Molecular basis and fitness implications of the interplay between light and the regulation of iron homeostasis in photosynthetic organisms

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Abstract

Photosynthetic organisms respond to changes in the external environment, including responses to changes in light color, intensity and nutrient availability. Iron is one of the nutrients that is critical for the function of photosynthetic organisms, primarily due to the high demand for iron in the photosynthetic photosystems, electron transport chains of plastids and mitochondria, and the structural and functional roles for iron in many critical proteins. Given the connection between iron demands and light-dependent processes such as photosynthesis, as well as the interplay between light and iron, there is a need for finely tuned co-regulation of light and iron acclimation responses to optimize photosynthesis and minimize potential dangerous interactions between iron and light. Such regulation is critical for balancing light and nutrient availability in the coordination of light-dependent aspects of organismal fitness. To accomplish this regulation, there is molecular crosstalk and/or common effectors in light signaling and iron acclimation control. We explore known paradigms that are central to light-iron interactions and the associated regulation of fitness in photosynthetic organisms.

Keywords: cyanobacteria, iron, light, photoreceptor, phytochrome, plants

1. Introduction

1.1. Iron and physiological responses in photosynthetic organisms. Iron (Fe) is a key element that has both structural and functional roles required for the growth of organisms from microbes (Hantke, 2001) to eukaryotes (Castagna et al., 2009; Connolly and Guerinot, 2002; de Silva et al., 1996; Walker and Connolly, 2008). In a variety of organisms, iron is required for a number of critical cellular functions ranging from the synthesis of heme and heme-dependent oxygen transport, to functions in redox and iron-dependent enzymatic reactions, to ribonucleotide synthesis, photosynthesis, and nitrogen fixation (Straus, 1994). In fact, Fe is an essential nutrient for plants and other photosynthetic organisms. The regulation of iron homeostasis and availability is central to optimal growth and productivity in these organisms as iron is essential for respiration and the activity of photosynthetic light-harvesting systems in many organisms capable of oxygenic photosynthesis, including cyanobacteria. Iron serves as a component of iron-sulfur (Fe-S) clusters, heme-containing cytochromes, and nonheme iron cofactors in photosynthetic protein complexes (reviewed by Shcolnick and Keren, 2006). Iron is also important as a cofactor in enzymes and a component of key substrates that are required for the production of phytochromes. Photosensory phytochromes are light-sensitive photoreceptors that impact plant growth and development beyond photosynthesis, including seed germination, cotyledon and leaf development, stem elongation and the photoperiodic induction of flowering, among others (e.g., Li et al., 2011a). Thus, apart from direct roles in the process of photosynthesis, in which light energy is converted to chemical energy, iron also impacts organismal fitness through impacting the production of components of photosynthetic organisms such as phytochromes needed for light-dependent regulation of growth, development and mitigation of damage.

1.2. Light-iron metabolism crosstalk. Photosynthetic organisms sense changes in and adapt to environmental fluctuations of light and nutrient availability. Whereas both light and iron reduce fitness when limited, in excess both iron and light can also limit fitness by resulting in the

generation of reactive oxygen species (ROS). Superoxide, hydrogen peroxide and other ROS molecules are generated and accumulate under stressful conditions in plants, including in response to excess light (Li et al., 2009) and when the overaccumulation of iron can result in the formation of highly reactive and potentially damaging hydroxyl radicals (Halliwell and Gutteridge, 1992). Thus, interplay between light absorption, excitation pressure, oxidative damage and iron homeostasis are critical for photosynthetic organisms to properly acclimate to variable environments. Light and iron are specifically linked as the ability to convert photons to chemical energy during photosynthesis requires iron-rich photosystems and other iron-dependent components (Kolber et al., 1994). Levels of ROS can increase when light excitation exceeds the photosynthetic capacity of cells, and iron limitation is one stress that can contribute to such an imbalance (for review see Latifi et al., 2009). In this sense, light can become co-limiting at low iron concentrations due to limitation of Fe-containing photosynthetic proteins in some cases (Thamatrakoln et al., 2013). Iron-dependent enzymes, such as catalases, peroxidases, and some superoxide dismutases, also contribute to mitigating damage that can occur when ROS are generated and accumulate in cells (Michel and Pistorius, 2004). Thus, iron limitation can indirectly support ROS accumulation due to reduced cellular ROS-mitigating capacity. Given the requirement of iron for light-dependent process such as photosynthesis, together with the potential for the generation of damaging products when iron and light are coordinately in excess, the need for finely-tuned regulation of plant responses to light and iron availability are tightly linked. Thus, balancing iron homeostasis with changes in the external photoenvironment and organismal iron demands is critical for fitness of plants and other photosynthetic organisms. Together, these observations suggest potential strong connections and/or overlap in the regulation of organismal responses to light and iron availability. Here, we discuss general mechanisms for iron uptake and transport in photosynthetic organisms, the interplay between iron homeostasis and light responses, and compare strategies used by plants and cyanobacteria for regulating light-iron interactions.

2. Iron transport strategies in photosynthetic organisms

Fe is abundant in soil; however, due to its low solubility, it is either assimilated by plants that exude compounds which contribute to acidifying the soil and thereby lead to reduction of iron to a form competent for uptake and conversion by roots (common in dicots such as *Arabidopsis*) or iron is chelated and taken up as iron-chelator complexes by roots (common in monocots) (Kim and Guerinot, 2007; Olsen et al., 1981) (Fig. 1). The widely studied model plant *Arabidopsis thaliana* (hereafter *Arabidopsis*) uses a reduction-based strategy, known as Strategy I, for iron uptake in response to iron-deficiency. Central to this strategy is a family of Fe-reductase enzymes encoded by the *Ferric Reductase Oxidase* (*FRO*) gene family and iron transporters encoded by the *Iron Regulated Transporter* (*IRT*) gene family (Jeong and Connolly, 2009). When the rhizosphere is acidified by secreting protons into the soil, the ferric to ferrous iron reduction is catalyzed by Fe(III)-chelate reductase (FROs) at the surface of roots before transfer into the plant (Fig. 1). Reductive iron uptake also has been reported in a cyanobacterial system (Kranzler et al., 2014). Other organisms, including grasses and many cyanobacteria, use a chelation-based strategy, i.e., Strategy II, for responding to iron deficiency (e.g. Abadía et al., 2011). In this strategy, organisms synthesize and secrete Fe-chelating siderophores under Fe-deficient conditions, followed by uptake of iron-siderophore complexes into the cell (Abadía et al., 2011). Siderophores are Fe(III) chelators with high binding affinity for iron (Kranzler et al., 2013). Yellow Stripe (YS) transporters contribute to this Strategy II iron acquisition response through the transport of siderophore-iron complexes which function to increase iron uptake (Jeong and Connolly, 2009).

2.1. IRT- and FRO- based iron reduction and uptake in plants. There are 8 FROs in *Arabidopsis* which are expressed at different levels and in different parts of the plant (Jain et al., 2014; Wu et al., 2005). *FRO2*, *FRO3*, *FRO6*, and *FRO7* are most highly abundant among the family members (Mukherjee et al., 2006). *FRO2* is expressed in roots, *FRO3* is expressed in

112 roots and leaves, and *FRO6* and *FRO7* are expressed in shoots (Mukherjee et al., 2006).
113 Expression of both *FRO2* and *FRO3* is induced by iron deficiency (Mukherjee et al., 2006).
114 *FRO1* is expressed at low levels in siliques and to a lesser degree in flowers, *FRO4* is expressed
115 at very low levels with some expression in siliques (Mukherjee et al., 2006). *FRO5* is expressed
116 in roots, similar to *FRO2* (Mukherjee et al., 2006), and has also been reported to be expressed in
117 leaves (Bauer et al., 2004). The final family member *FRO8* is enriched in shoots (Mukherjee et
118 al., 2006).

119 Perhaps, the most well-known ferric chelate reductase in *Arabidopsis* is *FRO2*, a root-
120 localized protein which facilitates reduction and uptake of iron from soil (Robinson et al., 1999)
121 (Fig. 1). Mutations in *FRO2* result in reduced ferric chelate reductase activity in roots in
122 response to iron limitation (Yi and Guerinot, 1996), whereas *FRO2* overexpression leads to
123 resistance to low iron levels (Connolly et al., 2003). *FRO6* has been reported to function as a
124 ferric chelate reductase localized to the plasma membrane (Jeong et al., 2008) and to facilitate
125 iron uptake in leaf cells (Li et al., 2011b). Closely related *FRO7* has been reported to be localized
126 to chloroplasts and to also contribute to Fe(III) chelate reductase activity (Jeong et al., 2008).
127 Although characterization of a *fro6* mutant has not been reported, *fro7* mutants have reduced
128 ferric reductase activity and lower iron levels in chloroplasts (Jeong et al., 2008). *FRO4* and
129 *FRO5* are more likely to be involved in copper acquisition (Bernal et al., 2012). The expression
130 of both of these genes is induced by copper deficiency and depletion of *FRO4* and *FRO5* results
131 in a reduction of copper-specific reductase activity and copper uptake by roots (Bernal et al.,
132 2012). *FRO3* and *FRO8* have been implicated in mitochondrial function (Jeong and Connolly,
133 2009). Both mitochondria and chloroplasts play central roles in cellular iron homeostasis due to
134 the role of iron in electron transport components in each of these compartments (Jain and
135 Connolly, 2013). Mutants for *FRO1*, *FRO3*, *FRO6*, and *FRO8* have yet to be reported.

136 Following iron reduction by FRO family proteins, IRT transporters function to take up
137 the reduced iron into the plant (Jeong and Connolly, 2009). There are three identified *IRT* genes

in Arabidopsis, of which *IRT1* and *IRT2* have been well characterized (Eide et al., 1996; Vert et al., 2001; Vert et al., 2002). *IRT1* was first identified based on its ability to restore functionality to a *fet3fet4* yeast mutant lacking low- and high-affinity iron uptake systems (Dix et al., 1994). Both *IRT1* and *IRT2* are root localized and induced by iron deficiency (Connolly et al., 2002; Vert et al., 2001; Vert et al., 2002). *IRT1* serves as a high-affinity transporter (Vert et al., 2002), whereas *IRT2* appears to have a distinct function, as its overexpression fails to complement an *irt1* mutant (Vert et al., 2009). *irt1* mutants exhibit chlorosis and reduced iron transport (Vert et al., 2002), whereas *irt2* mutants do not (Vert et al., 2009). These distinctions led to the observation that *IRT2* has a function in root-dependent responses to iron deficiency, but not a direct role in transport as does *IRT1* (Vert et al., 2009). It has been suggested that *IRT2* may function to sequester iron that has been transported into a plant cell to avoid deleterious impacts of free iron accumulation (Vert et al., 2009).

2.2. YSL functions in iron homeostasis in plants. Additional genes previously shown to impact iron homeostasis are the *Yellow Stripe-Like (YSL)* family, of which there are eight members in Arabidopsis (Chu et al., 2010). *YSL1*, *YSL2* and *YSL3* are purported to function as iron transporters (DiDonato et al., 2004; Waters et al., 2006). *YSL1* and *YSL3* are plasma membrane localized (Chu et al., 2010). *YSL1* localizes to shoots (Conte et al., 2013; DiDonato et al., 2004). *YSL2*, *YSL3*, and *YSL6* are localized in shoots and roots (Conte et al., 2013; DiDonato et al., 2004), whereas *YSL4* is ubiquitous (Conte et al., 2013). *YSL7* and *YSL8* appear to accumulate in leaves, but to a greater degree in roots (Hofstetter et al., 2013). Expression of *YSL1*, *YSL2*, and *YSL3* has been reported to be downregulated in shoots and roots in response to iron limitation (Conte et al., 2013; DiDonato et al., 2004; Waters et al., 2006). Based on mutation studies, the functions of some *YSL* family members have been assessed. Neither a *ysl1* mutant nor a *ysl3* mutant exhibits visible phenotypes as seedlings or adults (Le Jean et al., 2005; Waters et al., 2006); however, *ysl1* has reduced iron content in seeds (Le Jean et al., 2005). A *ysl1ysl3* double mutant does have an iron-deficient phenotype, including chlorosis and low levels of iron

(Waters et al., 2006). A *ysl2* mutant had no observable phenotype under either replete or iron-deficient conditions (DiDonato et al., 2004). YSL4 and YSL6 reportedly have roles in plant responses to iron excess (Chu et al., 2013; Divol et al., 2013), and/or heavy metal stress (Conte et al., 2013). Accordingly, the expression of neither *YSL4* nor *YSL6* is significantly impacted by iron limitation (Conte et al., 2013); however, the level of expression of these genes increases in the presence of excess iron (Divol et al., 2013). Neither single *ysl4*, *ysl6*, nor double *ysl4ysl6* mutants have observable phenotypes under normal conditions, but *ysl4ysl6* mutants are hypersensitive to excess iron (Conte et al., 2013; Divol et al., 2013). Mutants for *ysl7* and *ysl8* recently have been shown to exhibit defects in sensitivity to a bacterial virulence factor, which suggests that YSL7 and YSL8 function as transporters of a virulence factor (Hofstetter et al., 2013). However, a double *ysl7ysl8* mutant is dwarfed and exhibits reduced fertility under standard growth conditions; thus, it was proposed that these genes may also contribute to metal homeostasis, in addition to responses to pathogens (Hofstetter et al., 2013). A role for *YSL5* has yet to be confirmed in vivo.

2.3. Nramp functions in iron homeostasis in plants. Based on their ability to complement the mutant phenotype of a metal uptake deficient yeast strain, a family of six genes was identified in Arabidopsis, i.e., the *AtNramp* (*Natural resistance associated macrophage protein*) family (Thomine et al., 2000). *AtNramp1* was demonstrated to be expressed in shoots and roots and its expression is upregulated under low iron conditions (Curie et al., 2000). Overexpression of *AtNramp1* results in increased tolerance to high levels of iron (Curie et al., 2000). Recent characterization of an *nramp1* mutant, however, suggests a functional role for plasma-membrane localized *AtNramp1* in manganese homeostasis (Cailliatte et al., 2010). *AtNramp2* is root-enriched under iron sufficient conditions and its mRNA levels decreased under iron limitation (Curie et al., 2000). The function of *AtNramp2* in vivo has not been confirmed (Curie et al., 2000). Expression of *AtNramp3* and *AtNramp4* is observed in both roots and shoots and increases under iron starvation conditions (Thomine et al., 2000). Single *nramp3* or *nramp4*

mutants do not have significant observable iron-dependent phenotypes (Lanquar et al., 2005). A *nramp3* mutant, however, exhibits sensitivity to cadmium (Thomine et al., 2000). Double *nramp3nramp4* mutants are hypersensitive to low iron and fail to remobilize iron stored in seeds (Lanquar et al., 2005; Roschztardt et al., 2009). *AtNramp5* remains to be characterized. *AtNramp6* was recently shown through mutant and overexpression analyses to have a role in plant responses to cadmium (Cailliatte et al., 2009).

2.4. Ferritin functions in iron homeostasis. Ferritins function in iron storage in plastids and/or in protection against oxidative stress, the latter due in part to consumption of oxygen and hydrogen peroxide during the oxidation of ferrous iron to the storage form of ferric iron (reviewed by Briat et al., 2010). Protection against oxidative stress by ferritins may also be conferred by their contribution to cells avoiding the accumulation of free iron that could result in the production of ROS (Ravet et al., 2009). There are four ferritin genes in Arabidopsis, i.e., *AtFer1* to *AtFer4* (Petit et al., 2001). *AtFer1*, *AtFer3* and *AtFer4* are found in leaves and stems of mature plants, whereas *AtFER2* is found in seeds and seedlings (reviewed by Briat et al., 2010). *AtFer1* and *AtFer3* expression increases during iron overload (Petit et al., 2001). The impact of the absence of ferritins was assessed through mutant analyses. A *fer2* mutant does not have lower iron content, but does exhibit reduced germination under oxidative growth conditions, indicating a role for *AtFer2* in protection against oxidative stress during germination (Ravet et al., 2009). Characterization of a triple *fer1fer3fer4* mutant lacking leaf-localized ferritins indicated that the mutant has reduced growth when irrigated with excess iron and exhibits increased oxidative stress under standard growth conditions (Ravet et al., 2009). There are many ferritins and ferritin-like proteins in cyanobacteria that similarly function in iron storage (Keren et al., 2004) and organismal responses to oxidative stress (Ekman et al., 2014).

2.5. Other central regulatory genes which function in plant iron homeostasis. *FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT)* is a transcription factor gene whose expression is induced in iron-deficient growth (Colangelo and Gueriot,

2004). FIT impacts iron homeostasis through regulation of additional factors, including
impacting FRO2 and IRT1 accumulation (Colangelo and Guerinot, 2004). *POPEYE* (*PYE*) and
BRUTUS (*BTS*) are relatively recently identified transcription factors that impact root responses
to iron deficiency (Long et al., 2010). Both *PYE* and *BTS* are induced by iron deficiency and
regulate the expression of iron homeostasis genes, including *PYE*-regulated genes *IRT1*, *FRO3*,
Nramp4, and ferritin genes (Long et al., 2010). The gene *Permease in Chloroplasts1* (*PIC1*) has
been shown to function in iron transport in chloroplasts, and thereby also impacts iron
homeostasis (Duy et al., 2007). *PIC1* is ubiquitously expressed. Mutations of *PIC1* result in
chlorophyll deficiency, yet *pic* mutants exhibit increased levels of ferritins (Duy et al., 2007).

3. Light sensing and iron acclimation in plants

Iron transport is light-dependent in some photosynthetic systems (Bughio et al., 1997;
Fujii et al., 2011). Studies using radiolabelled iron demonstrated that iron transport from leaf
veins to mesophyll cells and into isolated chloroplasts is light-dependent in barley (Bughio et al.,
1997). In addition to direct light-dependent measurements of iron uptake, the expression of *IRT1*
and *FRO2* have been shown to increase during the day and to decrease at night under iron
sufficient conditions in *Arabidopsis* (Vert et al., 2003), suggesting light-dependent regulation of
iron uptake in this system as well. Notably, this diurnal regulation of *IRT1* and *FRO2* can be
overridden by plant iron status as the day vs. night difference in expression is not apparent under
iron-limited growth conditions (Vert et al., 2003). Strategy II-related siderophore production and
export is also impacted by light as iron-chelating siderophore secretion appears to be regulated
by light in some plants (Reichman and Parker, 2007; Takagi et al., 1984). The release of
siderophores has been detected under Fe-deficient conditions during the light phase of growth for
several plants (Gries and Runge, 1992; Ma et al., 2003; Reichman and Parker, 2007; Takagi et
al., 1984; Zhang et al., 1991). Thus, iron uptake can be impacted by light for both strategy I and
strategy II organisms. Furthermore, the high requirement for iron in photosynthetic photosystems

also supports a link between light-driven processes and cellular iron demands. Together, these observations and physiological requirements indicate that specific aspects of iron reduction and/or uptake are light regulated and suggest that some components in iron homeostasis are regulated by photoreceptors. Some prior associations of light with components of iron sensing and transport that impact the regulation of iron homeostasis have been noted.

3.1. Light and phytochrome-dependent regulation of FRO function. Among *FRO* family members found in Arabidopsis, some are regulated by light at the level of gene expression. For example, *FRO6* expression is enriched in green tissues, its expression is light-dependent and the *FRO6* promoter has light response elements (LRE) (Feng et al., 2006; Jeong et al., 2008). *FRO6* is present only in photosynthetically-active green tissues of the plant suggesting that it has a role in iron homeostasis that is required in light-dependent chlorophyll accumulation and/or chloroplast development, photosynthesis electron transfer and/or protection of cells from reactive oxygen species generated in photosystem (PS) I and PSII (Jeong et al., 2008). We confirmed by qRT-PCR that expression of *FRO6* is regulated by distinct wavelengths of light and observed that expression of closely related *FRO7* is also light regulated (Fig. 2A). It has been proposed that light-dependent regulation of *FRO6* occurs in a tissue-specific and/or developmentally regulated way in plants to coordinate iron acquisition with external developmental and environmental cues (Feng et al., 2006). Notably, a similar coordination of iron responses and external environmental cues may occur in other organisms including cyanobacteria (see below; Pattanaik et al., 2014). As *FRO6* mRNA levels are regulated by light, it is anticipated that specific photoreceptors contribute to its expression. In recent microarray analyses using lines in which we depleted phytochromes from mesophyll cells using transgenic expression of a gene encoding a phytochrome chromophore-degrading enzyme (Lagarias et al., 1997) driven by the *CAB3* promoter (i.e., *CAB3::BVR*) (Oh et al., 2013; Warnasooriya and Montgomery, 2009), we observed downregulation of the expression of a number of genes related to plant iron responses, including *FRO6* and/or its paralog *FRO7* (Oh et al., 2013). *FRO7* is

92.5% identical to *FRO6* and, thus, these genes are indistinguishable in the AtGenExpress visualization tool (Schmid et al., 2005). We thus used gene-specific primers to assess downregulation of *FRO6* and *FRO7* individually by quantitative PCR analyses. Both genes were significantly downregulated in mesophyll phytochrome-deficient lines (Fig. 2B). As the depletion of the phytochrome chromophore results in depletion of all five active phytochromes found in Arabidopsis, we analyzed single *phyA* and *phyB* mutants to assess which of these major phytochromes specifically contributes to the light-dependent expression of *FRO6* and *FRO7*. The expression of *FRO6* was not significantly altered in the dark in the absence of *phyA* (Fig. 2D), though *FRO6* expression was slightly upregulated in the absence of *phyB* relative to WT in the dark (Fig. 2C). By comparison, no differences in expression of *FRO7* were noted in the dark for either *phy* mutant relative to WT (Fig. 2C and 2D). Prior analyses did not indicate a significant misregulation of expression of *FRO6/7* in dark-grown WT, *phyA*, *phyB* or *phyAphyB* lines (Mazzella et al., 2005) largely in agreement with our analysis of dark-grown seedlings. No change was observed in expression of *FRO6/7* in FR-grown *phyA* or R-grown *phyB* mutants exposed to light up to 24 h (Tepperman et al., 2004). However, we observed a lack of significant induction of *FRO6* and a severely attenuated induction of *FRO7* in a *phyB* mutant grown for 7-days under R light relative to dark conditions (Fig. 2C). Also, neither *FRO6* nor *FRO7* were induced in a *phyA* mutant under FR light compared to dark-grown seedlings (Fig. 2D). Together, these data suggest that under FR light, *phyA* promotes accumulation of *FRO6* and *FRO7* mRNA (Fig. 2D), whereas, under R light, *phyB* promotes the accumulation of both of these genes (Fig. 2C). As *FRO7* is still induced to some degree under R light in a *phyB* mutant, additional R-absorbing phytochromes contribute to its induction in addition to *phyB*. Part of the role of phytochromes in regulating iron deficiency is likely due, at least in part, to their contribution to regulating *FRO6* and *FRO7* expression.

FRO6 was previously reported to be light regulated at the level of expression (Feng et al., 2006; Jeong et al., 2008) and given the close relationship of *FRO6* and *FRO7*, light regulation of

the *FRO7* gene was not unexpected. We also assessed the expression of additional *FRO* family members in the array data for lines depleted of phytochromes (Oh et al., 2013). Among the remaining six *FRO* genes, only *FRO3* and *FRO8* were present among genes that showed a statistically significant two-fold change in expression or greater (Oh et al., 2013). Both genes exhibited significantly altered expression, with the *FRO3* gene being significantly upregulated in phytochrome-deficient lines and *FRO8* being downregulated in mesophyll phytochrome-deficient lines (Table 1; Oh et al., 2013).

3.2. Light and phytochrome-dependent regulation of additional iron homeostasis genes. We also assessed expression of *YSL*, *Nramp*, and ferritin genes in phytochrome-deficient lines to determine if the significant impact of phytochromes on the expression of *FRO* genes was observed for other iron homeostasis genes. Notably, *YSL1* was downregulated by ~4-fold in lines depleted of mesophyll phytochromes, whereas *YSL3* and *YSL8* were significantly upregulated in phytochrome-deficient lines (Table 1; Oh et al., 2013). There was no impact on the expression the five remaining *YSL* genes in phytochrome-deficient lines (Table 1; Oh et al., 2013). Two *Nramp* genes were upregulated in mesophyll phytochrome-deficient lines, i.e. *Nramp1* and *Nramp3*, whereas the expression of other *Nramp* genes was not significantly impacted (Table 1; Oh et al., 2013). Two of the four ferritin genes found in Arabidopsis, i.e. *AtFer3* and *AtFer4*, were significantly down regulated in phytochrome-deficient lines (Table 1; Oh et al., 2013).

An interesting response was observed for other root-enriched, iron-response related genes. *IRT2* (Vert et al., 2002) and *PYE* (Long et al., 2010) were significantly upregulated in mesophyll phytochrome-deficient lines (Table 1). *IRT2* was upregulated by 2.2-fold and *PYE* by 1.6-fold. Notably, although no significant alteration in expression of *PYE* was observed for *phyA* or *phyB* single mutant seedlings grown for 6 days under R or FR light of much higher intensity than we used, R or FR exposure of wild-type seedlings resulted in a decreased accumulation of *PYE* mRNA (Ma et al., 2001). This finding indirectly implicates phyA and/or phyB and would suggest a correlation with no change or an increased expression in the absence of phyA or phyB,

the latter of which we observe in phytochrome-depletion lines. The increased expression of *IRT2* and *PYE* in phytochrome-depletion lines, thus, suggests that a deficiency in mesophyll phytochrome-dependent regulation of iron homeostasis genes in shoots results in a response in roots in an attempt to increase iron uptake. This finding correlates with prior observations that *IRT1* and *FRO2* are induced both locally and by a systemic shoot-derived signal in Arabidopsis (García et al., 2013; Mendoza-Cózatl et al., 2014; Vert et al., 2003). Notably, a shoot-to-root signal impacting iron uptake had been suggested even earlier for pea (Grusak and Pezeshgi, 1996) and has also been noted for tobacco (Enomoto et al., 2007, 2009) and rice (Enomoto et al., 2009). Recently, one component that may function in the systemic iron homeostasis signal was reported. The *OPT3* gene has been shown to function in the signaling network between shoots and roots that communicates iron status of leaves to roots (Mendoza-Cózatl et al., 2014). However, the expression of this gene is not impacted by phytochrome depletion (Table 1; Oh et al., 2013)

3.3. Arabidopsis, circadian rhythms, light sensing and plants. Prior studies demonstrated a tight link and reciprocal interactions between the function of the circadian clock and the regulation of iron homeostasis (for recent review see Tissot et al., 2014). For example, TIME FOR COFFEE (TIC), which functions as a regulator of the circadian clock, impacts expression of the *AtFer1* ferritin gene and thereby links the circadian clock to regulation of iron homeostasis (Duc et al., 2009). Iron deficiency also results in lengthening of the period of the clock in Arabidopsis (Chen et al., 2013; Hong et al., 2013; Salome et al., 2013). Clock component PRR7 is also linked to the regulation of three ferritins, i.e., FER1, FER3 and FER 4 (Liu et al., 2013). Notably, a *prr7* mutant is sensitive to excess iron, as is a *cca1lhy* double mutant (Liu et al., 2013). As *CCA1* (Circadian Clock Associated 1) and *LHY* (Late Elongated Hypocotyl) genes and encoded proteins comprise the core feedback loop of the circadian clock (Mizoguchi et al., 2002), a disruption in sensitivity to iron in *prr7* and *cca1lhy* mutants suggests a physiological implication of circadian regulation of iron homeostasis. In the dark, Fe-signaling

is uncoupled from circadian clock function while in light, functional plastids and phytochromes are required for Fe and circadian interactions (Salome et al., 2013). Photoreceptors phyA and phyB specifically have roles in coupling iron status to the circadian clock as the observed lengthening of the circadian period occurs via a mechanism that is phytochrome dependent (Salome et al., 2013). The connections between light signaling, clock function and iron homeostasis, together with prior connections which demonstrate that a clock period matched to the external environment enhances fitness of photosynthetic organisms (Dodd et al., 2005; Green et al., 2002; Ouyang et al., 1998), suggest impacts of coordinating complex signals in variable environments to optimize organismal fitness. The exact mechanisms by which these different signals are coordinated require additional investigations.

4. Light sensing and iron acclimation in cyanobacteria

Cyanobacteria exhibit oxygenic photosynthesis that is very similar to higher plants, but have much higher iron requirements than non-photosynthetic prokaryotes, i.e., as much as 10-fold more, and higher than some other photosynthetic organisms including plants (Kranzler et al., 2013). One potential reason for higher iron demands in these organisms may be related to the fact that iron availability affects both photosynthesis and nitrogen fixation in cyanobacteria (Berman-Frank et al., 2003; Henderson, 2005; Montgomery and Pattanaik, 2010). The use of either a reductive or chelation strategy for iron uptake has environmental implications for cyanobacteria. The specificity of high-affinity transport has been proposed to be effective in dense populations where cells do not readily diffuse away from an iron source, whereas the more generalist reduction strategy is likely to be utilized in dilute environments where different iron forms may be scarce (Kranzler et al., 2013). Similar to the impact of light on stimulating iron uptake in higher plants, iron uptake from the growth medium has been observed as light-dependent in some cyanobacteria, e.g. *Microcystis aeruginosa* (Dang et al., 2012; Fujii et al., 2011). This phenomenon is largely attributed to a photoreductive release of Fe^{3+} that leads to increased iron bioavailability (Fujii et al., 2011). Furthermore, as insoluble iron in aquatic

environments may be reduced by superoxide (reviewed by Kranzler et al., 2013) and superoxide can be light generated during electron transfer reactions in photosynthetic organisms (Sang et al., 2011; Zhang et al., 1999), additional mechanistic connections between light and iron availability are expected. Although cyanobacteria also produce siderophores, no link of siderophore production and/or secretion to light regulation has been reported for these organisms. However, similar to plants, additional aspects of the regulation of iron homeostasis are correlated with light perception and signaling.

4.1. Light and photoreceptor-dependent regulation of iron responses in cyanobacteria. Cross talk between light quality/intensity and iron limitation has been observed for *Fremyella diplosiphon* (Pattanaik et al., 2014; Pattanaik and Montgomery, 2010). Whereas wild-type cells grown under red light in iron-limited medium show classic iron-deficient symptoms such as photosynthetic pigment defects, wild-type cells grown under green light show minor responses to iron limitation (Pattanaik and Montgomery, 2010). RcaE, a cyanobacterial photoreceptor that is related to higher plant phytochromes (Kehoe and Grossman, 1996; Terauchi et al., 2004), impacts the observed light-regulated iron homeostasis responses in the cyanobacterium *F. diplosiphon* as an RcaE-deficient mutant shows resistance to iron limitation under both red and green illumination (Pattanaik et al., 2014). Light and iron also have impacts on cellular morphology (Pattanaik et al., 2014; Pattanaik and Montgomery, 2010). Red light appears to augment the effect of iron limitation on *F. diplosiphon* both at the level of growth and the regulation of stress-induced changes in cellular morphology (Pattanaik et al., 2014; Pattanaik and Montgomery, 2010). This response is regulated as the light quality-dependent difference in iron limitation-associated morphology changes does not occur in a mutant lacking the primary red and green light-responsive photosensory receptor RcaE (Pattanaik et al., 2014).

4.2. Light-dependent regulation of iron homeostasis-associated genes in cyanobacteria. The expression of the cyanobacterial *fur* gene, which is a transcriptional regulator that is responsible for maintaining iron homeostasis, is light dependent in *Microcystis*

aeruginosa PCC7806 (Martin-Luna et al., 2011). This observation supports additional light-iron links in cyanobacteria. Similar to the association of phytochrome function with the expression of *AtFer3* and *AtFer4* in Arabidopsis (Tables 1 and 2), phytochrome-related photoreceptor RcaE was shown to impact expression of numerous ferritin and ferritin-associated genes in the cyanobacterium *F. diplosiphon* (Pattanaik et al., 2014). A *PIC* homolog also has been described for the cyanobacterium *Synechocystis* sp. PCC 6803 (Duy et al., 2007). However, its function has not been fully explored, nor has light regulation or association with a photoreceptor been described.

5. Conclusions

In summary, a number of connections and insights are emerging regarding the molecular basis of the interplay of light and iron homeostasis in photosynthetic organisms, including prokaryotes and eukaryotes. These studies link photochromic photoreceptors which perceive changes in the external light environment with the regulation of responses of photosynthetic organisms to iron limitation and iron limitation-associated regulation of gene expression (Table 2). As photosensory photoreceptors respond to both light quality and light quantity (e.g., (Ballaré et al., 1991)), the linking of the function of these photoreceptors to iron homeostasis, may allow the organism to regulate energy inputs into responding to light fluctuation and iron limitation simultaneously. Such a response would limit the potential interactions that can lead to synergistic and deleterious effects on the organism when light and iron levels are suboptimal, which would impact organismal fitness.

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Figure Legends

Figure 1. Distinct strategies for iron uptake in plants. (A) Strategy I plants extrude protons into the extracellular environment to promote solubilization of insoluble ferric iron (Fe^{3+} insolub) to more soluble forms (Fe^{3+} solub), which are reduced to ferrous iron (Fe^{2+}) by ferric chelate reductases (FRO) at the surface of roots. Fe^{2+} is then taken into the cell by iron regulated transporters (IRT). (B) Strategy II plants chelate iron through synthesizing and excreting siderophores into the extracellular environment. Siderophores function as strong Fe^{3+} chelators and thus iron-siderophore complexes form, which are transported into cells via the Yellow Stripe (YS) family of transporters.

Figure 2. Impact of phytochromes on the expression of *FRO6* and *FRO7* genes in *Arabidopsis*. (A) *Arabidopsis thaliana* Col-0 wild-type (WT) seedlings were grown for 7 d in dark (D), continuous red (Rc; $50 \mu\text{mol m}^{-2} \text{s}^{-1}$), continuous far-red (FRc; $5 \mu\text{mol m}^{-2} \text{s}^{-1}$) or white (W; $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) light before extracting RNA for quantitative RT-PCR (qRT-PCR) as previously described (Oh et al., 2013); (B) No-0 WT, transgenic 35S::pBVR3 (Lagarias et al., 1997) or CAB3::pBVR2 (Warnasooriya and Montgomery, 2009) seedlings were grown for 7 d under FRc at $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ before extracting RNA from whole seedlings for qRT-PCR analyses; (C, D) Col-0 WT and T-DNA mutants for *phyA* and *phyB* were obtained from the Arabidopsis Biological Resource Center (ABRC; (Alonso et al., 2003). Seedlings were grown for 7 d in D, FRc or Rc illumination before RNA extraction and qPCR analyses. Relative gene expression level of the experimental genes *FRO6* and *FRO7* compared with *UBC21* is shown ($\pm\text{SD}$, $n=3$). Primers used were caaatggaccgctcttatcaaag/ctgaaaaacaccgccttcgt for *UBC21* (*At5g25760*), acgatgctttcaaggccaaag/cacatatcaaccatagctgtttcca for *FRO6* (*At5g49730*), and aacgcctccttaaaagccga/ggtttcatgatcatccatgtgt for *FRO7* (*At5g49740*). Statistics: Unpaired, two-tailed Student's t test comparing lines to dark-grown Col-0 WT (A, C, D) or far red-grown No-0

736 WT (B) (unless two bars are connected by a line with a symbol), *, $p < 0.05$, **, $p < 0.01$, ***,
737 $p < 0.001$.

Table 1. Expression levels of Arabidopsis gene families impacting iron homeostasis in wild-type and phytochrome-deficient lines.

Gene name	Locus	Average of Signal values (expression) $\pm$ SD		
		WT	35S::pBVR3	CAB3::pBVR2
<i>FRO1</i>	<i>AT1G01590</i>	NP	NP	NP
<i>FRO2</i>	<i>AT1G01580</i>	NP	NP	NP
<i>FRO3</i>	<i>AT1G23020</i>	482 $\pm$ 28	851 $\pm$ 158*	855 $\pm$ 137**
<i>FRO4</i>	<i>AT5G23980</i>	NP	NP	NP
<i>FRO5</i>	<i>AT5G23990</i>	NP	NP	NP
<i>FRO6/FRO7</i>	<i>AT5G49730/ AT5G49740</i>	2817 $\pm$ 98	1959 $\pm$ 115***	143 $\pm$ 29***
<i>FRO8</i>	<i>AT5G50160</i>	39 $\pm$ 8	38 $\pm$ 22	16 $\pm$ 6*
<i>IRT1</i>	<i>AT4G19690</i>	447 $\pm$ 554	425 $\pm$ 389	1341 $\pm$ 507
<i>IRT2</i>	<i>AT4G19680</i>	42 $\pm$ 7	53 $\pm$ 34	94 $\pm$ 29*
<i>YSL1</i>	<i>AT4G24120</i>	312 $\pm$ 60	215 $\pm$ 37	76 $\pm$ 51**
<i>YSL2</i>	<i>AT5G24380</i>	174 $\pm$ 59	237 $\pm$ 109	230 $\pm$ 100
<i>YSL3</i>	<i>AT5G53550</i>	479 $\pm$ 10	617 $\pm$ 83*	555 $\pm$ 43*
<i>YSL4</i>	<i>AT5G41000</i>	174 $\pm$ 8	165 $\pm$ 23	163 $\pm$ 32
<i>YSL5</i>	<i>AT3G17650</i>	524 $\pm$ 140	580 $\pm$ 8	529 $\pm$ 48
<i>YSL6</i>	<i>AT3G27020</i>	1031 $\pm$ 184	1155 $\pm$ 152	1254 $\pm$ 230
<i>YSL7</i>	<i>AT1G65730</i>	184 $\pm$ 10	185 $\pm$ 66	177 $\pm$ 61
<i>YSL8</i>	<i>AT1G48370</i>	143 $\pm$ 21	179 $\pm$ 26	235 $\pm$ 18**
<i>ATNRAMP1</i>	<i>AT1G80830</i>	1547 $\pm$ 251	2759 $\pm$ 1108	3702 $\pm$ 494**
<i>ATNRAMP2</i>	<i>AT1G47240</i>	479 $\pm$ 107	462 $\pm$ 19	411 $\pm$ 93

<i>ATNRAMP3</i>	<i>AT2G23150</i>	480±31	468±134	640±40**
<i>ATNRAMP4</i>	<i>AT5G67330</i>	1194±42	1444±265	1413±198
<i>ATNRAMP5</i>	<i>AT4G18790</i>	3±2	16±11	8±8
<i>ATNRAMP6</i>	<i>AT1G15960</i>	23±10	32±14	50±32
<i>FER1</i>	<i>AT5G01600</i>	11309±2422	13391±1275	11932±671
<i>FER2</i>	<i>AT3G11050</i>	236±99	300±120	282±57
<i>FER3</i>	<i>AT3G56090</i>	2617±310	1585±169**	1795±48*
<i>FER4</i>	<i>AT2G40300</i>	1076±32	770±103**	621±157**
<i>PIC1</i>	<i>AT2G15290</i>	766±47	619±112	581±194
<i>FIT1</i>	<i>AT2G28160</i>	NP	NP	NP
<i>POPEYE</i> (<i>PYE</i>)	<i>AT3G47640</i>	158±7	192±3**	257±22**
<i>BRUTUS</i> (<i>BTS</i>)	<i>AT3G18290</i>	421±63	441±124	437±100
<i>OPT3</i>	<i>AT4G16370</i>	554±94	530±42	517±94

Data were extracted from microarrays described in (Oh et al., 2013)

NP, not present on array

Statistics: Unpaired, two-tailed Student's t test comparing transgenic lines to WT, *, p<0.05; **, p<0.01; ***, p<0.001.

744 **Table 2.** Localization and function of phytochrome-impacted iron homeostasis genes in
745 *Arabidopsis*.

Gene name	Locus	Location	Function	Ref.
<i>FRO3</i>	<i>AT1G23020</i>	roots, leaves, mitochondria	Not yet determined	(Jeong and Connolly, 2009; Mukherjee et al., 2006)
<i>FRO6</i>	<i>AT5G49730</i>	shoots, plasma membrane	Ferric chelate reductase	(Jeong et al., 2008; Mukherjee et al., 2006)
<i>FRO7</i>	<i>AT5G49740</i>	shoots, chloroplasts	Ferric chelate reductase	(Jeong and Connolly, 2009; Mukherjee et al., 2006)
<i>FRO8</i>	<i>AT5G50160</i>	shoots, mitochondria	Not yet determined	(Jeong et al., 2008; Mukherjee et al., 2006)
<i>IRT2</i>	<i>AT4G19680</i>	roots	Iron sequestration	(Vert et al., 2009; Vert et al., 2001)
<i>YSL1</i>	<i>AT4G24120</i>	shoots, plasma membrane	Iron transporter	(Chu et al., 2010; Conte et al., 2013; DiDonato et al., 2004; Waters et al., 2006)
<i>YSL3</i>	<i>AT5G53550</i>	shoots and roots, plasma membrane	Iron transporter	(Chu et al., 2010; Conte et al., 2013; Waters et al., 2006)
<i>YSL8</i>	<i>AT1G48370</i>	leaf-enriched, roots	Transporter of a virulence factor, metal homeostasis	(Hofstetter et al., 2013)
<i>ATNRAMP1</i>	<i>AT1G80830</i>	shoots, roots,	Iron homeostasis,	(Cailliatte et al., 2010;

		plasma membrane	manganese homeostasis	Curie et al., 2000)
<i>ATNRAMP3</i>	<i>AT2G23150</i>	shoots, roots	Cadmium homeostasis	(Lanquar et al., 2005; Roschztardt et al., 2009; Thomine et al., 2003)
<i>FER3</i>	<i>AT3G56090</i>	adult leaves and stems	Iron storage, protection against oxidative stress	(Briat et al., 2010; Petit et al., 2001)
<i>FER4</i>	<i>AT2G40300</i>	adult leaves and stems	Iron storage, protection against oxidative stress	(Briat et al., 2010; Petit et al., 2001)
<i>POPEYE</i>	<i>AT3G47640</i>	roots	Iron homeostasis transcription factor	(Long et al., 2010)

Figure 1

Figure 2