

MtNramp1 mediates iron supply to rhizobia-infected *Medicago truncatula* nodule cells

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All known organisms need iron to accomplish important biological processes for life, ranging from gene transcription to respiration. Particularly in symbiotic nitrogen fixation (SNF) iron plays a critical role since the activity of key proteins involved in this process, such as nitrogenase, leghemoglobin, Fe-superoxide dismutase and other proteins involved in energy transduction, directly depends on the presence of iron as cofactor in their active centre. In the model legume *Medicago truncatula*, iron is delivered by the vasculature and released in the apoplast on the zone II of the nodule (infection/maturation zone). Then iron moves into rhizobia-infected cells and it is used in the synthesis of iron-containing proteins. Therefore, different iron transporters should mediate iron traffic through the plasma membrane of plant cells and the symbiosome membrane. However, no candidates were available to be responsible for iron transport across the plasma membrane from the nodule apoplast to rhizobia-infected cells.

In the present work, we have identified a *Nramp* member gene from *M. truncatula* (*MtNramp1*) as responsible for iron transport from nodule apoplast into rhizobia-infected cell. *MtNramp1* shows the highest expression in the nodule among the seven *Nramp* genes present in *M. truncatula* genome. Immunolocalization studies show that MtNramp1 is located in the plasma membrane of zone II nodule cells. A loss-of-function *nramp1* mutant presented impaired growth specifically under symbiotic conditions, concomitant with a lower nitrogenase activity compared to wild-type plants. This phenotype was rescued by the addition of iron to the nutritive solution or by complementation of a mutant with a wild-type *Nramp1* copy. Furthermore yeast complementation assays using mutant affected on iron transport pointed to a role of MrNramp1 in iron transport toward the cytosol. All together, these results point to a role of MtNramp1 in iron supply to nodule cells connected to SNF, and represent an important step toward the understanding of iron incorporation and homeostasis in plant nitrogen-fixing tissues.

Acknowledgments

This work was supported by ERC Starting Grant (ERC-2013-StG-335284) and MINECO Grant (AGL-2012-32974) to M.G-G.