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Review

Getting a sense for signals: Regulation of the plant iron deficiency response ☆

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ABSTRACT

Understanding the Fe deficiency response in plants is necessary for improving both plant health and the human diet, which relies on Fe from plant sources. In this review we focus on the regulation of the two major strategies for iron acquisition in plants, exemplified by the model plants *Arabidopsis* and rice. Critical to our knowledge of Fe homeostasis in plants is determining how Fe is sensed and how this signal is transmitted and integrated into a response. We will explore the evidence for an Fe sensor in plants and summarize the recent findings on hormones and signaling molecules which contribute to the Fe deficiency response. This article is part of a Special Issue entitled: Cell Biology of Metals.

1. Introduction

Fe is essential for both plant growth and crop yields and most importantly, humans rely on dietary Fe from plant sources. According to the World Health Organization, the current most common nutritional disorder in the world is Fe deficiency, with over 30% of the world's population affected [http://www.who.int/nutrition/topics/ida/en/index.html]. Due to the limited solubility of Fe in most neutral or basic soils, there is not a readily accessible supply of Fe in the rhizosphere and plants are often limited in Fe content. Thus, increasing the ability of plants to acquire and store Fe could have significant effects on plant and human nutrition. With this goal in mind, it is important to uncover the mechanisms of how plants sense and respond to Fe availability.

When faced with Fe limitation, plants employ a set of responses to boost Fe mobilization and uptake from soil so they can ensure there is enough Fe for critical cellular processes [1]. Fe is an essential cofactor in metabolic processes such as the respiratory electron transport chain. Additionally, as photosynthetic organisms, plants require Fe for chlorophyll biosynthesis and for the reactions of photosynthesis. There are two main strategies plants use for Fe acquisition. First, Strategy I, based on reduction of Fe, is used by non-grasses such as *Arabidopsis*. Second, grasses (also known as graminaceous plants) such as rice use Strategy II, a chelation based strategy. We will briefly outline the basic components of each of these strategies and then discuss in detail the regulation of each of these strategies. For a more comprehensive overview of the two strategies as well as discussion of how our knowledge about these strategies has led to transgenic crops with enhanced tolerance to iron deficiency or with increased iron content, the reader is referred to Kobayashi and Nishizawa [2]. This review will also explore the possibilities for how Fe is sensed and how different signals are integrated into the response, with particular attention to the recent advances in the field.

2. The reduction strategy

Upon Fe limitation, plants which use the reduction strategy release protons via root plasma membrane H^+-ATPases belonging to the AHA family [3,4]. As exemplified by *Arabidopsis*, several AHAs are induced in Fe-deficient roots, but analysis of loss of function mutants suggests that AHA2 is the chief player [5]. This acidification by proton release serves to increase the solubility of Fe in the soil. One unit drop in pH increases the solubility of Fe by 1000 fold [6]. Following acidification, Fe^{3+} is reduced to Fe^{2+} by a membrane-bound ferric-chelate reductase enzyme, i.e. by AtFRO2 in *Arabidopsis* or PsFRO1 in pea [7,8]. Reduction seems to be a rate-limiting step in Fe uptake because transgenic overexpression of ferric chelate reductases in *Arabidopsis*, rice, tobacco, and soybeans increases tolerance to low iron [9–12]. The reduced form of Fe is transported into the root by the plasma-membrane divalent cation transporter IRT1 [13,14], the founding member of the ZIP family [15]. IRT1 is an essential gene because *irt1* mutants are severely chlorotic and seedling-lethal unless supplied with large amounts of exogenous Fe [16–18]. Expression of *IRT1* and *FRO2* indicates that Fe uptake occurs predominantly in epidermal layers [16,19].

Besides these physiological mechanisms, plants respond to Fe deficiency through morphological changes that result in increased root

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surface area for the reduction and uptake of Fe. Examples include increased formation and branching of root hairs, root-tip swelling, and enhanced lateral root formation [20,21].

3. The chelation strategy

Grasses release phytosiderophores (PSs), such as mugineic acids (MAs), which bind Fe^{3+} with high affinity, in order to acquire Fe from the rhizosphere in Fe-limited conditions [22]. Phytosiderophores are synthesized from nicotianamine (NA), a non-proteinogenic amino acid formed by condensation of three molecules of S-adenosyl methionine. Although all plants can synthesize NA, which serves as a transition metal chelator, only the grasses go on to convert NA to PS. The chelated complexes of Fe(III)–PS are subsequently transported into the roots through yellow stripe (YS)/yellow stripe-like (YSL) family transporters, named for YS1 of maize [23,24]. For example, OsYSL15 is the major transporter responsible for Fe(III)–PS uptake in rice [25,26]. Other members of the YSL family transport metal–NA complexes in both grasses and non-grasses.

Although the biosynthetic pathway and the uptake transporters have been well studied [2], the mechanism by which PS are released remained unknown. The missing piece was recently identified: two transporters of the major facilitator superfamily (MFS), TOM1 and HvTOM1 from rice and barley respectively, were shown to be involved in the efflux of the PS deoxymugineic acid [27]. *Xenopus* oocytes expressing either transporter were able to release ^{14}C-labeled deoxymugineic acid but not ^{14}C-labeled NA, suggesting that TOM1 and HvTOM1 are PS efflux transporters.

In the same study, two other rice MFS members, ENA1 and ENA2, were identified as NA transporters by their ability to transport ^{14}C-labeled NA, but not ^{14}C-labeled deoxymugineic acid [27]. ENA1 is similar to AtZIF1, which localizes to the vacuolar membrane and was shown to be involved in Zn detoxification [28]. Although originally thought to be a Zn transporter given its localization and the zinc sensitive phenotype of an *atzif1* loss of function mutant, its similarity to ENA1 suggested that AtZIF1 might be a NA transporter. Recently, overexpression of *ZIF1* has been shown to enhance NA accumulation in vacuoles [29]. Additionally, heterologous expression of ZIF1 increases NA content in yeast cells expressing nicotianamine synthase, but does not complement a Zn-hypersensitive mutant that lacks vacuolar Zn transport activity. Similarly, ENA1 may participate in metal detoxification by transporting NA into the vacuole.

Despite being a Strategy II plant by uptake of Fe(III)–PS, rice possesses a ferrous transporter, OsIRT1, and can take up Fe^{2+} [30,31]. Evidence in support of the importance of being able to take up Fe^{2+} comes from a study of rice that cannot synthesize PS due to a mutation in the *NICOTIANAMINE AMINOTRANSFERASE (NAAT)* gene. This mutant can still grow normally when supplied with Fe^{2+} [30]. Furthermore, lines carrying a T-DNA insertion in the YSL15 transporter gene are viable. Strategy II plants, however, do not have an inducible ferric-chelate reductase activity that is a classic part of the Strategy I response. This is likely the result of an adaptation to waterlogged rice paddies, in which Fe^{2+} is more prevalent than Fe^{3+} due to reduced oxygen levels [31].

4. Regulation of the reduction strategy

There have been a number of transcriptomic and proteomic studies aimed at determining gene expression and protein profile changes upon Fe deficiency. Proteomic studies are beyond the scope of this review, but for a recent discussion of the Fe deficient protein profile in *Arabidopsis*, refer to Schmidt and Buckhout [32]. Several recent transcriptomic studies have aimed at identifying regulatory networks involved in Fe homeostasis in *Arabidopsis* [32–35]. Here, we will discuss two main networks that are currently at the forefront of the field: the FIT network and the POPEYE network. For another recent review on regulation of the reduction strategy, refer to Ivanov et al. [36]. Both Ivanov et al. [36] and the study by Long et al. [37] use co-expression analysis to show regulatory networks involved in Fe deficiency responses.

4.1. The FIT network

In *Arabidopsis*, FIT (FER-like iron-deficiency-induced transcription factor), is required for regulation of the Strategy I Fe-deficiency response [38–41]. FIT is the functional ortholog of FER, a bHLH transcription factor essential for the Fe-deficiency response in tomato [38–41]. In addition to *IRT1* and *FRO2*, *FIT* is induced in the epidermis of the root upon Fe-deficiency and like the *irt1* mutant, *fit* is seedling lethal unless watered with supplemental Fe [38]. FIT controls the Fe uptake machinery at multiple levels: *FRO2* is transcriptionally regulated by FIT, while *IRT1* is both transcriptionally and posttranscriptionally regulated by FIT [38,39]. Recent studies have examined how FIT itself is regulated [42–44]. Sivitz et al. [44] demonstrated a dual regulation of FIT by Fe starvation. At the transcriptional level, *FIT* is induced by Fe starvation and FIT protein subsequently accumulates. The second mode of regulation is post-transcriptional. Using proteasomal and translational inhibitors, they showed that in Fe-limited conditions, FIT is actively destabilized and turned over by 26S proteasomal degradation. The authors suggest that in Fe deficiency, FIT binds to its target promoters and then this “exhausted” FIT is rapidly degraded. “Fresh” FIT is synthesized to allow for subsequent transcriptional cycles and amplification of FIT target gene transcription. This idea, that a limited half life of transcriptional activators may promote continuous gene expression by delivery of “fresh” activator after “fatigued” activator is spent, has previously been described [45]. Alternatively, a more simple explanation is that the decreased stability and increased proteasomal degradation of FIT in Fe-deficient conditions is to ensure that once synthesized, FIT can be rapidly removed from the cell. The function of this would be to prevent FIT from activating gene expression when it is no longer needed (i.e. when Fe supply increases).

Regulated turnover and activity of FIT strengthen the notion that FIT is a major transcriptional regulator of the Fe deficiency response. Evidence suggests that FIT is a key regulator in integrating incoming hormonal and other intracellular signals. For example, ethylene signaling effects FIT abundance via two transcription factors in the ethylene signaling pathway [42]. This model for FIT turnover will be discussed in Section 7.1.3. Another signaling molecule, NO, has also been implicated in posttranslational regulation of FIT [43]. It is proposed to play a role in stabilization of FIT protein. These findings will be discussed with further detail in Section 7.1.5. Overall, control of FIT turnover and activity may be the major way plant roots respond to changing Fe availability in the rhizosphere and meet the nutritional demand for Fe.

Overexpression of *FIT* does not affect *FRO2* and *IRT1* expression in the root, suggesting that FIT may act with a binding partner to form a heterodimer [38–40]. qRT-PCR experiments have shown that mRNA for four additional bHLH genes (*bHLH38*, *bHLH39*, *bHLH100*, and *bHLH101*) are induced by Fe-deficiency [40,46,47]. Using bimolecular fluorescence complementation, FIT was shown to interact with both bHLH38 and bHLH39 [47]. In plants constitutively overexpressing both *FIT* and *bHLH38* or *bHLH39*, *FRO2* and *IRT1* expression are high and plants exhibit greater Fe accumulation than WT [47]. These data support a role for FIT heterodimer formation with either bHLH38 or bHLH39 for induction of the Strategy I response.

In a recent report [48], co-overexpression of *FIT* and *bHLH38* or *bHLH39* was shown to increase expression of *HMA3* and *IRT2* in addition to *MPT3* and *IREG2*/*FPN2*, which are previously published FIT targets [38]. These genes encode proteins that function in sequestration of heavy metals in vacuoles and vesicles. One result of such overexpression is increased Cd sequestration in roots and therefore less Cd