The bifunctional transporter-receptor IRT1 at the heart of metal sensing and signalling

Virginia Cointry, Grégory Vert

► To cite this version:

Virginia Cointry, Grégory Vert. The bifunctional transporter-receptor IRT1 at the heart of metal sensing and signalling. *New Phytologist*, 2019. hal-02387370

HAL Id: hal-02387370

<https://hal.science/hal-02387370>

Submitted on 29 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**The bifunctional transporter-receptor IRT1 at the heart of metal
sensing and signaling**

Virginia Cointry and Grégory Vert[#]

Laboratoire de Recherche en Sciences Végétales, UMR5546 CNRS/Université Toulouse 3, 24
chemin de Borde Rouge, 31320 Castanet-Tolosan, France.

[#]corresponding author : Gregory.Vert@lrsv.ups-tlse.fr ; +33534323810

Twitter account : @GregVertLab

ORC ID Grégory Vert : 0000-0002-0844-9991

Word count : 2,406

Figures to be published in color : all figures

18	Abstract
19	
20	Content
21	Summary
22	I. Introduction
23	II. Transcriptional regulatory networks driving <i>IRT1</i> expression
24	III. Ubiquitin-mediated endocytosis and degradation of IRT1
25	IV. From IRT1 transporter to IRT1 transceptor
26	V. Conclusions
27	Acknowledgements
28	References
29	
30	

Summary

Transporters are at the center of regulatory modules allowing the optimal assimilation, distribution or efflux of substrate molecules. The IRT1 root metal transporter represents a textbook example where detailed regulatory networks have been shown to integrate several endogenous and exogenous cues at various levels to regulate its expression and to fine tune iron uptake. Here, we summarize recent advances in the dissection of the transcriptional and post-translational control of *IRT1* by its various metals substrates and discuss the emerging role of IRT1 in the direct sensing of non-iron metals flowing through IRT1 to drive its metal-triggered degradation. We propose that transceptors are likely a common theme in the regulation of nutrient transport by sensing local nutrient concentrations.

Key words

Iron, metal, transporter, IRT1, regulation, transcription, endocytosis, transceptor

The plasma membrane represents a selective barrier between the extracellular medium and the cytoplasm for ions, small molecules and macromolecules in all living cells. Membrane transport proteins control fluxes of their substrates across membranes, and as such, their activities underlie physiological processes as diverse as nutrition, detoxification of xenobiotics, hormone transport, electrical activity, etc. The Arabidopsis genome encodes about 1,200 transporters involved in the uptake, the short- and long-distance distribution, the compartmentalization, the remobilization or the excretion of a wide variety of substrates in the plant. Their functional importance in plant growth, development and stress responses is now widely accepted, as revealed by the many reports of severe phenotypes from corresponding loss-of-function mutants. As such, transport proteins are the major determinants of what comes in and out of a cell, when it is safe to transport substrates and in what amount, and thus representing the target of many signaling pathways. The precise control of transport proteins requires sophisticated sensing and signaling mechanisms to perceive specific cues, such as substrate concentration or environmental conditions (e.g. salt, drought, wounding, etc.), and to transduce the information to regulate transporter genes. The study of the Arabidopsis IRON-REGULATED TRANSPORTER-1 (IRT1) root metal transporter has revealed an exquisite network of transcriptional and post-translational regulation that are integrated in the root epidermis to fine tune the uptake of essential iron ions (Brumbarova *et al.*, 2015). Recent work also uncovered that IRT1 is not only the final target of these regulatory networks, but is also part of the metal sensing machinery (Dubeaux *et al.*, 2018). Below, we will review and discuss the molecular basis of metal uptake regulation and the intricate mechanisms allowing the IRT1 transporter to act as the major root iron transporter to take up iron from the soil and to directly perceive metal availability.

Transcriptional regulatory networks driving *IRT1* expression

The regulation of nutrient transporters is usually part of a homeostatic control to provide enough of its substrate to cells and to avoid an excess that can be deleterious. This is a fine balance between substrate availability and the plant need, the latter being highly influenced by plant physiological state and environmental conditions. As the major root iron transporter responsible for iron uptake from the soil, *IRT1* expression is strongly and rapidly upregulated when plants are experiencing iron limitation (Eide *et al.*, 1996; Vert *et al.*, 2002). The transcriptional regulation of *IRT1* by iron uses an elegant dual control that integrates local information about soil iron availability and a systemic signal translating the shoot iron status (Vert *et al.*, 2003). This ensures that *IRT1* is expressed not only when plants need iron but

also when iron is found in non-negligible amounts in soils. A wealth of upstream regulators required for *IRT1* induction by low iron have been identified (Brumbarova *et al.*, 2015). These include RING E3 ligases from the BRUTUS (BTS) family and a repertoire of bHLH transcription factors acting in cascade downstream of BTS, culminating in the direct binding of heterodimers between the master regulator FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) and group Ib bHLHs to *IRT1* promoter and promoters of many iron-regulated genes (Colangelo & Guerinot, 2004; Yuan *et al.*, 2008; Sivitz *et al.*, 2012; Selote *et al.*, 2015; Zhang *et al.*, 2015; Li *et al.*, 2016; Liang *et al.*, 2017). FIT activity is itself controlled in a complex way in response to iron starvation, involving nuclear translocation, destabilization, homo- and heterodimerization with group Ib bHLHs, part of it being controlled by CALCINEURIN B-LIKE-INTERACTING PROTEIN KINASE-11 (CIPK11)-dependent phosphorylation of FIT (Meiser *et al.*, 2011; Sivitz *et al.*, 2011; Gratz *et al.*, 2019).

The nature of the iron sensing machinery at stake in the local and long-distance signaling cascade, and how these pathways are integrated however remain obscure. The fact that *BTS* gene is transcriptionally induced by low iron, combined to the ability of BTS protein to bind iron and act as an E3 ubiquitin ligase targeting group IVc bHLHs, suggests that BTS could integrate local and long-distance iron signaling pathways (Selote *et al.*, 2015) (Fig. 1). Another candidate for integrating both pathways is FIT itself, provided that its activation by calcium and CIPK11 would be confined as a local response. Further dissection of the regulatory mechanisms involved in iron-deficiency responses using split-root experiments is required to disentangle the contribution of the local and systemic.

Iron uptake and *IRT1* expression are highly connected to other pathways, with several endogenous and exogenous cues (e.g. circadian clock or hormones) impinging on *IRT1* transcription (Brumbarova *et al.*, 2015). This is achieved mostly through the integration of many signaling pathways converging at the level of *IRT1* promoter to provide the plant with an optimized and robust iron uptake machinery. Well-characterized examples include the interaction between FIT and the ethylene signaling transcription factors ETHYLENE INSENSITIVE-3 (EIN3)/EIN3 LIKE-1 (EIL1) or the gibberellin signaling DELLA repressors that both modulate FIT activity and iron-deficiency responses (Lingam *et al.*, 2011; Wild *et al.*, 2016).

Ubiquitin-mediated endocytosis and degradation of IRT1

Several nutrient transporters from model organisms have been shown to be downregulated at the protein level upon substrate excess. Paramount to this downregulation is the role of ubiquitin(Ub)-mediated endocytosis and degradation in the vacuole. In the case of IRT1, iron excess does not trigger IRT1 degradation (Barberon *et al.*, 2011; Dubeaux *et al.*, 2018). Rather, *IRT1* is post-translationally regulated by non-iron divalent metals (Zn, Mn, Co) that are also substrates of IRT1 due to their similarity with iron and that massively accumulate in plant cells upon iron deficiency (Eide *et al.*, 1996; Vert *et al.*, 2002). IRT1 is gradually removed from the plasma membrane by differential ubiquitination with exposure to increasing concentrations of these highly reactive non-iron metals. Moderate non-iron metal concentrations trigger the multimono-ubiquitination of IRT1 on lysine(K) K154 and K179 by an unknown plasma membrane-localized E3 Ub ligase (Barberon *et al.*, 2011; Barberon *et al.*, 2014; Dubeaux *et al.*, 2018). This triggers IRT1 internalization towards early endosomes and a decrease in the plasma membrane pool of the transporter. Higher concentrations of non-iron metals lead to the extension of multimono-ubiquitination into K63-linked polyUb chains by the RING E3 Ub ligase IRON DEGRADATION FACTOR-1 (IDF1) (Dubeaux *et al.*, 2018), and presumably by the E2 UB-CONJUGATING ENZYMES-35/36 (UBC35/36) that are responsible for K63 polyUb chain formation in Arabidopsis (Li & Schmidt, 2010). The conversion of multimono-ubiquitin decorating IRT1 into K63 polyUb chains occurs somewhere between the cell surface and early endosomes, and is thought to increase the avidity for the recognition by the ENDOSOMAL SORTING COMPLEX REQUIRED FOR TRANSPORT (ESCRT) necessary for vacuolar targeting (Dubeaux & Vert, 2017). Besides the conserved subunits from the ESCRT complex found in eukaryotes, a plant-specific protein ESCRT-associated factor named FAB1, YOTB, VAC1, EEA1-1 (FYVE1) has been shown to interact with both IRT1 and Ub to drive proper intracellular sorting of endocytosed IRT1 (Barberon *et al.*, 2014; Gao *et al.*, 2014). Another endosomal located protein, SORTING NEXIN-1 (SNX1), also controls the fate of IRT1 by promoting its recycling to the cell surface (Ivanov *et al.*, 2014). K63 polyubiquitination of IRT1 likely tugs the balance between vacuolar targeting and recycling to push IRT1 towards the lytic vacuole for degradation.

Overall, the dual substrate-dependent control of IRT1 ensures that plants limit the accumulation of potentially noxious heavy metals. However, this would occur at the expense of the uptake of essential iron without a trick taking advantage of different signaling mechanisms. Plants indeed use a systemic control of *IRT1* transcription by low iron via a shoot-borne signal, and a local regulation by non-iron metal excess underlying metal transport rates (Vert *et al.*, 2003; Dubeaux *et al.*, 2018). Consequently, plants experiencing iron

limitation will accumulate IRT1 protein in roots only where non-iron metals are low to optimize the uptake of essential iron (Fig. 2).

The metal-dependent destabilization of IRT1 by IDF1 raises important questions about the mechanisms allowing the recruitment of IDF1 to IRT1 upon metal stress, considering that *IDF1* expression appears to be rather constitutive. The phosphorylation of target to be ubiquitinated is often a trigger to recruit E3 Ub ligases. IRT1 follows this rule with the recruitment of IDF1 to multimonoubiquitinated IRT1 upon metal excess requiring phosphorylation of IRT1 in serine/threonine residues neighboring Ub sites (Dubeaux *et al.*, 2018). This is achieved by the CIPK23 kinase, a well-known kinase phosphorylating other plant transport proteins such as ARABIDOPSIS K⁺ TRANSPORTER-1 (AKT1) and HIGH AFFINITY K⁺ TRANSPORTER-5 (HAK5), CHLORATE RESISTANT-1/NITRATE TRANSPORTER 1.1 (CHL1/NRT1.1) or AMMONIUM TRANSPORTER 1 (AMT1) (Xu *et al.*, 2006; Ho *et al.*, 2009; Ragel *et al.*, 2015; Straub *et al.*, 2017). Although the reported mechanisms of transporter regulation appear to be radically different for AKT1, CHL1/NRT1.1 and IRT1, CIPK23 is a central regulator in all cases. Interestingly, no crosstalk appears to exist between these CIPK23-dependent processes, suggesting that these responses are likely spatially restricted so that locally activated CIPK23 specifically modifies the correct target. This is in part explained, in the case of IRT1, by the original mechanism of CIPK23 triggered by direct metal binding to IRT1 (see below).

From IRT1 transporter to IRT1 transceptor

A major advance in our understanding of how plants regulate IRT1 and plant metal responses came from the discovery that IRT1 acts as a bifunctional transporter, capable of directly binding and sensing non-iron metal availability (Grossoehme *et al.*, 2006; Dubeaux *et al.*, 2018). IRT1 perceives intracellular non-iron metal excess using a histidine-rich motif in an intrinsically disordered cytosolic loop (Dubeaux *et al.*, 2018) (Fig. 1). Mutation of the corresponding histidine residues does not impair metal transport but abolishes downstream responses to high non-iron metal levels culminating in the degradation of IRT1 (Kerkeb *et al.*, 2008; Dubeaux *et al.*, 2018). Mechanistically, the binding of non-iron metals to such histidine residues triggers the recruitment of CIPK23, which phosphorylates IRT1 (Dubeaux *et al.*, 2018). The sensing loop has been proposed to sit at the exit of the permeation domain, thus perceiving the flux of metals going through IRT1. This suggests that the histidine-rich motif is loaded with non-iron metal upon high metal flux to recruit CIPK23 and trigger IRT1 degradation by IDF1-dependent K63 polyubiquitination. One may speculate that metal

binding to the histidine-rich domain in IRT1 may fold this otherwise unstructured loop (Fig. 3), offering an interacting platform to specifically interact with CIPK23. A less likely, but not exclusive hypothesis, may reside in metal-loaded IRT1 serving as Mn donor to directly activate the peculiar Mn-dependent CIPK23 kinase (Hashimoto *et al.*, 2012). This would however not explain the ability of Zn alone to trigger IRT1 Ub-mediated endocytosis. Regardless, these two processes would result in high spatial restriction of CIPK23 recruitment and activation, thus providing specificity to the metal response to IRT1. Future structural studies of IRT1, unbound and bound to metals and possibly in complex with CIPK23, will help unravel the precise molecular mechanisms employed by IRT1 to sense and recruit downstream factors required for its own degradation.

IRT1 is the only reported target of its own sensing/signaling activity in the control of plant metal uptake and responses to metal overload. Other transceptors in yeast and plants have however been shown to drive various aspects of nutrient responses (Steyfkens *et al.*, 2018). For example, the CHL1/NRT1.1 Arabidopsis nitrate transceptor controls : i) the fast induction of early nitrate-responsive genes, ii) the long-term repression of nitrate responses by high nitrogen provision, and iii) the modification of root system architecture (Ho *et al.*, 2009; Bouguyon *et al.*, 2015). Whether IRT1 is also part of other aspects of plant metal responses, such as the genomic reprogramming upon changes in non-iron metal availability, will have to be investigated using plants expressing mutant versions for the histidine-rich motif to uncouple transport and sensing.

Conclusions

The downstream signaling mechanisms of transporter regulation are rapidly emerging, but the early events of sensing and initial signal transduction remain elusive in most cases. These may use a genuine receptor, as described for nutrient-sensing G Protein-Coupled Receptors (GPCRs) in yeast and mammals, or a more direct sensing/signaling mechanism by transporter themselves (Holsbeeks *et al.*, 2004). Receptors have been proposed to originate from nutrient transporters, due to the similarity between ligand such as neurotransmitters and nutrients (Boyd, 1979). Transceptors would represent an intermediate evolutionary step between transporters and receptors capable of sensing substrates, which have been conserved to directly detect nutrient molecules in the environment or inside the cell. Transceptors represent a perfect option to participate in the detection of substrate availability, intracellular substrate concentration or even substrate flux, thus acting as local sensors.

Transceptors are either actively transporting nutrient permeases as receptor like GENERAL AMINO ACID PERMEASE-1 (GAP1) in yeast (Donaton *et al.*, 2003), or proteins homologous to transporters capable of sensing but devoid of transport activity such as the SUCROSE NON-FERMENTING-3 (SNF3) yeast sucrose transceptor (Ozcan *et al.*, 1996). In plants, no GPCR-type nutrient sensor has been described to date and only a handful of transceptors have been demonstrated or proposed (Steyfkens *et al.*, 2018). All the reported examples of plant transceptors fall into the actively transporting category. Besides the well-characterized CHL1/NRT1.1 and IRT1, other possible transceptors comprise the AMT1 family of ammonium transporters and the SULFATE TRANSPORTER 1;2 (SULTR1;2) (Lima *et al.*, 2010; Rogato *et al.*, 2010; Zhang *et al.*, 2014).

To be considered as a transceptor, a transporter must bind substrates either in the binding pocket used for entry into the translocation channel or to another location to trigger divergent substrate-dependent conformational changes to initiate signaling (Diallinas, 2017). While for IRT1 the identity of the metal binding site used for perception could be anticipated by the ability of histidine to directly bind metals, binding sites for most substrates cannot be predicted. While more transceptors likely exist in plants, their identification will likely emerge from forward genetic screens, through the isolation of mutations uncoupling transport and sensing functions, and from biochemical studies using non-transported substrates analogs able to activate signaling. These approaches, combined to the flourishing number of structural studies on membrane proteins including transporters/transceptors, will certainly shed light on the atomic determinants of substrate binding sites, of substrate transport and selectivity, and finally of early signaling mechanisms.

Acknowledgements

We apologize to authors whose relevant works have not been cited because of length constraints. We thank Maria Naranjo Arcos for critical reading of the manuscript and helpful suggestions. The support of the French National research Agency (ANR, ANR-13-JSV2-0004-01), CNRS (ATIP and ATIP Plus program), the Fondation pour le Recherche Médicale (FRM, ECO20170637545), and the French Laboratory of Excellence “TULIP” (ANR-10-LABX-41; ANR-11-IDEX-0002-02) is gratefully acknowledged.

References

- Barberon M, Dubeaux G, Kolb C, Isono E, Zelazny E, Vert G. 2014.** Polarization of IRON-REGULATED TRANSPORTER 1 (IRT1) to the plant-soil interface plays crucial role in metal homeostasis. *Proc Natl Acad Sci U S A* **111**(22): 8293-8298.
- Barberon M, Zelazny E, Robert S, Conejero G, Curie C, Friml J, Vert G. 2011.** Monoubiquitin-dependent endocytosis of the iron-regulated transporter 1 (IRT1) transporter controls iron uptake in plants. *Proc Natl Acad Sci U S A* **108**(32): E450-458.
- Bouguyon E, Brun F, Meynard D, Kubes M, Pervent M, Leran S, Lacombe B, Krouk G, Guiderdoni E, Zazimalova E, et al. 2015.** Multiple mechanisms of nitrate sensing by *Arabidopsis* nitrate transceptor NRT1.1. *Nat Plants* **1**: 15015.
- Boyd CA. 1979.** Chemical neurotransmission: an hypothesis concerning the evolution of neurotransmitter substances. *J Theor Biol* **76**(4): 415-417.
- Brumbarova T, Bauer P, Ivanov R. 2015.** Molecular mechanisms governing *Arabidopsis* iron uptake. *Trends Plant Sci* **20**(2): 124-133.
- Colangelo EP, Guerinot ML. 2004.** The essential basic helix-loop-helix protein FIT1 is required for the iron deficiency response. *Plant Cell* **16**(12): 3400-3412.
- Diallinas G. 2017.** Transceptors as a functional link of transporters and receptors. *Microb Cell* **4**(3): 69-73.
- Donaton MC, Holsbeeks I, Lagatie O, Van Zeebroeck G, Crauwels M, Winderickx J, Thevelein JM. 2003.** The Gap1 general amino acid permease acts as an amino acid sensor for activation of protein kinase A targets in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* **50**(3): 911-929.
- Dubeaux G, Neveu J, Zelazny E, Vert G. 2018.** Metal Sensing by the IRT1 Transporter-Receptor Orchestrates Its Own Degradation and Plant Metal Nutrition. *Mol Cell* **69**(6): 953-964 e955.
- Dubeaux G, Vert G. 2017.** Zooming into plant ubiquitin-mediated endocytosis. *Curr Opin Plant Biol* **40**: 56-62.
- Eide D, Broderius M, Fett J, Guerinot ML. 1996.** A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proc Natl Acad Sci U S A* **93**(11): 5624-5628.
- Gao C, Luo M, Zhao Q, Yang R, Cui Y, Zeng Y, Xia J, Jiang L. 2014.** A unique plant ESCRT component, FREE1, regulates multivesicular body protein sorting and plant growth. *Curr Biol* **24**(21): 2556-2563.
- Gratz R, Manishankar P, Ivanov R, Koster P, Mohr I, Trofimov K, Steinhorst L, Meiser J, Mai HJ, Drerup M, et al. 2019.** CIPK11-Dependent Phosphorylation Modulates FIT Activity to Promote *Arabidopsis* Iron Acquisition in Response to Calcium Signaling. *Dev Cell* **48**(5): 726-740 e710.
- Grossoehme NE, Akilesh S, Guerinot ML, Wilcox DE. 2006.** Metal-binding thermodynamics of the histidine-rich sequence from the metal-transport protein IRT1 of *Arabidopsis thaliana*. *Inorg Chem* **45**(21): 8500-8508.
- Hashimoto K, Eckert C, Anschutz U, Scholz M, Held K, Waadt R, Reyer A, Hippler M, Becker D, Kudla J. 2012.** Phosphorylation of calcineurin B-like (CBL) calcium sensor proteins by their CBL-interacting protein kinases (CIPKs) is required for full activity of CBL-CIPK complexes toward their target proteins. *J Biol Chem* **287**(11): 7956-7968.
- Ho CH, Lin SH, Hu HC, Tsay YF. 2009.** CHL1 functions as a nitrate sensor in plants. *Cell* **138**(6): 1184-1194.

- Holsbeeks I, Lagatie O, Van Nuland A, Van de Velde S, Thevelein JM. 2004.** The eukaryotic plasma membrane as a nutrient-sensing device. *Trends Biochem Sci* **29**(10): 556-564.
- Ivanov R, Brumbarova T, Blum A, Jantke AM, Fink-Straube C, Bauer P. 2014.** SORTING NEXIN1 is required for modulating the trafficking and stability of the *Arabidopsis* IRON-REGULATED TRANSPORTER1. *Plant Cell* **26**(3): 1294-1307.
- Kerkeb L, Mukherjee I, Chatterjee I, Lahner B, Salt DE, Connolly EL. 2008.** Iron-induced turnover of the *Arabidopsis* IRON-REGULATED TRANSPORTER1 metal transporter requires lysine residues. *Plant Physiol* **146**(4): 1964-1973.
- Li W, Schmidt W. 2010.** A lysine-63-linked ubiquitin chain-forming conjugase, UBC13, promotes the developmental responses to iron deficiency in *Arabidopsis* roots. *Plant J* **62**(2): 330-343.
- Li X, Zhang H, Ai Q, Liang G, Yu D. 2016.** Two bHLH Transcription Factors, bHLH34 and bHLH104, Regulate Iron Homeostasis in *Arabidopsis thaliana*. *Plant Physiol* **170**(4): 2478-2493.
- Liang G, Zhang H, Li X, Ai Q, Yu D. 2017.** bHLH transcription factor bHLH115 regulates iron homeostasis in *Arabidopsis thaliana*. *J Exp Bot* **68**(7): 1743-1755.
- Lima JE, Kojima S, Takahashi H, von Wiren N. 2010.** Ammonium triggers lateral root branching in *Arabidopsis* in an AMMONIUM TRANSPORTER1;3-dependent manner. *Plant Cell* **22**(11): 3621-3633.
- Lingam S, Mohrbacher J, Brumbarova T, Potuschak T, Fink-Straube C, Blondet E, Genschik P, Bauer P. 2011.** Interaction between the bHLH transcription factor FIT and ETHYLENE INSENSITIVE3/ETHYLENE INSENSITIVE3-LIKE1 reveals molecular linkage between the regulation of iron acquisition and ethylene signaling in *Arabidopsis*. *Plant Cell* **23**(5): 1815-1829.
- Meiser J, Lingam S, Bauer P. 2011.** Posttranslational regulation of the iron deficiency basic helix-loop-helix transcription factor FIT is affected by iron and nitric oxide. *Plant Physiol* **157**(4): 2154-2166.
- Ozcan S, Dover J, Rosenwald AG, Wolfl S, Johnston M. 1996.** Two glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression. *Proc Natl Acad Sci U S A* **93**(22): 12428-12432.
- Ragel P, Rodenas R, Garcia-Martin E, Andres Z, Villalta I, Nieves-Cordones M, Rivero RM, Martinez V, Pardo JM, Quintero FJ, et al. 2015.** The CBL-Interacting Protein Kinase CIPK23 Regulates HAK5-Mediated High-Affinity K⁺ Uptake in *Arabidopsis* Roots. *Plant Physiol* **169**(4): 2863-2873.
- Rogato A, D'Apuzzo E, Barbulova A, Omrane S, Parlatti A, Carfagna S, Costa A, Lo Schiavo F, Esposito S, Chiurazzi M. 2010.** Characterization of a developmental root response caused by external ammonium supply in *Lotus japonicus*. *Plant Physiol* **154**(2): 784-795.
- Selote D, Samira R, Matthiadis A, Gillikin JW, Long TA. 2015.** Iron-binding E3 ligase mediates iron response in plants by targeting basic helix-loop-helix transcription factors. *Plant Physiol* **167**(1): 273-286.
- Sivitz A, Grinvalds C, Barberon M, Curie C, Vert G. 2011.** Proteasome-mediated turnover of the transcriptional activator FIT is required for plant iron-deficiency responses. *Plant J* **66**(6): 1044-1052.
- Sivitz AB, Hermend V, Curie C, Vert G. 2012.** *Arabidopsis* bHLH100 and bHLH101 control iron homeostasis via a FIT-independent pathway. *PLoS One* **7**(9): e44843.
- Steyfkens F, Zhang Z, Van Zeebroeck G, Thevelein JM. 2018.** Multiple Transceptors for Macro- and Micro-Nutrients Control Diverse Cellular Properties Through the PKA

- Pathway in Yeast: A Paradigm for the Rapidly Expanding World of Eukaryotic Nutrient Transceptors Up to Those in Human Cells. *Front Pharmacol* **9**: 191.
- Straub T, Ludewig U, Neuhauser B. 2017.** The Kinase CIPK23 Inhibits Ammonium Transport in *Arabidopsis thaliana*. *Plant Cell* **29**(2): 409-422.
- Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat JF, Curie C. 2002.** IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* **14**(6): 1223-1233.
- Vert GA, Briat JF, Curie C. 2003.** Dual regulation of the *Arabidopsis* high-affinity root iron uptake system by local and long-distance signals. *Plant Physiol* **132**(2): 796-804.
- Wild M, Daviere JM, Regnault T, Sakvarelidze-Achard L, Carrera E, Lopez Diaz I, Cayrel A, Dubeaux G, Vert G, Achard P. 2016.** Tissue-Specific Regulation of Gibberellin Signaling Fine-Tunes *Arabidopsis* Iron-Deficiency Responses. *Dev Cell* **37**(2): 190-200.
- Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, Wu WH. 2006.** A protein kinase, interacting with two calcineurin B-like proteins, regulates K⁺ transporter AKT1 in *Arabidopsis*. *Cell* **125**(7): 1347-1360.
- Yuan Y, Wu H, Wang N, Li J, Zhao W, Du J, Wang D, Ling HQ. 2008.** FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in *Arabidopsis*. *Cell Res* **18**(3): 385-397.
- Zhang B, Pasini R, Dan H, Joshi N, Zhao Y, Leustek T, Zheng ZL. 2014.** Aberrant gene expression in the *Arabidopsis* SULTR1;2 mutants suggests a possible regulatory role for this sulfate transporter in response to sulfur nutrient status. *Plant J* **77**(2): 185-197.
- Zhang J, Liu B, Li M, Feng D, Jin H, Wang P, Liu J, Xiong F, Wang J, Wang HB. 2015.** The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in *Arabidopsis*. *Plant Cell* **27**(3): 787-805.

Figure legends

Figure 1. Metal sensing and signaling in the root epidermis.

When plants are in demand for iron, the *IRON-REGULATED TRANSPORTER-1* (*IRT1*) gene is transcriptionally upregulated in the root epidermis in response to local and long-distance signals underlying iron availability and the shoot iron status, respectively. How these signals are integrated is currently unknown. Downstream of BRUTUS (BTS) are found group IVc bHLH transcription factors (bHLH34, 105, 105, 115) that are positive regulators of iron deficiency responses. bHLH_{IVc} bind to the promoters of bHLH_{IVb} (bHLH38, 39, 100, 101) transcription factors to activate their transcription in iron-starved plants. bHLH_{IVb} heterodimerize with the FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) bHLH and bind to *IRT1* promoter, thus driving *IRT1* gene expression in iron-deficient conditions. FIT activity is positively regulated by CALCINEURIN B-LIKE-INTERACTING PROTEIN KINASE-11 (CIPK11)-dependent phosphorylation, represented by a phosphate group attached to FIT. Non-iron metals (Zn, Mn, Co) transported by IRT1,

due to their similarity with iron, are directly sensed by IRT1 in root epidermal cells through a stretch of histidine residues in a cytosolic loop to negatively regulate IRT1.

Figure 2. Spatial separation of metal-dependent signaling pathways targeting *IRT1*.

IRT1 transcription in response to low iron is dually controlled by local and long-distance signals (red arrows) to allow *IRT1* gene expression in roots. When roots encounter a non-iron metal-rich soil patch, IRT1 protein is locally degraded in that area of the soil. IRT1 protein is therefore only found in the zone depleted for non-iron metals to take up traces of essential iron and limit the absorption of highly-reactive non-iron metals.

Figure 3. Molecular basis of IRT1 Tranceptor function.

When plants need to take up iron and express high levels of IRT1 in a soil that contains non-iron metals, the flux of the latter is directly sensed by a histidine-rich stretch in IRT1 sitting in an unstructured cytosolic loop at the exit of IRT1 permeation domain. Metal-bound IRT1 recruits the CIPK23 kinase, which in turn phosphorylates serine and threonine residues in the vicinity of the histidine-rich motif. Phosphorylated IRT1 has the ability to interact with the E3 ubiquitin ligase IRON DEFICIENCY FACTOR-1 (IDF1) to promote lysine(K)-63 polyubiquitin-mediated endocytosis and degradation of IRT1. We propose that non-iron metal binding to histidine residues folds the otherwise unstructured loop (tentatively represented here by a helix) to allow the recruitment of the CIPK23 kinase.