of PML3 demonstrates the importance of Mn influx into the secretory system. It establishes a link between Mn entry in the Golgi, the presence of Mn dependent glycosyl transferase in this compartment and the formation of the cell wall. Both protein glycosylation and glycosylation of cell wall polymers such as homogalacturonans, rhamnogalacturonans, xylogalacturonans and xyloglucans involve Mn-dependent glycosyl transferases (He *et al.*, 2021). The data presented show that arabinose, xylose and mannose contents are specifically decreased in the *pml3* mutant cell walls under Mn deficiency. This supports the notion that Mn

plays an important role in the synthesis of secreted cell wall polymers. Yang et al. also demonstrates a strong decrease in the levels of glycosylation with xylose and fucose. Especially the amount of RSW2, a protein involved in cell wall organization which is the target of Mn-dependent MNS1/2 glycosyl transferase, is decreased in the *pml3* mutant under Mn limiting conditions. Together, these defects indirectly affect cellulose deposition and ultimately cell wall expansion accounting for the growth defect of *pml3* under Mn deficiency, while photosynthesis apparently remains unaffected.

Previously, other Mn transporters have been implicated in Mn loading into the secretory system. The Ca/Mn pump ECA3 resides in the Golgi and endosomes (Fig. 1) (Mills *et al.*, 2008; Li *et al.*, 2008). Like *pml3*, the *eca3* mutant displays growth defects under Mn deficiency. The CDF transporter MTP11 localizes in the Golgi and in the PCV/late endosome (Peiter *et al.*, 2007; Delhaize *et al.*, 2007). However, in contrast to *plm3* and *eca*3, the *A. thaliana mtp11* mutant is more tolerant to Mn deficiency. All 3 genes *PML3*, *ECA3* and *MTP11* are involved in the tolerance to Mn excess. This raises the question of the respective functions of these transporters. Each transporter may act in different tissues or developmental stages. Furthermore, these transporters could act at different steps of the secretory pathway. Our knowledge of this complex and dynamic system has considerably evolved in the last decade with the identification of functional sub-compartments within the trans-Golgi network (TGN). In this view, it would be useful to refine the localization of ECA3 and MTP11 and compare it to that of PML3. It would also be interesting to test whether ECA3 or MTP11 loss of function affects protein glycosylation and cell wall formation as observed in *pml3*. Since these transporters play roles in both Mn deficiency and excess, it would be important to further test if their localization is affected by fluctuation of Mn supply, as recently shown for the Mn uptake transporter NRAMP1 (Castaings *et al.*, 2021).

Once in the secretory system, Mn will end up either in the apoplast or in the vacuole following membrane fusion (Fig. 1). It thus appears necessary to recover Mn from the secretory system for other functions (*eg* photosynthesis) at least under Mn deficiency. In contrast to the loading step, only one transporter involved in Mn retrieval has been identified so far. The NRAMP2 transporter was shown to reside in the TGN (Alejandro *et al.*, 2017; Gao *et al.*, 2018). The *nramp2* mutant is more sensitive to Mn deficiency for root growth and photosynthesis. Accordingly, the mutation of *NRAMP2* also affects Mn supply to chloroplasts. Interestingly, NRAMP1, that functions in Mn uptake at the plasma membrane, was also localized in the TGN under sufficient Mn supply and could thus be also involved in Mn release from this

compartment (Agorio *et al.*, 2017; Castaings *et al.*, 2021). The *pml3* mutation partially rescues the root growth phenotype and the chlorophyll content of the *nramp2* mutant under Mn deficiency. This underlines the functional link between Mn loading in the Golgi and its downstream retrieval from the TGN due to the dynamic nature of the secretory pathway (Figure 1).

The import of Mn in the secretory pathway and the higher tolerance of the *pml3* mutant to Mn excess raise the question of the role of the secretory pathway in the sequestration of Mn. The *mtp11* mutant accumulates more Mn in roots. The MTP11 transporter was proposed to mediate Mn secretion leading to increased tolerance. However, this role is expected to be deleterious under Mn deficiency. Downstream of the TGN, the flux of vesicles might also be targeted to the vacuole. This pathway would contribute to the storage of Mn in the vacuole together with Mn transporters that reside on the vacuolar membrane such as MTP8 and CAX2 (He *et al.*, 2021). Collectively, these transporters are required for tolerance to Mn excess. Conversely, under Mn deficiency, Mn needs to be remobilized from the vacuole to provide Mn to photosystem II in chloroplasts (Lanquar *et al.*, 2010).

The study by Yang *et al.* provides an important piece in the jigsaw puzzle of Mn homeostasis in plant cells. It highlights the need to load Mn in the Golgi as a cofactor for glycosyl transferases that are essential for cell wall formation. This model is inferred from genetic and biochemical evidence. However, it would need to be substantiated by direct detection of Mn in organelles using Mn specific fluorescent sensors or high resolution XRF microscopy (Das *et al.*, 2019). These approaches may allow to visualize a Mn concentration gradient through the secretory pathway and how it is spatially modulated by the activities of PML3, MTP11, ECA3 and NRAMP2. Different members of UPF0016/GTD family are thus involved in targeting Mn in two different organelles hosting enzymes that require this element as cofactor. Differential regulation and differences in affinity for Mn of PAM71 and CMT1 or PML3 could provide means to prioritize Mn use for photosynthesis or cell wall synthesis according to plant needs. Finally, further research is needed to delineate the pathways for the supply of Mn for other important functions, as the superoxide dismutase in mitochondria and peroxisomes.

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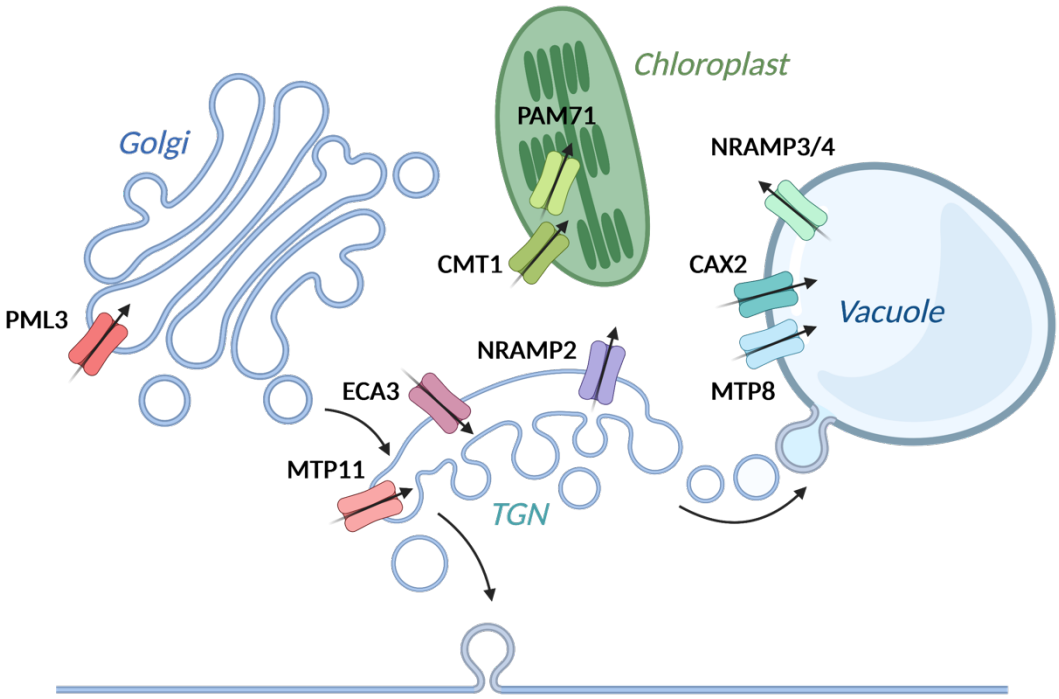


Figure 1: Transporters involved in the intracellular distribution of Mn in plant cells. The transporters involved in influx and efflux from the secretory pathway (PML3, MTP11, ECA3, NRAMP2), into the plastids and thylakoids (CMT1, PAM71) and in the vacuole (MTP8, CAX2, NRAMP3 and NRAMP4) are shown. Straight arrows through the transporters indicate the direction of the transport. Curved arrows indicate the flow of vesicles through the secretory pathway. Created in BioRender.c