Review

Iron sensors and signals in response to iron deficiency

Takanori Kobayashi [a,b], Naoko K. Nishizawa [b,*]

[a] Japan Science and Technology Agency, PRESTO, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[b] Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi, Ishikawa 921-8836, Japan

ABSTRACT

The transcription of genes involved in iron acquisition in plants is induced under iron deficiency, but our understanding of iron sensors and signals remains limited. Iron Deficiency-responsive Element-binding Factor 1 (IDEF1) and Hemerythrin motif-containing Really Interesting New Gene- and Zinc-finger proteins (HRZs)/BRUTUS (BTS) have recently emerged as candidate iron sensors because of their functions as potent regulators of iron deficiency responses and their iron-binding properties. IDEF1 is a central transcriptional regulator of graminaceous genes involved in iron uptake and utilization, predominantly during the early stages of iron deficiency. HRZs/BTS are E3 ubiquitin ligases and negative regulators of iron deficiency responses in both graminaceous and non-graminaceous plants. Rice OsHRZ1 and OsHRZ2 are also potent regulators of iron accumulation. Characterizing these putative iron sensors also provides clues to understanding the nature of iron signals, which may involve ionized iron itself, other metals, oxygen, redox status, heme and iron-sulfur clusters, in addition to metabolites affected by iron deficiency. Systemic iron responses may also be regulated by phloem-mobile iron and its chelators such as nicotianamine. Iron sensors and signals will be identified by demonstration of signal transmission by IDEF1, HRZs/BTS, or unknown factors.

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1. Introduction

Fe is an essential element in virtually all living organisms. Fe is required in many metabolic processes, including photosynthesis and respiration. Despite its high abundance in the soil, Fe is only slightly soluble especially under alkaline and aerobic conditions. Plants grown under low Fe availability, such as in calcareous soils, often suffer from Fe deficiency, which reduces growth, crop yield and quality [1]. Thus, the development of crops tolerant to low Fe

availability, or with high levels of Fe for human nutrition has long been pursued.

Living organisms have evolved elaborate systems that acquire adequate amounts of Fe from the environment and transport it throughout the body. Higher plants take up Fe from the rhizosphere using two major strategies: the reduction strategy (Strategy I) and a chelation strategy (Strategy II) [2]. Non-graminaceous plants utilize Strategy I, whereas graminaceous plants possess a specific ability to synthesize potent Fe(III) chelators designated mugineic acid family phytosiderophores and utilize Strategy II [2,3]. Molecular components of these strategies have been characterized [4–7]. These strategies have previously been considered mutually exclusive, but some exceptions have recently been reported in which Strategy II plants possess partial Strategy I uptake systems and *vice versa* [4,8,9].

Excessive Fe is also deleterious, as ionized Fe^{2+} catalyzes the generation of reactive oxygen species in the Fenton reaction where H_2O_2 is converted to highly reactive hydroxyl radicals, promoting oxidative stress [1]. Because of this toxic nature of Fe, Fe uptake mechanisms are induced only under low Fe availability, and repressed under Fe sufficiency. Fe is thought to be chelated by various biomolecules in the plant body in both ferrous and ferric forms to keep solubility and prevent toxicity. Only a small portion of ionized Fe^{2+} and Fe^{3+} is thought to be dissociated from the chelating molecules by equilibrium reaction, and these free Fe ions, preferably Fe^{2+}, are thought to be incorporated into Fe proteins and other biomolecules. Fe overload induces expression of Fe-storage protein ferritin, which sequesters Fe in non-toxic form [4,10].

Genes involved in Fe acquisition strategies are transcriptionally upregulated in response to Fe deficiency [4–6]. This is in contrast to the animal system, in which the primary components of Fe acquisition are post-transcriptionally regulated [11,12]. Key transcription factors regulating Fe acquisition-related genes have been identified in both non-graminaceous and graminaceous plants [4,5]. However, signal substances and the sensors regulating this response have not been identified. In this review, we summarize recent findings which shed light on Fe sensors and signals. Although numbers of metabolites affected by Fe nutritional conditions could act as Fe signaling molecules, we mainly focus on possibilities of more direct Fe sensing which might be performed by binding Fe and other metals by Fe regulators.

2. Candidate iron sensors

2.1. Definition of iron sensors

Oxford dictionaries (http://oxforddictionaries.com/) define the word "sensor" (noun) as "a device which detects or measures a physical property and records, indicates, or otherwise responds to it". From a biochemical and physiological standpoint, we propose a definition of Fe sensor in a living system as a biomolecule that (i) binds Fe or an intimately related molecule(s) (input); (ii) thereby changes its function (transmission and conversion); and (iii) regulates Fe homeostasis (output).

Known Fe sensors conforming to these criteria include the bacterial ferric uptake regulation (Fur) protein [13] and the mammalian iron regulatory protein (IRP)/iron responsive element (IRE) system [11] (Fig. 1). Fur is a ferrous Fe-binding transcriptional repressor [criteria (i)]. When the Fur lacks Fe, it loses its DNA-binding activity [criteria (ii)]. Consequently, Fur is unable to repress Fe acquisition-related genes under Fe-deficient conditions [criteria (iii)] (Fig. 1A). On the other hand, IRP1 and IRP2 post-transcriptionally regulate mammalian Fe responses by binding to IRE, which is a stem-loop structure found in various mRNAs involved in Fe homeostasis. When bound to IRP, IRE located in

Table 1
Comparison of IDEF1 and HRZs/BTS as candidate Fe sensors.

Functions as a Fe sensor	IDEF1	HRZs/BTS
(i) Binding Fe or an intimately related molecule(s) (input)		
Binding Fe	Yes (Fe^{2+})	Yes
Binding other metals	Yes (Zn^{2+} *etc.*)	Yes (Zn)
Binding other molecules	?	?
(ii) Thereby changing its function (transmission and conversion)		
DNA binding	No change?[a]	?
Transactivation	Increased without metals?[a]	?
Degradation/acumulation	?[b]	No change?[c]
Modification	?	?
Binding co-regulators	?[b]	?[d]
Localization	?	?
Ubiquitination activity	–	?
(iii) Regulating Fe homeostasis (output)		
Fe deficiency response	Yes (positive)	Yes (negative)[e]
Fe deficiency tolerance	Yes (positive)	Yes (negative)
Fe accumulation	No?	Yes (negative)[c]

[a] Based on *in vitro* and yeast results. In contrast, transgenic rice plants overexpressing *IDEF1* without metal-binding regions fail to induce target genes at an early stage of Fe deficiency [19], suggestive of a positive involvement of metal binding in DNA binding and/or transactivation *in planta*.

[b] An IDEF1-binding Bowman-Birk trypsin inhibitor IBP1 protects IDEF1 from protein degradation, and the *IBP1* transcript level is induced under Fe deficiency [25].

[c] Results reported only for rice HRZs but not for *Arabidopsis* BTS [20].

[d] BTS interacts with bHLH transcription factors involved in regulation of Fe homeostasis [21].

[e] Results reported precisely for rice HRZs but only preliminarily for *Arabidopsis* BTS [20,21].

the 5′-untranslated regions represses translation, while IRE in 3′-untranslated regions stabilizes its mRNA. Under Fe-replete conditions, IRP1 binds an Fe–sulfur (S) cluster [criteria (i)] and loses its ability to bind IRE [criteria (ii)], negating its IRE-mediated regulation [criteria (iii)]. IRE in the 5′-untranslated regions also binds ferrous Fe, changing its binding affinities with both IRP and translation initiation factors [14]. IRP2 is another IRE-binding protein lacking a Fe–S cluster. This protein loses its activity under Fe sufficiency because of Fe-dependent proteasomal degradation mediated by another Fe sensor, the F-box leucine rich repeat protein 5 (FBXL5) [15,16]. FBXL5 binds Fe *via* the hemerythrin domain [criteria (i)] and mediates ubiquitination of IRP2 [criteria (iii)]. Under low Fe conditions, FBXL5 itself is subjected to proteasomal degradation, which is associated with the absence of Fe in the hemerythrin domain [criteria (ii)] [15–17] (Fig. 1B).

Neither the Fur and IRP/IRE systems, nor a biomolecule conforming to all of these three criteria, have not been identified in plants. Nevertheless, our recent studies identified two types of regulatory proteins that conform to (i) and (iii) of the above-mentioned criteria; namely, Iron Deficiency-responsive Element-binding Factor 1 (IDEF1) [18,19] and Hemerythrin motif-containing Really Interesting New Gene (RING)- and Zinc-finger proteins (HRZs) [20] in rice (Table 1 and Fig. 2). HRZs are homologous to the previously identified protein BRUTUS (BTS) in *Arabidopsis thaliana* [21].

2.2. IDEF1

IDEF1 has been identified as a rice transcription factor that specifically binds the CATGC sequence within the Fe deficiency-responsive *cis*-acting element IDE1 [18], and it is a positive regulator of the majority of rice genes responsible for Fe uptake and utilization especially during early stages of Fe deficiency [18,22] (Fig. 2). IDEF1-binding sequence was also predicted as one of the most predominantly accumulated sequences within 500 bp-upstream regions of Fe deficiency-responsive genes in rice roots [23], suggesting that IDEF1 plays an important role in the response to Fe deficiency. *IDEF1* transcript levels do not change in response to Fe availability [18,22,24], in contrast to the majority of other