Metallic Action! The Dynamics of a Tripartite Iron Uptake Complex in Arabidopsis Roots

Plants generally obtain mineral nutrients from the soil through their roots; however, the availability of a specific soil nutrient may limit plant growth and productivity and, conversely, the presence of too much of a specific soil nutrient may be toxic to the plant. Iron (Fe) is an essential micronutrient that fulfills numerous roles in plant cellular processes, serving as a key component for respiration and photosynthesis and as a cofactor for many enzymes (Barker and Pilbeam, 2015). Although relatively abundant in the Earth’s crust, iron is normally found in soils in its ferric form (Fe$^{3+}$), which is poorly bioavailable due to its low solubility. To cope with this difficulty, nongramineous plants like the model plant Arabidopsis (Arabidopsis thaliana) have evolved an Fe$^{3+}$ reduction-based mechanism that involves acidification of the rhizosphere by the proton pump H$^+$-ATPASE2 (AHA2), reduction and solubilization of Fe$^{3+}$ to Fe$^{2+}$ by FERRIC REDUCTION OXIDASE2 (FRO2), and finally transport of Fe$^{2+}$ into the cell by IRON REGULATED TRANSPORTER1 (IRT1; Jeong et al., 2017; Rajniak et al., 2018). In addition to acting as the high-affinity iron uptake transporter (Jeong et al., 2017), IRT1 also acts as a broad-spectrum transporter, mediating the absorption of a variety of metals such as cadmium, cobalt, and zinc. Plants alter the expression, subcellular localization, and turnover of IRT1 to finely tune the uptake of iron and other metals from the soil (Cointry and Vert, 2019). Surprisingly, aside from the importance of IRT1 for metal homeostasis, little is known about other proteins associated with IRT1 dynamics and activity.

In this issue of Plant Physiology, Martin-Barranco et al. (2020) shed light on this topic by exploring the interactome of IRT1 in Arabidopsis root epidermal cells. To copurify IRT1-associated proteins, they used an Arabidopsis irt1-1 null mutant expressing an IRT1-mCitrine fusion protein under the control of the IRT1 promoter. To induce IRT1-mCitrine expression, transgenic plants were grown on media containing iron and then transferred to media with no iron but containing an excess of noniron metal substrates (zinc, manganese, and cobalt). Root protein extracts were used to immunopurify IRT1-mCitrine and its interacting proteins, which were then analyzed by mass spectrometry. Among the 142 putative interacting proteins identified, two were particularly notable for their participation with IRT1 in the acidification-reduction-transport of iron uptake in Arabidopsis: AHA2 and FRO2. These results were confirmed by coimmunoprecipitation and split-ubiquitin assays, providing strong evidence that IRT1 directly interacts with AHA2 and FRO2. Moreover, these experiments also revealed that AHA2 and

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**Figure 1.** Dynamics of the IRT1-AHA2-FRO2 iron uptake complex in the model plant Arabidopsis. IRT1, AHA2, and FRO2 form a tripartite complex that solubilizes Fe$^{3+}$ through a three-step mechanism: (1) rhizosphere acidification (AHA2); (2) Fe$^{3+}$ reduction (FRO2); and (3) transport of Fe$^{2+}$ into the root epidermal cell (IRT1). Martin-Barranco et al. (2020) demonstrate that, in optimal concentrations of noniron metals, IRT1, AHA2, and FRO2 colocalize to the outer polar domain of the plasma membrane (PM) and intracellular vesicles. AHA2 localizes to the inner polar plasma membrane. However, the tripartite complex is dissociated in conditions of noniron metal excess, where IRT1 is trafficked (via endocytosis) to the intracellular vesicles. This mechanism likely reduces the amount of metals absorbed by the root epidermal cells, avoiding toxicity caused by high concentrations of nutrients in the soil.
FRO2 physically associate with each other. Taken together, these observations indicate the existence of an IRT1-AHA2-FRO2 tripartite protein complex that modulates iron uptake in Arabidopsis root epidermal cells.

High concentrations of mineral nutrients outside and inside plant cells can lead to toxicity due to oxidative and osmotic stress and, thus, hinder plant growth and development. As a possible mechanism to protect plants from toxic concentrations of metal nutrients, IRT1 is polyubiquitinated in the presence of noniron metal excess, leading to its removal from the plasma membrane and degradation in the vacuole (Dubeaux et al., 2018). As AHA2 and FRO2 belong to the same IRT1 protein complex, Martín-Barranco et al. (2020) wondered whether these two proteins were also ubiquitinated under conditions of high availability of noniron metals. Using antiubiquitin immunoblots, the authors observed that whereas the excess of noniron metals lead to a strong increase in IRT1 ubiquitin levels, the pools of ubiquitinated AHA2 and FRO2 remain unchanged when plants are treated with optimal and high concentrations of these nutrients. These results provide striking evidence that, despite belonging to the same protein complex and participating in a common physiological mechanism (i.e. iron uptake), IRT1, AHA2, and FRO2 are differentially regulated by metal availability.

To better understand the intracellular dynamics of the IRT1-AHA2-FRO2 complex, Martín-Barranco et al. (2020) evaluated the subcellular localization of these three proteins in the presence of metal nutrients. For this purpose, plants expressing fluorescently tagged versions of IRT, AHA2, and FRO2 were subjected to treatment with noniron metal substrates, followed by visualization of root tip epidermal cells by confocal microscopy. The authors observed that, in the presence of physiologically relevant amounts of noniron metal substrates, IRT1 and FRO2 colocalize at the outer polar domain of the plasma membrane (facing the rhizosphere) and in intracellular vesicles (early endosomes). Under these same conditions, AHA2 displayed an apolar localization to the plasma membrane, with weak fluorescent signals in the intracellular vesicles. Upon treatment with an excess of noniron metals, IRT1 was depleted from the cell surface and accumulated in the intracellular vesicles. By contrast, treatment with excess noniron metals did not change the localization of AHA2 and FRO2, which still mostly localized at the plasma membrane and in the intracellular vesicles (Fig. 1). These results indicate that, distinct from IRT1, AHA2 and FRO2 are not endocyctosed in response to high concentrations of metal nutrients, suggesting that the IRT1-AHA2-FRO2 complex dissociates prior to IRT1 endocytosis and that the members of the tripartite complex are differentially regulated by metal availability.

The capacity of plants to regulate nutrient absorption is of great interest, since it has a major impact on crop productivity. Boosting mineral nutrients with fertilizers to maintain plant growth carries high environmental risk due to soil salinization and nutrient leakage (Barker and Pilbeam, 2015). Therefore, the development of crops that are more efficient at absorbing nutrients is imperative. The work by Martín-Barranco et al. (2020) describes, at the molecular level, how plants finely tune the uptake of essential metal nutrients in different conditions of availability. Although future work is needed to precisely determine how the activity of the IRT1-AHA2-FRO2 complex may influence the activity of each of its members and how it is optimized for nutrient uptake in plants, this study provides useful information for efforts to improve plant nutrient efficiency.

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1Author for contact: marcelolattarulo@ufmt.br.
2Senior author.

www.plantphysiol.org/cgi/doi/10.1104/pp.20.01271

Plant Physiol. Vol. 184, 2020