Iron Acquisition and Transcriptional Regulation

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1. Introduction: Role and Importance of Iron

Iron is an essential element for all eukaryotes. The facile ability of iron to gain and lose electrons permits its participation in a wide variety of oxidation−reduction reactions. Further, the ability of iron as a component of heme to bind oxygen makes it indispensable as an oxygen carrier and sensor, particularly in vertebrates where the size of the organism provides a barrier to simple oxygen diffusion requiring specialized oxygen transport. Iron is found as a prosthetic group on proteins in various forms: elemental iron, oxoiron or oxoiron−zinc, heme, and iron−sulfur clusters. While the diversity of iron-containing proteins and the reactions they participate in highlights the usefulness of iron in biochemical reactions, iron is problematic for two reasons. First, the same facile property of electron gain and loss that permits iron to participate in oxidation−reduction reactions permits iron to donate electrons to oxygen or hydrogen peroxide, generating toxic oxygen radicals, superoxide anion and hydroxyl radical. The existence of these oxygen radicals has led to the evolution of enzyme systems capable of either preventing or removing oxygen radicals once formed. Loss of these enzymes, such as superoxide dismutase, leads to increased cellular damage and compromised growth. Because iron can be toxic, all iron acquisition systems are highly regulated in order to restrict the concentration of iron within biological fluids, extending from the cytosol of yeast to the plasma of vertebrates. Furthermore, regulation of iron acquisition is one of the most critical steps in maintaining iron homeostasis because eukaryotes do not have regulated mechanisms of iron egress.

The second reason that iron is problematic is that bioavailable iron is scarce. Iron is the fourth most abundant element on earth but is not uniformly distributed. Iron is scarce both because there are vast geographical areas, such as the oceans, which are iron-poor and because iron, when abundant, is found in a biologically inaccessible form. Iron's proclivity to react with oxygen has resulted in the predominant form of iron being the insoluble ferric hydroxide. Great investment of cellular resources is required to make iron bioavailable. Even with such effort, the external concentration of iron is often limiting, leading to cellular responses that "triage" iron to biochemical pathways that most require it. We have come to understand that not only is the acquisition and storage of iron highly regulated, but the processes that divert iron among intracellular biochemical pathways are also

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highly regulated. This review will focus on processes involved in iron acquisition, storage, and dispersal in eukaryotes and their regulation at many levels, focusing most on transcriptional control.

1.1. Regulation of Iron-Related Genes in *S. cerevisiae*

Saccharomyces cerevisiae, bakers' yeast, is among the simplest of the eukaryotes. The importance of iron to this model organism is illustrated in the number of genetically separable iron acquisition pathways and the concerted mechanisms that regulate the transcription of these pathways. For example, *S. cerevisiae* has four different systems that permit it to transport iron across the plasma membrane. Iron acquisition by yeast illustrates the two major mechanisms that most organisms employ to make iron biologically available (Figure 1). Ferric iron (Fe^{3+}) is sparsely soluble (reported solubility of 10^{-18} M). One mechanism widely used by bacteria, fungi, and plants is production of organic molecules, collectively referred to as siderophores, that have a high affinity for iron (for review, see ref 1). For example, desferrioxamine, a siderophore produced by *Streptomyces pilosus*, has an affinity for iron of 10^{-33} M, permitting it to bind Fe^{3+} in aqueous solutions. Siderophores may be secreted into the environment followed by reuptake of iron-bound forms for the capture of environmental Fe^{3+}. Capture of siderophore−iron complexes is mediated through cell surface transporters. Transporters are specific for different siderophores, and *S. cerevisiae* can express up to four different siderophore transporter genes. Siderophore synthesis and transport are regulated by iron demand and are increased under conditions of iron scarcity. Yeast, such as *S. cerevisiae*, that do not make their own siderophores can still express siderophore transporter genes, permitting them to capture siderophores produced by other organisms.[2,3]

The second major approach to make iron bioavailable requires two steps. The first is to acidify the local environment, as the solubility of ferric iron is highly pH dependent. The solubility of iron changes from 10^{-18} M at pH of 7 to 10^{-3} M at pH of 2.0. Most organisms attempt to lower the pH of the external environment to facilitate iron solublization and iron transport. The optimal pH for growth of cultured *S. cerevisiae* is 4.5, which is also the luminal pH of certain cellular compartments such as vacuoles and lysosomes. Vacuoles in yeast and plants are storage organelles for iron, and in vertebrates lysosomes are organelles where iron-containing compounds are recycled. The pH of these organelles ranges from 4.5 to 5.5, which permits increased iron solubility. Vacuolar and cell surface pH are regulated by ATP-driven H^+ transporters, which transport H^+ from the cytosol across the plasma membrane or vacuolar membrane. The activity or synthesis of these transporters is independent of iron. In contrast, the second required component of the iron-solublization system, a ferrireductase, is regulated by iron.[4,5] Ferrireductases are heme-containing transmembrane proteins present on the cell surface or vacuole that utilize cytosolic NADPH to export electrons across membranes[6] with the exported electron converting Fe^{3+} to Fe^{2+}. Ferrireductase is a misnomer, as the same enzymes can also reduce Cu^{2+} to Cu^+ and reduction of copper is required for copper acquisition.[7,8] *S. cerevisiae* has seven ferrireductases localized to the cell surface or the vesicular apparatus including the vacuole.[9]

Fe^{2+} is the substrate for all known elemental iron transporters. *S. cerevisiae* has three elemental iron transporters. First, there is a high-affinity iron transport system composed of a multicopper oxidase Fet3p[10] and a transmembrane permease Ftr1p.[11] In addition to the high-affinity iron transport system, *S. cerevisiae* has two transporters that can transport iron as well as other transition metals such as Mn^{2+}, Cu^+, and Zn^{2+}. One transporter, Fet4p.,[12] is unique to the phylum *Ascomycota*. The second low-affinity transporter, Smf1p, is a member of the NRAMP family that has homologues in many kingdoms including bacteria, plants, and vertebrates.[13] This transporter is an H^+/transition metal symporter, utilizing the pH gradient to transport iron.[14] Smf family members are localized not only at the plasma membrane but also in acidic compartments such as endosomes, vacuoles, or lysosomes.

S. cerevisiae will not grow on iron-limited medium in the absence of the Fet3p/Ftr1p transport system.[10] These two proteins are found in a complex and must be synthesized simultaneously for both to be appropriately transported to the cell surface.[11] Fet3p is biochemically similar to the mammalian multicopper oxidase ceruloplasmin. These enzymes use Fe^{2+} as a substrate and oxidize it to Fe^{3+}.[15,16] The protein oxidizes three atoms of iron sequentially, storing the extracted electrons. When a fourth atom of iron is oxidized, the multicopper oxidase, in one concerted reaction, reduces molecular oxygen to water, preventing the formation of oxygen radicals.

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + H_2O$$

As opposed to low-affinity transport systems, the Fet3p/Ftr1p transport system is specific for iron. It is thought that binding of ferric iron to the transmembrane permease Ftr1p imbues specificity on the transport system.

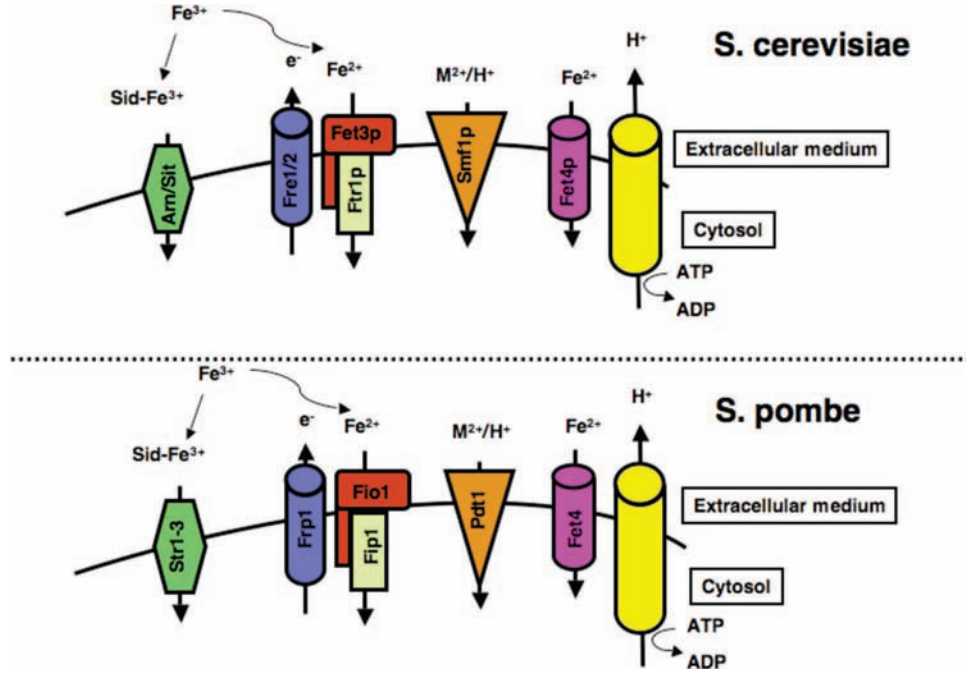

Figure 1. Cell surface iron acquisition in *S. cerevisiae*. Proteins involved in *S. cerevisiae* iron transport are shown in the top panel, and relevant *S. pombe* homologues are shown below. Fe^{3+} can be bound to small organic molecules termed siderophores (Sid) and the iron−siderophore complex taken up by specific transporters. *S. cerevisiae* contains multiple transporters (Arn/Sit proteins, in green)) for siderophore−iron complexes (Sid−Fe^{3+}). Each transporter shows differences in the siderophores they will accept as substrates. Fe^{3+} can be reduced to Fe^{2+} by electrons donated by transmembrane heme containing proteins referred to as ferrireductases (Fre1p/Fre2p). These proteins can also reduce Cu^{2+} to Cu^{+}. In *S. cerevisiae*, there are two major cell surface ferrireductases (blue), of which *FRE2* is regulated primarily by iron and *FRE1* is regulated by copper and iron. Fe^{2+} is taken up by a high-affinity transport system encoded in *S. cerevisiae* by *FET3* and *FTR1* (red and cream, respectively). Fet3p is a multicopper enzyme that oxidizes Fe^{2+} to Fe^{3+}. Fe^{3+} is transported across the plasma membrane by Ftr1p. *S. cerevisiae* also has two low-affinity iron transporters, Fet4p and Smf1p (magenta and orange, respectively). Smf1p is a H^{+}/M^{2+} transporter that can transport transition metals such as Mn^{2+}, Zn^{2+}, and Fe^{2+}, and homologues are present in all eukaryotes. Acquisition of elemental iron across the plasma membrane in all species usually involves acidification of the media to increase iron solubility. Acidification is accomplished by an ATP-driven H^{+} transporter (yellow).

1.1.1. Transcriptional Regulation of Genes Required for Iron Acquisition

Iron acquisition requires gene products to both solubilize iron and then to transport it across membranes. In yeast, the gene products that solubilize iron and acquire it are coordinately regulated in response to iron need. Transcripts are high in low-iron medium and are low in high-iron medium. Transcription of siderophore transporters, ferrireductases, and the high-affinity iron transport system are regulated coordinately through the action of a low-iron-sensing transcription factor Aft1p.[2] Several studies have revealed the overall picture of Aft1p function in responding to iron conditions and cooperation with other transcription factors for fine-scale regulation of the iron regulon, while questions relating to the exact molecular mechanism of iron sensing remain open. *AFT1* was first identified through a genetic screen that identified yeast mutants exhibiting high levels of ferrireductase even in the presence of high-iron medium.[17] The screen utilized a genetically engineered strain in which a histidine biosynthetic enzyme (*HIS3*) was placed under the control of the *FRE1* promoter. In the presence of iron, the strain was a histidine auxotroph and would not grow, but would grow in the absence of iron due to low iron-dependent induction of the *FRE1* promoter and concomitant expression of *HIS3*. Yamaguchi-Iwai et al. identified a mutant strain that was a histidine prototroph in the presence of iron. This strain contained a missense allele of *AFT1*, encoding a constitutively active Aft1p. Critical results that showed that Aft1p was the predominant transcriptional regulator of iron gene transcription included the following: deletion of *AFT1* prevents expression of target genes, a dominant Aft1p mutant leads to constitutive expression of target genes, and Aft1p binds to a specific DNA recognition sequence in the promoters of target genes.[17−19] Aft1p regulates the transcription of target genes, collectively referred to as the iron regulon (Table 1). Most of these genes have recognizable functions in iron metabolism and encode siderophore transporters, reductases, and other proteins that make iron accessible, such as *HMX1*, encoding a heme oxygenase that can liberate iron from heme.

S. cerevisiae also has a paralogue to *AFT1*, *AFT2*, whose function is unclear.[20,21] Deletion of *AFT2* has no effect on the ability of yeast to grow on iron-limited medium or on transcription of iron-regulated genes. Yet, a double deletion of *AFT1* and *AFT2* is more sensitive to iron-limited growth than either single deletion. Overexpression of *AFT2* in an *aft1*Δ strain can regulate the iron regulon in an iron-dependent manner, and Aft2p can be found bound to Aft1p-target genes.[22,23] Analysis of microarray data obtained from strains with deletions of *AFT1* or *AFT2* have shown that Aft2p directs the transcription of the vacuolar iron transporter *SMF3* and a mitochondrial iron transporter *MRS4* in an iron-dependent manner, indicating that Aft2p might regulate transcription of genes involved in intracellular iron homeostasis. Aft2p might play a role in regulating genes that utilize iron under anaerobic conditions. It was suggested that, while Aft1p is more specific to the canonical iron-responsive DNA sequence TGCACCC than Aft2p, Aft2p is able to bind to variant sequences.[22]

Table 1. Iron Regulon of *S. cerevisiae*[a]

gene	location of gene product	function
FET3	cell surface	multicopper oxidase
FTR1	cell surface	iron permease
FRE1	cell surface	ferrireductase
FRE2	cell surface	ferrireductase
FRE3	cell surface	ferrireductase
ARN1	cell surface	siderophore transporter
ARN2/TAF1	cell surface	siderophore transporter
ARN3/SIT1	cell surface	siderophore transporter
ARN4/ENB1	cell surface	siderophore transporter
FIT1	cell surface	siderophore binding
FIT2	cell surface	siderophore binding
FIT3	cell surface	siderophore binding
AKR1	cell surface	palmitoyl transferase
VHT1	cell surface	biotin transporter
FET5	vacuole	multicopper oxidase
FTH1	vacuole	iron permease
FRE6	vacuole	ferrireductase
COT1	vacuole	Co^{2+}/Zn^{2+} transporter
CCC2	Golgi	Golgi copper-transporter required for assembly of Fet3p/Fet5p
ATX1	Golgi	CCC2 copper chaperone required for assembly of Fet3p/Fte5p
HMX1	endoplasmic reticulum	heme oxygenase
CTH1	cytosol	mRNA binding protein
CTH2/TIS11	cytosol	mRNA binding protein
BNA2	cytosol	putative tryptophan 2,3-dioxygenase
ECM4	cytosol	glutathione transferase
ISU1	mitochondria	iron−sulfur cluster assembly protein
MRS4	mitochondria	Fe^{2+} transporter

[a] This table identifies genes regulated by Aft1p and Aft2p. The data is compiled from Rutherford et al.,[123] Courel et al.,[22] and Philpott and Protchenko.[124]

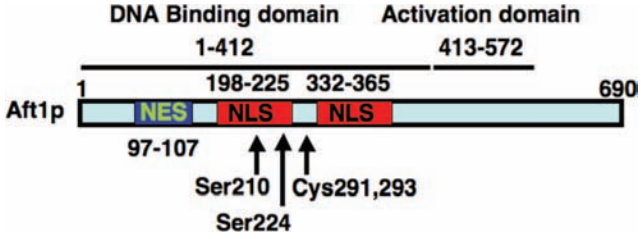

Figure 2. Topology of Aft1p, the low iron-sensing transcription factor in *S. cerevisiae*. The low iron-sensing transcription factor Aft1p from *S. cerevisiae* contains a DNA binding domain, an activation domain, a nuclear localization sequence, and a nuclear export sequence. Listed are two cysteines that, when mutated, lead to constitutive transcriptional activation. Also listed are two serine residues that, when phosphorylated, permit Aft1p to interact with the nuclear export machinery. NES is a sequence of amino acids that is responsible for export of proteins from the nucleus while NLS is a sequence of amino acids that is required for protein import into the nucleus.

While both Aftp1 and Aft2p respond to iron deprivation by activating transcription, the molecular basis of iron sensing remains to be clarified. The amino termini of Aft1p and Aft2p, thought to contain the DNA binding domains, are 39% identical (Figure 2).[18] The amino-terminal domains of both Aft1p and Aft2p contain a homologous CXC motif. Mutation of either cysteine results in constitutive activation of Aft1p/Aft2p targets.[17] Most transcription factors have a DNA binding domain (DBD) and a transcription activation domain or domains (AD(s)). Hypothetically, iron could affect properties of these distinct domains of Aft1p/Aft2p, either DNA binding or transcriptional activation, subsequent to DNA binding. The authors took advantage of the modular Gal4p transcription factor to distinguish these possibilities. The Gal4p transcription factor has clearly defined and separable DBD and AD regions that can function autonomously. Fusion of the carboxyl-terminal portion of Aft1p to the heterologous Gal4p DBD conferred activation to a Gal4p-dependent *GAL1*-reporter gene.[17] These data were interpreted to suggest that the carboxyl-terminal of Aft1p/Aft2p contained the activation domain. Transcription due to the fusion protein was not iron-regulated, suggesting that the iron-regulated step required the binding of the amino terminal domain of Aft1p/Aft2p to DNA. Because of the abundance of information regarding Aft1p, we will focus much of our comments on Aft1p. In conditions of iron sufficiency, Aft1p is primarily a cytosolic protein. In conditions of iron deficiency, Aft1p moves from the cytosol to the nucleus, where it then occupies promoters of the iron regulon.[24] When cells reach iron sufficiency, Aft1p dissociates from promoters and is exported back to the cytosol. There is no evidence that Aft1p binds iron, although the lack of evidence may reflect the difficulties in expressing or purifying Aft1p.

1.1.1.1. Aft1p Regulation: Nuclear-Cytoplasmic Shuttling. Regardless of how Aft1p is activated, a first step in the activation process requires the movement of Aft1p from cytosol to nucleus. The entry of Aft1p into the nucleus is due to two discrete portions of Aft1p.[25] These regions are rich in basic amino acids, and when these sequences were fused to reporter constructs, such as green fluorescent protein (GFP), they could induce nuclear localization of the reporter. It was further demonstrated that the nuclear importer Pse1p was required for import of Aft1p.[25] A temperature-sensitive allele of *PSE1* blocked Aft1p localization to the nucleus as well as transcription of the iron regulon under restrictive growth conditions. Aft1p was shown to bind to Pse1p in vitro in an iron-independent fashion, consistent with a model where iron does not affect the ability of Pse1p to import Aft1p into the nucleus. Furthermore, Aft1p also contains nuclear export signal (NES) sequences. The NES was identified as a single discrete amino acid sequence.[26] Mutation of amino acids within this sequence resulted in the localization of Aft1p in the nucleus even in iron-containing medium, suggesting that Aft1p shuttles between the cytosol

and the nucleus regardless of iron levels. Further, NES-Aft1p mutant proteins were constitutively transcriptionally active. Additional studies showed that export of nuclear Aft1p required the activity of the exportin Msn5p, one of a family of four exportins required for export of different cargo from the nucleus. The authors utilized a yeast two-hybrid analysis to identify protein interactions. This analysis takes advantage of the observation that binding of two proteins can be detected through transcriptional activation. One of the proteins is fused to a heterologous DBD of a transcription factor, and the other is fused to a heterologous AD. An interaction between the two test proteins results in the creation of a transcriptional activator complex made up of two distinct hybrid fusion proteins, one bearing DNA binding capability and the other bearing transcription activation capability, and is a highly sensitive measure of protein interaction. The yeast two-hybrid analysis indicated that Msn5p binds to Aft1p in an iron level-sensitive fashion. Two different domains in Aft1p were shown to both bind to Msn5p and are required for the nuclear export of Aft1p. It is of interest that neither of the domains contained the putative NES. One of the domains, however, contained the amino acid Cys291 that, when mutated, results in nuclear localization and constitutive activation of Aft1p. Aft1p was shown to form a homomultimer in the presence of iron. Ueta et al. also observed that phosphorylations of two serines (S210 and S224) were required for nuclear export but phosphorylation was not iron-dependent. These observations led Ueta et al. to speculate that iron promoted the dimerization of Aft1p, leading to its nuclear export and termination of transcription.[26] It was not reported, however, if deletion of Msn5p led to increased expression of the iron-regulon. Deletion of *MSN5* does not affect viability, yet *MSN5* was not identified in screens for constitutive activation of the iron regulon.[27,28]

1.1.1.2. Aft1p Regulation: Linkage to Fe−S Cluster Synthesis. It seems clear that Aft1p shuttles between the cytosol and nucleus and that nuclear export may be iron regulated. There is also evidence suggesting that the initial movement of Aft1p into the nucleus is regulated. First, overexpression of Aft1p in iron-replete cells results in its movement into the nucleus and activation of the iron regulon. This result would not be expected if the binding of iron to Aft1p were responsible for its retention in the cytosol, although the possibility exists that perhaps some species of iron or an iron-conjugate may become limiting. Second, mutations in genes involved in iron−sulfur cluster synthesis affect Aft1p, resulting in activation of the iron-regulon.[29,30] Mitochondrial iron−sulfur (Fe−S) cluster synthesis requires the activity and participation of a number of proteins (for review, see ref 31). These proteins are transcribed by nuclear encoded genes and are imported into the mitochondria. Fe−S clusters are synthesized in the mitochondria and are utilized within the mitochondria as prosthetic groups on proteins, such as aconitase. Fe−S groups are also exported to the cytosol where a large protein complex assembles Fe−S groups on proteins that are active in both the cytosol and nucleus. Activation of the iron regulon due to mutations in Fe−S encoded enzymes occurs under conditions in which cytosolic iron is high.[32] This observation suggests that activation of the iron regulon is responsive to deficits in mitochondrial Fe−S cluster formation, but not directly to cytosolic iron. Fe−S clusters are an indispensible function of mitochondria and therefore represent an essential role for cellular iron. Among Fe−S cluster-containing proteins is Rli1p, an essential protein located in the cytosol, which is required for rRNA processing and translation initiation.[33]

Loss of Fe−S cluster synthesis leads to activation of transcription of the iron-regulon, which suggests that Aft1p may indirectly sense iron levels through deficits in Fe−S cluster synthesis. Iron deprivation of cells is known to affect the formation of Fe−S clusters as measured by reduced activity in mitochondrial aconitase as well as other Fe−S containing proteins. The question of how Aft1p senses Fe−S cluster synthesis has been addressed by a genetic screen, which identified yeast mutants that showed activation of an iron-sensitive reporter construct (*FET3p::lacZ*) under conditions of iron sufficiency.[28] This reporter construct contains a β-galactosidase-encoding gene (*lacZ*) that has been fused to the promoter for *FET3* (*FET3p*). Conditions that lead to increased transcription of *FET3p* can be detected by an increase in the easily assayed β-galactosidase activity. A number of mutants were identified that led to increased transcription of *FET3*, a component of the high-affinity elemental iron transport system. Among the genes identified were mutant forms of *NFS1*, *ISU1*, and *GSH2*. These three genes encode proteins whose products are required for Fe−S cluster synthesis. The screen also uncovered a mutant form of *MTM1*. Deletion of *MTM1* had previously been shown to lead to increased mitochondrial iron and activation of iron regulon, although Fe−S cluster synthesis was not altered.[34] The screen also identified two other genes, termed *FRA1* and *FRA2*. Deletion of either gene did not affect mitochondrial iron accumulation or Fe−S cluster synthesis but led to activation of the iron regulon, indicating a possible novel role in Aft1p activation. Activation of the iron regulon by deletion of these genes was independently observed in a genome-wide screen, which assayed strains with specific gene deletions for increased uptake of siderophores.[27]

Deletion of both *FRA1* and *FRA2* together did not lead to increased transcription of *FET3* beyond that seen in single gene deletion strains. Fra1p and Fra2p are cytosolic proteins and have been found in a complex with cytosolic glutaredoxins, Grx3p and Grx4p. Grx3p and Grx4p are involved in sulfhydryl chemistry, either reducing disulfides or resolving mixed disulfides. Deletion of *GRX3* or *GRX4* individually had little effect on cellular activities.[35,36] Deletion of both genes, however, resulted in decreased cell growth and increased activation of the iron regulon under iron-replete conditions. While these experiments support a role for a Fe−S sensing machine composed of Fra1p, Fra2p, and Grx3p/Grx4p, the mechanism of Fe−S sensing is unclear. Fra1p and Fra2p can form a complex with Grx3p and/or Grx4p, while Grx3p and/or Grx4p can form a complex with Aft1p, but data is lacking for either a Fra1p or Fra2p-Aft1p complex. The role of the Fra/Grx complex in regulating Aft1p activity therefore requires clarification. Deletion of *FRA1* or *FRA2* did not have synergistic effects with deletion of genes required for Fe−S cluster synthesis, suggesting they function in the same pathway. However, deletion of either *FRA* genes or genes required for Fe−S cluster synthesis only resulted in submaximal activation of the iron regulon relative to the level of activation seen when cells are iron-starved. These results suggest that Fra1p and Fra2p interpret the Fe−S cluster signal but that iron depletion can lead to a greater activation of transcription. In contrast, double deletion of *GRX3* and *GRX4* led to complete activation of the iron regulon. Finally, it remains unclear how the Fra/Grx complex

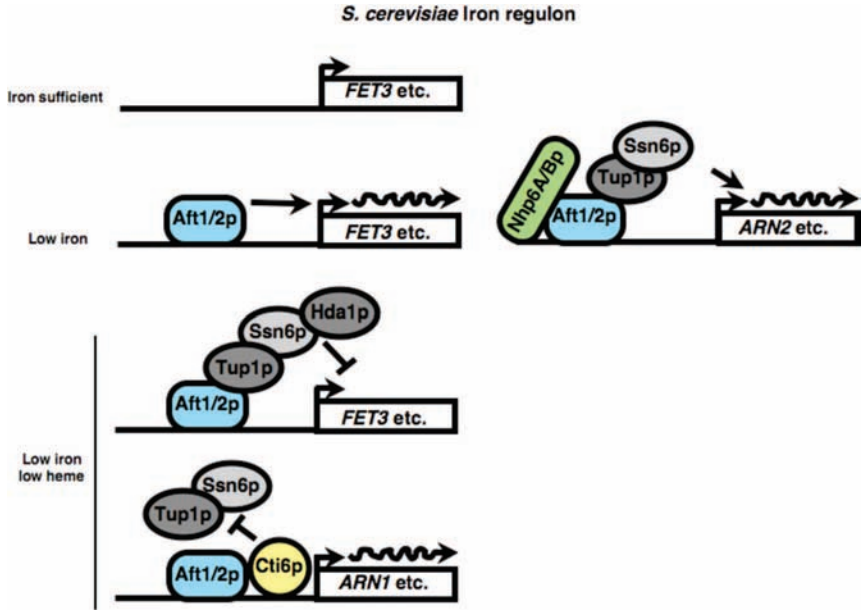

Figure 3. Regulation of transcription by Aft1p. In iron-sufficient conditions, Aft1p resides in the cytosol. In iron-deficient conditions, Aft1p translocates to the nucleus, where it binds to the promoters of genes in the iron regulon and activates their transcription. Transcription of some genes in the iron regulon requires the presence of additional general transcription factors such as Tup1p/Ssn6p and Nhp6A/Bp. In the absence of heme, members of the iron regulon whose functions require heme or oxygen are repressed by the presence of Tup1p/Ssn6p and Hda1p. Other members of the iron regulon, whose function is oxygen/heme independent, are still transcribed. Transcription of these genes requires the presence of Cti6p, which by binding to promoter sequences removes Tup1p/Ssn6p.

interprets the Fe−S cluster signal. As mentioned above, Fe−S clusters exported to the cytosol are assembled on apoproteins by a cytosolic machine. Deletion of genes that encode components of this machine prevent the formation of Fe−S containing cytosolic or nuclear proteins.[31] Yet, deletion of these genes did not lead to increased expression of the iron regulon.[23] These results suggest that Aft1p is not a Fe−S cluster containing protein, and thus, it remains to be determined how Aft1p senses mitochondrial Fe−S clusters.

1.1.1.3. Transcriptional Regulation of the Iron Regulon in *S. cerevisiae* beyond Aft1p. Within the group of Aft1p-regulated genes, regulation can vary between environmental conditions or through mutation of other transcription factors (Figure 3). Differential gene regulation of an Aft1p target was first observed in a mutant strain where the Aft1p-regulated ferric reductase, *FRE2*, was not transcribed, although other Aft1p-targets were. *NHP6A* was identified by transforming yeast with plasmids containing various pieces of chromosomal DNA (a DNA library) obtained from a wild type yeast strain. Each transformed cell will have multiple copies of the same plasmid. One of these plasmids was able to cause reestablished expression of *FRE2* in transformed mutant cells, and the transforming DNA contained the *NHP6A* gene.[37] Nhp6ap and its homologue Nhp6bp associate with chromatin and appear to bend DNA, altering DNA structure.[38] Deletion of either gene has little effect on viability, but deletion of both genes will lead to reduced cell growth. Nhp6a/bp interacts with Ssn6p and Tup1p, transcriptional repressors of a large number of genes.[39] Further work demonstrated that, depending on context, the Ssn6p/Tup1p complex was involved in gene activation as well as repression. Deletion of *SSN6* or *TUP1* prevented activated Aft1p from inducing transcription of *FRE2* and *ARN2* but did not prevent induction of *FET3*.[37] Rather, deletion of *SSN6* or *TUP1* resulted in an increased expression of *FET3* under iron-sufficient conditions. Deletion of both *NHP6A* and *NHP6B* reduced expression of *FRE2* but had no effect on *FET3*. Finally a strain with deletions in *NHP6A* and *NHP6B* and *SSN6* prevented iron-regulated expression of *FRE2* but not *FET3*.

A second demonstration that members of the iron regulon could be differentially regulated came from examining effects of oxygen depletion on transcription of the iron regulon. Transport of elemental iron by the high-affinity iron transport system is completely dependent on oxygen. First, reduction of Fe^{3+} to Fe^{2+}, a requirement for high-affinity iron transport, is dependent on ferrireductases, which are heme-containing enzymes. Synthesis of heme requires oxygen.[40] Second, transport of iron by Fet3p/Ftr1p will not occur in the absence of oxygen, as oxygen is the terminal electron acceptor of electrons extracted in the oxidation of Fe^{2+} to Fe^{3+}. Crisp et al. demonstrated that, in the absence of heme, which in yeast is a sensor for oxygen, transcription of both the high-affinity iron and copper transport systems did not occur.[41] They further showed that, under anoxic or heme-deficient conditions, Aft1p could still sense iron deficiency and translocate from cytosol to nucleus. Under these conditions, Aft1p was bound to promoter regions of iron regulon genes, yet transcription of some (but not all) genes was inhibited. Most notably, transcription of the genes that encode the elemental iron transport system, *FET3* and *FTR1*, was inhibited, but genes that encode siderophore transporters were still transcribed in an iron-regulated manner.[42] The underlying physiology is that many proteins encoded by genes in the iron and copper regulons are not functional under anaerobic conditions. Most proteins that bind copper are involved in oxygen detoxification or metabolism. Examples include cytochrome oxidase, Fet3p, and superoxide dismutase. Similarly, many proteins that utilize iron also use oxygen as a substrate (e.g., methyl sterol oxidase, lipoxygenase, lipid desaturases, ferrochelatase) and are not functional under anaerobic conditions. Thus, the demand for redox-active metals is reduced under anaerobic conditions.

Crisp et al. identified the mechanism by which the absence of heme leads to differential expression of iron-regulated genes via transcriptional repression. Reduction of the distance between the Aft1p binding site and the TATA box maintained iron−regulated expression in the absence of heme (that is, expression became insensitive to heme). A genetic screen identified *TUP1* and the histone deacytelase *HDA1* as being required for the inhibitory effect on Aft1p, as deletion of either gene resulted in expression of *FET3* in the absence of heme. These observations suggest an additional role for the general repressor Tup1p in control of the iron regulon. It was noted that deletion of *TUP1* had a much greater effect in repressing *FET3* transcription than did deletion of *SSN6*, encoding a Tup1p partner with other roles in the iron regulon (discussed above).

Siderophore transport is oxygen-independent, and though *S. cerevisiae* does not make siderophores, it can acquire siderophores produced by other organisms. The ability to accumulate siderophores permits *S. cerevisiae* to compete with other microorganisms for iron under anaerobic conditions. The siderophore transport gene *ARN1*, which exhibits iron regulation even in the absence of heme, does so because of a DNA sequence adjacent to the Aft1p binding site. Mutation of this sequence in *ARN1* or insertion of this sequence in the promoter region of *FET3* indicated this sequence is necessary and sufficient to confer iron regulation in the absence of heme. Cti6p, a PHD finger domain containing protein that interacts with the Tup1p/Ssn6 complex, was identified as a protein factor conferring heme-independence of *ARN1* regulation through the identified DNA sequence. PHD (plant homeo domain) are zinc finger-like motifs which are found in many proteins including transcription factors. Cti6p binding to this sequence resulted in antagonism of Tup1p binding, resulting in loss of gene repression. The nature of the heme sensor remains unknown, be it Aft1p, Tup1p, or an unidentified protein.

A role for the transcription coactivator complex, Mediator, in the iron regulon has also been identified.[43] Mediator is an extremely large multiprotein complex conserved in eukaryotes that functions in linking transcriptional activators to RNA Polymerase II and the general transcription machinery (for review, see refs 44−47). Structurally, Mediator appears to be composed of several modules, including a dissociable four-protein subcomplex containing a cyclin-dependent kinase called the Srb/Cdk module. In general, Mediator functions as a coactivator for DNA-binding transcription factors. To date, there are a number of cases in which deletion of particular Mediator subunits can abrogate transcription by particular transcription activators, indicating that activators likely contact any of a number of different Mediator subunits (for example, see ref 48). Mediator may also function in transcriptional repression for a subset of Mediator-dependent promoters.[49] Data for Mediator function in repression comes from the fact that a number of genes are upregulated upon mutation/deletion of components encoding the Srb/Cdk module.[50] In yeast, deletion of *SSN3*, the gene encoding the cyclin-dependent kinase within Mediator (usually referred to as Cdk8p or Srb10p in the literature), causes upregulation of a number of genes, including many within the iron regulon.[43] Intriguingly, mutation of a Cdk8p-phosphorylation target within the Med2p protein of Mediator itself (*med2-S208A*) also has similar effects on the low iron regulon. It is not yet clear if Mediator directly represses the low-iron regulon or if compromise of Cdk8p function alters cytosolic iron levels or the sensing of those levels or some other iron-related physiology.

1.1.2. Transcriptional Remodeling in Response to Low Iron

Iron limitation results in increased transcription of mRNAs that encodes proteins involved in iron acquisition or in iron-dependent metabolic processes. These changes result in a rearrangement of metabolism and synthesis to processes that utilize less iron-containing proteins. For example, under iron-limiting conditions, glutamine synthesis is no longer catalyzed by Glt1p, a Fe−S containing enzyme.[51] Instead glutamine is synthesized by the actions of Gdh1p/Gdh3p and Gln1p, enzymes that do not require iron. Most notably, iron-deprivation leads to a reduction in respiration, which is rich in iron-containing enzymes, that is compensated for by an increase in glycolysis. Microarray analysis revealed extensive transcriptional changes resulting from iron deprivation that affected the mRNA levels of at least 200 genes. Recent studies identified post-transcriptional processes that effect changes in the levels of mRNA involved in iron acquisition and metabolism. Puig et al. determined that two homologous genes, *CTH1* and *CTH2*, induced by Aft1p/Aft2p under iron-limiting conditions, were responsible for regulating a wide variety of genes that encode proteins involved in iron- and oxygen-based metabolism.[52] Cth1p /Cth2p binds to specific elements in the 3′ untranslated region (UTR) of mRNAs, causing a decrease in their half-life. Under low iron conditions, mRNAs that increase iron storage or are high-end iron consumers are reduced in abundance. The result is that pathways that are parsimonious in iron usage are dramatically upregulated in preference to those that use iron. Of interest is that Cth1p and Cth2p, while highly homologous, are only partially redundant. The two genes are expressed differently: Cth1p is only expressed transiently in response to iron limitation while Cth2p is expressed longer.[53] Cth1p is thought to preferentially destabilize mRNAs for mitochondrial proteins involved in respiration or amino acid synthesis.

The mechanism of Cth1p/Cth2p mediated mRNA destabilization has been elucidated in great detail. Guided by studies in mammalian systems, Puig et al. noted that Cth2p contains a tandem zinc finger (TZF) motif, a domain that typifies the tristetraprolin (TTP) family of RNA binding proteins. In the mammalian proteins TTP and BRF-1, this domain binds AU-rich elements present in rapidly degraded mRNAs. Puig et al. showed that Cth2p binds similar elements in its mRNA targets and that these elements are required for Cth2p-dependent destabilization. Domains outside the TZF domain in TTP and BRF-1 interact with mRNA decapping, deadenylation, and exonucleolytic activities, thereby recruiting these activities to target mRNAs. Cth2p-dependent destabilization of a target mRNA, *SDH4*, appears to occur with a 5′ to 3′ polarity, suggestive of a requirement for the mRNA-decapping machinery in the Cth2p mechanism. Consistent with these results, Puig and co-workers identified the Dhh1p DEAD box RNA helicase, an activator of mRNA decapping that also helps recruit target mRNAs to cytosolic P-bodies, the sites of mRNA degradation, as required for Cth2p destabilization of *SDH4* mRNA.[53] Direct interactions between Cth1p or Cth2p and the carboxy-terminus of Dhh1p were shown via yeast two-hybrid screen. Interestingly, deletion of genes encoding specific components of the mRNA-decapping and degradation machinery, *XRN1*, *DCP1*, or *DCP2*, also affected Cth2p intracellular localiza-

tion, causing it to be trapped in P-bodies, the normal site of 5′ to 3′ mRNA decay.[53] These results together suggested the model where Cth2p normally transiently interacts with P-bodies, likely through Dhh1p, to recruit target mRNAs to the mRNA decapping machinery. This model does not appear to be the entire story for Cth1p/2p, as it has been reported that an additional Cth2p target, *ISA1*, is degraded independently of Dhh1p.[53]

An additional mRNA-degradation pathway has been linked to iron-regulated genes—a pathway dependent on the sole yeast RNase III homologue, Rnt1p.[54] Rnt1p is a double-stranded RNA endonuclease that relies on RNA-hairpin capped with an AGNN-tetraloop sequence within its targets to specify cleavage.[55,56] Deletion of *RNT1* leads to upregulation of some iron acquisition genes under iron-replete conditions, while *rnt1*Δ cells are hypersensitive to excess iron.[54] Furthermore, mislocalization of Rnt1p to the cytoplasm blocked the low-iron dependent induction of iron regulon genes to various extents.[54] The relationship of Rnt1p to the iron regulon remains mechanistically opaque, but of note is that Lee et al. identified several extended mRNA transcripts for some iron regulon mRNAs such as *FIT3*, *ARN2*, and *ARN3* when examining strains defective for various mRNA-degradation pathways in combination with *rnt1*Δ. These observations suggest that surveillance of particular extended iron regulon transcripts may be important for normal iron regulon control under iron-replete conditions.

1.1.3. Transcriptional Regulation of Genes Required for Iron Storage

Acquisition of iron occurs by iron transport from the media but also via transport of iron from the vacuole to the cytosol. Fungi and plants store iron in the vacuole and employ two iron-export systems in the vacuolar membrane, one high-affinity and one low-affinity. These export systems are homologues of the cell surface iron transport systems; thus, the *S. cerevisiae* vacuolar high-affinity system is composed of a multicopper oxidase, Fet5p,[57] and a transmembrane permease, Fth1p.[58] The low-affinity transport system is encoded by *SMF3* and is a H^+/M^{2+} symporter that is not specific to iron and can transport other transition metals.[59,60] This transporter is homologous to the cell surface transporter Smf1p and a mammalian transporter, SLC11A1/DMT1. Both vacuolar transport systems in *S. cerevisiae* are regulated in response to iron deprivation by Aft1p and Aft2p. *SMF3* appears to be one of the genes of the iron regulon that is a preferential target of Aft2p.[22]

In the presence of iron, there is decreased expression of the vacuolar iron transporters resulting from decreased Aft1p regulated transcription. In the face of continued iron accumulation, cytosolic iron levels are maintained at a homeostatic level by sequestration of iron into the vacuole via increased expression of the vacuolar iron importer Ccc1p. This transporter is a member of a unique family found in fungi and plants.[61,62] In yeast, Ccc1p can transport Fe^{2+} and Mn^{2+}.[61,63] Like many genes involved in iron metabolism, *CCC1* is regulated both transcriptionally and post-transcriptionally. Transcription of *CCC1* is regulated by iron through the activity of the Yap5p transcription factor, which binds to a Yap consensus DNA binding site in the promoter region of *CCC1*.[64] Yap5p is a member of the yeast activator protein family of bZIP transcription factors, which are homologues of the human AP-1 transcription factor. These transcription factors contain a leucine zipper motif that mediates dimerization adjacent to a basic DNA binding domain. The *YAP* family in *S. cerevisiae* consists of eight homologous genes that affect transcriptional changes in response to environmental stimuli.[65] The most studied member of the *YAP* family is *YAP1*, which coordinates a transcriptional response to oxidants such as H_2O_2.[66] Yap1p is a cytosolic protein that contains a DNA-binding domain and an activation domain with two-cysteine rich regions. Oxidants cause changes in the cysteines in the cysteine-rich domains, either by inducing intradomain disulfides or by inducing disulfide bonds with other proteins, such as periredoxins.[67] The change in disulfide status allows translocation of Yap1p into the nucleus, where it then activates transcription of genes that mediate an antioxidant response.

Figure 4. Topology of Yap5p, the high iron-sensing transcription factor in *S. cerevisiae*. The high iron-sensing transcription factor Yap5p is composed of two domains, a DNA binding domain and an activation domain. DNA binding by Yap5p is not affected by iron, but activation is iron-regulated. The activation domain has two cysteine-rich modules, and the cysteines are required for iron-regulated transcription. Each module acts additively; mutations in both modules are required to completely prevent iron-regulated transcription.

Yap5p has a similar architecture to Yap1p, a DNA binding domain and an activation domain containing two cysteine-rich regions (Figure 4). Similar to the regulation of Yap1p, cysteines within those regions of Yap5p are critical for iron-responsive transcription, as mutation of cysteines to alanines affects transcription of *CCC1*. The two cysteine-rich regions act as independent modules in which mutation of cysteines in the amino-terminal module leads to a 70% reduction in activity, mutation in the carboxyl-terminal module affects a 30% activity, and mutation of cysteines in both modules causes complete loss of transcriptional activity.[64] In the case of Yap5p, its regulation by iron is subsequent to its DNA binding, as Yap5p is found constitutively bound to *CCC1* regardless of iron conditions, in contrast to the regulation of Yap1p by stimuli at the level of nuclear localization.[64] It is currently unclear how iron leads to regulation of Yap5p, although there is some evidence that excess iron in cells engenders alteration or reorganization of free sulfhydryls in Yap5p.

Under low-iron conditions, iron transport into the vacuole is prevented, as it would result in further depletion of cytosolic iron. A decrease in the iron-dependent transcription of *CCC1* is only part of the mechanism that reduces the level of *CCC1* mRNA. The 3′-terminus of *CCC1* contains Cth1p /Cth2p target sites, and *CCC1* mRNA degradation is promoted by Cth1p and Cth2p. Under normal iron conditions when an iron-independent promoter transcribes *CCC1*, Cth1p/Cth2p destabilization of *CCC1* mRNA results in a 50% decrease in steady-state *CCC1* mRNA levels.[64] In summary, iron movement into and out of the vacuole is regulated by the level of high iron-sensing transcription factor Yap5p, which activates *CCC1* transcription, and by the low iron-sensing transcription factors Aft1p/Aft2p that destabilize *CCC1* mRNA through the actions of their targets Cth1p/Cth2p.[52] Aft1p/Aft2p at the same time induces the transcription of vacuole iron exporters, reversing iron flow from vacuole to cytosol. The coordinated activity of the high and

low iron-sensing transcription factors coordinates vacuolar iron storage with cytosolic iron requirements.

1.2. Regulation of Iron-Related Genes in *S. pombe* and Other Yeast

The transporters that mediate iron acquisition in other yeast are highly homologous to those of *S. cerevisiae* and are also regulated at the transcriptional level in response to iron. What is different is that most yeast utilizes a completely different iron-sensing transcriptional regulator systems. Iron regulation through the Aft1p transcription factor is restricted to only some genera in the family *Saccharomycetes*. Other species of yeast and fungi not in that family utilize an iron transcription factor that belongs to the GATA transcription family. The GATA family of transcription factors was named due to their binding to a DNA binding sequence, which contains the GATA motif. These transcription factors are related by a high degree of amino acid sequence identity within their zinc finger DNA-binding domains and are prevalent in both lower and higher eukaryotes. The best studied of the GATA family iron-regulated transcription factors are in fungi, and in particular the fission yeast *Schizosaccharomyces pombe* (for review, see ref 68). *S. cerevisiae* and *S. pombe* are strongly evolutionarily divergent. One of the obvious features is that *S. cerevisiae* reproduces by asymmetric budding while *S. pombe* undergoes binary fission. *S. pombe* and *S. cerevisiae* are as evolutionarily divergent from each other as each is from vertebrates. There are many features of *S. pombe* that are more similar to vertebrates than in *S. cerevisiae*. For example, *S. cerevisiae* has very few genes with introns, whereas introns are the rule in *S. pombe*. Additionally, *S. pombe* maintains chromatin modifications, RNA-dependent RNA silencing machinery, and centromeric structure that are conserved in higher eukaryotes but lacking in *S. cerevisiae*. It is of interest that the iron-sensing transcription factor in *S. pombe* is similar to transcription factors in higher vertebrates, but the transporters that mediate iron acquisition are highly homologous between *S. cerevisiae* and *S. pombe*. The high-affinity elemental iron transport system in *S. pombe* consists of a multicopper oxidase encoded by *fio1*$^+$ (ferrous iron oxidase) and a partner transmembrane iron permease encoded by *fip1*$^+$ (ferriferous permease).[69] Expression of both genes in *S. cerevisiae* can complement a strain with deletions in *FET3* and *FTR1*. The substrate for the Fio1/Fip1 transport system is ferrous iron generated by a ferrireductase Frp1, which is highly homologous to the *S. cerevisiae* ferrireductases.[70] In addition to the elemental iron transport system, *S. pombe* also has three siderophore transporter genes *str1*$^+$, *str2*$^+$, and *str3*$^+$. These genes are also regulated by iron demand and are increased under low iron conditions.[71]

1.2.1. Transcriptional Regulation of Genes Required for Iron Acquisition

In *S. cerevisiae*, Aft1p is an iron-induced activator of transcription. Deletion of Aft1p results in the absence of transcriptional activity. In *S. pombe* and in other yeast that utilize a GATA-based iron-sensing transcription factor, the transcription factor is a repressor and reduced expression of the repressor allows transcription of low-iron regulated genes (Figure 5). The *S. pombe* GATA-factor Fep1 was identified based on analysis of the promoter sequence of the iron-regulated genes *fio1*$^+$, *fip1*$^+$, and *frp1*$^+$. The *fip1*$^+$ and *fio1*$^+$ genes share the same promoter, with the *fip1*$^+$−*fio1*$^+$ genes divergently transcribed. Mapping of the intergenic region identified two GATA-containing elements that, when mutated, led to constitutive expression of *fip1*$^+$ and *fio1*$^+$.[71] Two similar elements were found in the promoter regions of the iron-regulated ferrireductase, *frt1*, and in the iron-regulated siderophore transporters, *str1*$^+$, *str2*$^+$, and *str3*$^+$. Examination of the *S. pombe* genome for GATA-type transcription factors indicated four candidates of which *fep1*$^+$ was shown to be responsible for iron-regulated expression. Deletion of *fep1*$^+$ resulted in constitutive expression of *fio1*$^+$, *fip1*$^+$, *frt1*$^+$, and *str1*$^+$. A recombinant amino-terminal Fep1 fragment interacted with GATA sequences in an iron-dependent manner. The amino-terminal sequence of Fep1p is homologous to the amino terminal sequence of other iron-sensitive fungal GATA transcription factors, which include Urbs1 (from *Ustilago maydis*),[72] SRE (from *Neurospora crassa*), SREA (from *Aspergillus nidulans*),[73] Sfu1 (from *Candida albicans*),[74] Sre1 (from *Histoplasma capsulatum*),[75] and Fep1 (from *Pichia pastoris*).[76] The carboxyl-terminal portion of Fep1 contains the repression domain, while the amino-terminal portion contains the DNA-binding domain.[77] Fusion of the amino-terminal portion of Fep1 to heterologous activation domains confers iron-regulated expression on reporter constructs. This result suggests that DNA binding is iron-dependent. Analysis of GFP-tagged Fep1 showed that it localized in the nucleus independently of iron status. Chromatin cross-linking studies showed that it was bound to DNA in iron-replete cells and was not bound in iron-limited cells. These results suggest that iron effected changes in Fep1 structure, leading to its association with DNA.

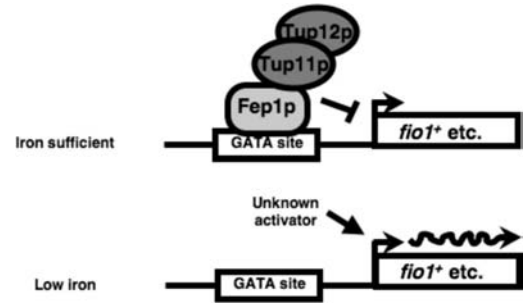

Figure 5. Model for the regulation of genes required for iron acquisition in *S. pombe*. Fep1 is an iron-sensitive transcription factor that binds to GATA promoter elements in the presence of iron and represses target genes. Repression requires the participation of the general repressor factors Tup11 and Tup12. Fep1 is released from the promoter in the absence of iron, permitting expression of genes required for iron acquisition, possibly through an unidentified transcriptional activator.

The amino-terminal DNA binding domain of other fungal iron-sensitive GATA transcription factors has a similar architecture. There are two zinc finger domains separated by a domain that contains four highly conserved cysteines. Disruption, truncation, or mutation of the zinc finger domains reduces DNA-binding activity.[77] Depending on the specific iron-regulated GATA transcription factor, loss of one of the zinc fingers may lead to partial or total loss of activity. Mutation of the highly conserved cysteines to alanines in the cysteine-rich region reduces iron-dependent binding to DNA and inhibits the repressive activity of the transcription factors. Fep1 and the GATA iron-sensing transcription factors SRE from *Neurospora crassa* isolated as recombinant proteins expressed in bacteria are reddish-brown, which suggests the likely presence of iron.[77,78] The most compelling

evidence for the presence of iron binding by these GATA transcription factors comes from a study of Sre1 from the pathogenic fungus *Histoplasma capulatum*. Chao et al. expressed the amino-terminal portion of Sre1 as a maltose binding protein chimera in bacteria. The isolated protein bound both zinc, in near stoichiometric amounts (1.6 atoms/monomer), and iron, in substiochometric amounts (0.5−1.0 atom/monomer).[75] The authors point out that the less than expected metal content may reflect the fact that only a portion of the native protein was present in the chimera. Importantly, the authors showed that DNA binding activity of the Sre1-chimera was iron-dependent, as removal of iron decreased binding affinity. Additionally, mutation of the cysteines to alanines reduced iron binding and reduced DNA binding affinity. It was noted that the cysteine mutations reduced zinc binding and mutations in the zinc fingers reduced iron binding. It is thought that the zinc fingers maintain the structure of the protein, rather than zinc playing a more active role in metal sensing. These data indicate that binding of iron affects the conformation of iron sensing transcription factors, reducing their affinity for their cognate DNA-binding sites.

The ability of GATA binding transcription factors to repress transcription involves their interaction with the products of genes that encode the general transcription factors *tup11*$^+$ and *tup12*$^+$.[79] The encoded proteins, which are highly homologous to each other, are homologues of the *S. cerevisiae* Tup1p. Deletion of both *tup11*$^+$ and *tup12*$^+$ results in derepression of *fio1*$^+$ transcription, rendering it independent of iron. Some data exist concerning the mechanism of repression. Tup11 and Tup12 can assemble together as a complex. The carboxyl termini of Tup11, Tup12 (and Tup1p) contain WD40 repeats, which are well-recognized protein interaction domains. Using a variety of different approaches (yeast two-hybrid, or by expressing the recombinant proteins in bacteria and determining if immunoprecipitation of one protein results in the coprecipitation of the second protein, "bacterial pull-downs"), Znaidi et al. showed that the WD40-containing carboxyl termini potions of Tup11 and Tup12 interact with the carboxyl terminal portion of Fep1.[79] The amino terminal portions of the homologous Tup1p from *S. cerevisiae* interact with histones. Thus, a model for iron-regulated control of the *S. pombe* iron regulon suggests that iron induces binding of the amino terminal portion of Fep1 to DNA, which leads to recruitment of a Tup11/Tup12 complex through their WD40 domains' interaction with the carboxyl terminal domain of Fep1. The amino terminal domains of Tup11 and Tup12 are then free to interact with histones and recruit histone-modifying factors, such as histone deacetylases to inhibit transcription. While removal of Fep1 repression permits transcription of genes required for iron acquisition, the transcription factor that is responsible for activation has not yet been identified.

While differences exist between the regulatory logic of *S. cerevisiae* and *S. pombe* in respect to the iron regulon, there are similarities between the two strains in the mode by which copper-sensing transcription factors regulate iron-acquisition genes. In both *S. pombe and S. cerevisiae*, the high-affinity iron transport system is dependent on copper. Both Fio1 (*S. pombe*) and Fet3p (*S. cerevisiae*) are copper enzymes for which copper is required to oxidize iron and store the extracted electrons. Copper deficiency in both species of yeast results in iron deficiency due to the lack of functional multicopper oxidase. In *S. pombe*, copper depletion results in the inhibition of iron transport through decreased transcription of *fio1*$^+$ and *fip1*$^+$.[80] Copper depletion also results in increased expression of the high-affinity copper transporter Ctr4. Transcription of *ctr4*$^+$ is due to the copper-sensing transcription factor Cuf1, which is a transcriptional activator. Cuf1 binds to copper-sensitive elements in the *ctr4*$^+$ promoter. Deletion of *cuf1*$^+$ results in the inability to induce *ctr4*$^+$ transcription in the absence of copper. Cuf1, however, represses transcription of *fio1*$^+$ and *fip1*$^+$ even in iron-deficient medium where Fep1 is not bound to DNA. The common promoter region of *fio1*$^+$ and *fip1*$^+$ contains copper-sensitive elements that are responsible for Cuf1 binding, which results in repression of *fio1*$^+$ and *fip1*$^+$ transcription in the absence of iron. Thus, Cuf1 can act as either a transcriptional activator or a repressor. The repression of *fio1*$^+$ in the absence of copper prevents the transcription and translation of what would be an ineffective protein, as Fio1 is nonfunctional without copper. The iron regulon of *S. cerevisiae* can also be responsive to copper depletion, as Labbe et al. noted that the promoter region of *FET3* contains binding sites for the copper-sensing transcriptional activator of *S. cerevisiae*, Mac1p.[80] They demonstrated that deletion of *MAC1* led to increased expression of *FET3* under conditions of low copper. These results suggest that Mac1p can also repress the high-affinity iron transport system in *S. cerevisiae* in the absence of copper, analogous to Cuf1 in *S. pombe*. However, repression is not nearly complete, as an apoFet3p can be found on the surface of copper-starved *S. cerevisiae*.

1.2.2. Transcriptional Regulation of Genes Involved in Iron Utilization and Storage

In both *S. cerevisiae* and *S. pombe*, iron limitation increases mRNAs for proteins involved in iron-acquisition and decreases mRNA for proteins involved in iron-storage and -utilization (Figure 6). In *S. cerevisiae*, this "metabolic remodeling" occurs through both lack of transcriptional activation (e.g., vacuolar transporter *CCC1*) and destabilization of mRNAs involved in iron utilization by Cth1p/Cth2p. In *S. pombe*, decreased levels of mRNA for the *S. pombe CCC1* homologue, *pcl1*$^+$, and the mRNA for the iron-containing protein Sdh4, in response to iron limitation, do not appear to involve specific post-transcriptional regulation. While there are proteins homologous to Cth1p and Cth2p in *S. pombe*, they do not appear to regulate mRNAs of iron-storage or iron-utilization genes. Instead, a transcriptional activator that is regulated by Fep1 in an iron-dependent manner regulates this class of genes. The promoter regions of *sdh4*$^+$, *isu2*$^+$, and *pcl1*$^+$, along with 70 other genes, contain CCCAT sequences.[81,82] In *S. cerevisiae*, the function of CCCAT sequences in gene expression has been worked out for some time. The CCCAT sequence is found in promoters for many genes involved in oxidative phosphorylation. A complex of four *HAP* proteins, Hap2p−Hap5p, binds to CCCAT sites and activates gene transcription.[83] Activation results when glucose is depleted and yeast require respiration for their energy needs. The expression of Hap4p is glucose regulated and *HAP4* transcription is repressed by glucose.[84] In the absence of glucose, Hap4p is expressed and binds to the Hap2p/Hap3p/Hap5p complex, leading to activation of CCAT-containing genes involved in respiration.

The story in *S. pombe* is slightly different and illustrates how conserved regulatory machinery may be adapted for different purposes. *S. pombe* has homologues of Hap2p,

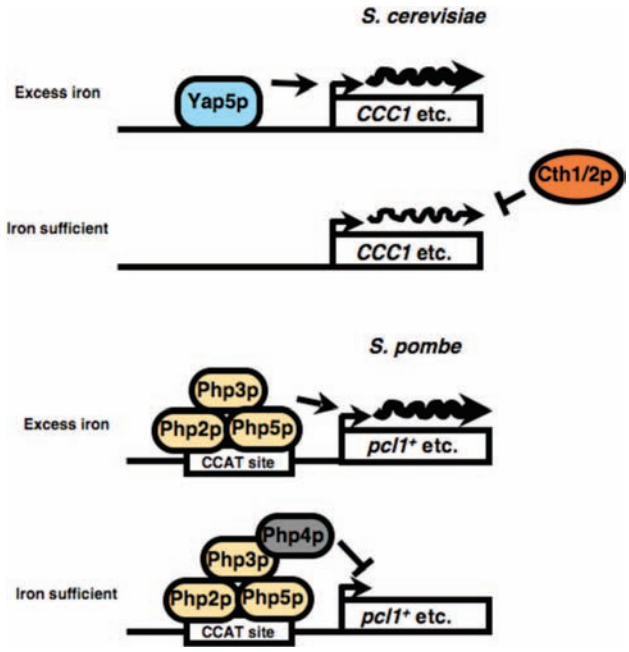

Figure 6. Coordinate regulation of genes involved in iron acquisition and utilization. In *S. cerevisiae*, transcription of mRNA for the vacuolar iron transporter, the iron-dependent transcriptional activator Yap5p, regulates Ccc1p. When iron levels decrease, Yap5p is no longer transcriptionally active. *CCC1* mRNA is destabilized in low iron conditions through the binding of Cth1p and Cth2p to the 3′-UTR of the *CCC1* mRNA. The low iron-sensing transcription factor Aft1p transcriptionally activates *CTH1 and CTH2*. Cth1p and Cth2p destabilize mRNA involved in iron-utilizing processes. In *S. pombe*, transcriptional activation of genes for iron storage and utilization results from a transcriptional activator complex composed of Php2, Php3, and Php5. Under low iron conditions, *php4*$^+$ is no longer repressed by Fep1. Php4 binds to the Php complex and inhibits gene transcription.

Hap3p, and Hap5p, termed Php2, Php3, and Php5. These proteins bind to CCAT sites in *S. pombe* genes and, when expressed in *S. cerevisiae*, can replace their *S. cerevisiae* homologues and regulate CCCAT-containing genes. A fourth component of the Php complex in *S. pombe*, Php4, has limited sequence homology *to S. cerevisiae HAP4*. Additionally, Php4 is regulated by iron but not by glucose.[81] The promoter sequence of *php4*$^+$ contains GATA sites and is repressed by Fep1 binding when iron levels are high. In the absence of Php4, the Php complex (Php2, Php3, Php5) resides on the promoter of iron-utilization genes and activates their transcription. In the absence of iron, repression by Fep1 is relieved, leading to the expression of Php4. Php4 binds to promoter-bound Php complexes and represses transcription of genes involved in iron storage and utilization. Thu, *S. pombe* and *S. cerevisiae*, through different mechanisms, inversely coordinate the levels of mRNA for genes involved in iron acquisition and utilization.

1.3. Transcriptional Regulation of Genes involved in Heme Acquisition and Iron Storage in *C. elegans*

The mechanisms by which the nematode *C. elegans* imports iron have not been clarified. Nematodes, like all other eukaryotes, require iron and most probably obtain their iron from ingestion of bacteria, their major food source. Nematodes, and the broader group of helminthes, both parasitic and free-living, have one aspect of iron metabolism not seen in other eukaryotes: they are heme auxotrophs.[85] Nematodes do not contain the genes for porphyrin biosynthesis, which in addition to iron is the substrate for heme. They cannot synthesize heme and must obtain it from their diet. Microarray analysis of *C. elegans* grown in heme-replete and heme-deficient axenic medium resulted in the identification of mRNA whose expression was inversely correlated with heme sufficiency.[86] Many of the mRNAs encoded proteins whose deduced sequence appeared to encode membrane-bound transporters. Depletion of hrg-1, or its paralogue hrg-4, resulted in alteration in heme sensing as defined by changes in transcription for heme-responsive genes and in decreased growth of mutant *C. elegans*. Decreased expression of the zebrafish hrg-1 homologue using RNA-mediated interference (RNAi) leads to morphological defects and a severe deficiency in hemoglobinization of zebrafish red blood cells.[86] These results suggest that hrg-1 encodes a heme transporter whose function is conserved between worms and vertebrates. The mechanism by which hrg-1 mRNA levels in either *C. elegans* or zebrafish respond to heme deficiency is unknown.

C. elegans does regulate the storage of iron. *C. elegans* utilizes the iron storage protein ferritin to store iron under high-iron conditions. Ferritin is a multimer consisting of 24 subunits of different amounts of either H-ferritin or L-ferritin monomers. The H-ferritin monomer contains the ferroxidase activity that is required to insert iron into the nanocage. The L-ferritin monomer helps provide stability to the assembled nanocage. Ferritin's principal function is the storage of iron in a nontoxic, but bioavailable, form. The assembled ferritin molecule, often referred to as a nanocage, can store up to 4 500 atoms of iron. Iron is found within ferritin as a mineral ferrihydrite, which is nontoxic as it cannot participate in the production of oxygen radicals. In mammalian cells, expression of ferritin is primarily regulated at the translational level (for review, see ref 87). There is some transcriptional regulation of ferritin in higher vertebrates, mostly in response to cytokine signaling or to oxidative damage.[88] In *C. elegans*, there is no evidence of translational regulation of ferritin; rather, ferritin mRNA levels are increased in response to iron at the transcriptional level.[89] An iron-responsive DNA element was identified as a common sequence present in the two *C. elegans* ferritin genes.[90] The identified minimum iron-regulatory sequence of 63 base pairs contains two GATA-binding motifs and three octameric direct repeats. The octomeric sequences are predicted to bind bHLH transcription factors, while the relevant GATA-binding factor, ELT-2, was identified by a variety of approaches, including RNAi. ELT-2 itself does not show iron-regulated expression, and ELT-2 is broadly involved in regulating a large number of genes that have little to do with iron utilization or acquisition. These results led to the suggestion that an iron-regulated bHLH factor(s), through the octameric direct repeat DNA sequences, might be responsible for the iron-dependent transcriptional control of *C. elegans* ferritin genes.

1.4. Transcriptional Regulation of Genes Required for Iron Acquisition in Plants

Plants, like all eukaryotes, require iron and have to deal with the issue of bioavailability. For plants, the problem is particularly acute as 30% of plants live in alkaline soil.[91] Alkaline conditions promote the insolubility of iron, increasing the difficulty and energy that plants must expend to make

iron bioavailable. Plants utilize two different strategies to obtain iron. Grasses, which are monocots, secrete metabolites of mugineic acid that tightly bind iron, which are termed phytosiderophores.[92] The iron−phytosiderophore complexes are taken up by specific transporters present in roots.[93] The transporters responsible for taking up iron−phytosiderophore complexes were identified through cloning of a gene responsible for iron deficiency in maize. A maize mutant, termed "yellow stripe" due to severe yellowing, iron deficiency between the veins, lacked the ability to take up Fe(III)−PS complexes. The gene (*YS1*) that was defective in the yellow stripe mutant was shown to encode a membrane protein that was a phytosiderophore−iron proton-coupled symporter, as shown by physiological studies of the protein expressed in *Xenopus* oocytes.[94] In a given species, there are many genes that encode YS transporters that function in both iron acquisition into roots and distribution of iron within plant tissues.[95]

The genes that encode the enzymes involved in mugenic acid synthesis, as well as genes that encode the transporters, are transcriptionally increased as a result of iron deficiency. Transcription of iron-responsive genes in rice is controlled by OsIRO2, which is a member of the bHLH family of transcription factors.[96] Expression of OsIRO2 in plants as a transgene increased mRNAs for both phytosiderophore synthesis and for uptake of iron−phytosiderophores. Conversely, RNAi silencing of osIRO2 resulted in decreased expression of phytosiderophore genes and decreased iron uptake. The binding site for osIRO2 in promoter of many iron-regulated genes was identified as CACGTGG. A large number of genes are regulated by osIRO2, but only a subset of those genes have binding sites for osIRO2. Examination of the promoter region in IDS2, which encodes a dioxygenase involved in phytosiderophore biosynthesis, revealed two iron-responsive elements termed iron deficiency element 1(IDE1) and IDE2. Proteins that bound to each of these elements were identified as transactivating factors that regulated iron-deficiency response. The transcription factor iron deficiency element factor 1 (IDEF1) binds to IDE1, and transcription factor IDEF2 binds to IDE2. Plants carrying IDEF1 as a transgene showed resistance to iron deficiency.[98] Silencing of IDF2 by RNAi resulted in aberrant iron homeostasis with decreased expression of iron transporters and enzymes required for phytosiderophore synthesis and transport.[99] Many of the genes suppressed in IDEF2 RNAi plants were also regulated by osIRO2, suggesting a complex relationship among the iron-responsive transcription factors. The levels of IDF1 and IDF2 mRNA were not affected by iron, but the promoter region for osIR2 has an IDE2 binding site and may be regulated by IDF2. The mechanism by which any of these transcription factors sense iron, however, remains to be determined.

The approach used primarily by dicots (nongrasses) to solubilize iron involves a cell surface ferrireductase for reduction of Fe^{3+} to Fe^{2+} followed by transport into cells through a Fe^{2+} transmembrane permease Irt1 (for review, see refs 100 and 101). The expression of Irt1 and root ferrireductases is regulated by iron, and these activities are increased by iron scarcity. A transcriptional regulator for the iron acquisition genes in plants was identified through the positional cloning of a mutant gene in tomato. Mutations in the *fer* gene result in an inability to induce transcription of the iron starvation response, resulting in iron-starved (chlorotic) plants.[102] Positional cloning of the *fer* locus identified

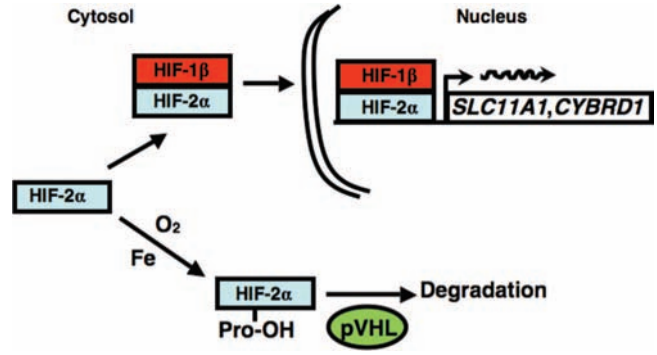

Figure 7. Transcriptional regulation of the vertebrate intestinal iron transport system. HIF-2α is a bHLH protein that is synthesized in intestinal cells. Prolines within HIF-2α are hydroxylated by cytosolic iron-containing prolyl hydroxylases, and hydroxylation requires oxygen as a substrate. The von Hippel-Lindau tumor suppressor (pVHL), which is an E3-ubiquitin ligase protein, ubiquitinates hydroxylated HIF-2α Ubiquitination of HIF-2α results in its degradation by the proteasome. In the absence of oxygen or iron, HIF-2α is not hydroxylated and not degraded. Increased amounts of HIF-2α permit it to bind to cytosolic HIF-2β (also known as ARNT), another bHLH protein. The heterodimeric complex translocates into the nucleus, where it activates transcription of the ferrireductase CYBRD1/DCYTB and the iron transporter SLC11A1/DMT1.

a bHLH transcription factor as the *fer* gene.[103] Transposon insertions into the native *fer* locus reproduced the phenotype of chlorosis, providing proof that this gene was the responsible transcription factor. The mutant phenotype of chlorosis could be reproduced by mutation of the homologous gene in other species of plants.[104] Finally, ectopic expression of the *fer* gene or its homologues from other plants could rescue the phenotype of *fer* mutants.

The *fer* gene is part of a very large family of plant bHLH transcription factors. There may be at least 161 members of this family in *Arabidopsis*. While expression of *fer* mRNA in tomato was reported to be iron-insensitive, the levels of the *fer* homologue *FIT1* in *Arabidopsis* mRNA are iron regulated.[105] Overexpression of *FIT1* in transgenic plants, driven by an iron-insensitive promoter, did not lead to increases in the iron-acquisition system. This result suggests that the activity of *FIT1*, in addition to its expression, may be iron-regulated. It has been suggested that *FIT1*, like other bHLH transcription factors, dimerizes with a second transcription factor; the second factor, or the heteromeric complex, might then have iron-regulated properties.

1.5. Transcription Regulation of Genes Involved in Iron Acquisition in Vertebrates

Vertebrates like all other organisms regulate iron acquisition. Iron uptake into vertebrates occurs primarily in the proximal portion of the intestine, the duodenum. Iron absorbed by the apical surface of the duodenal enterocyte is either stored within the enterocytes or exported from the enterocyte into plasma by iron transporters present on the basolateral surface (Figure 8). Iron acquisition across the apical plasma membrane is mechanistically similar to iron acquisition in yeast and plants. Iron uptake occurs in a relatively acidic environment. The duodenum is the first part of the intestine to receive the contents of the stomach. The pH of the stomach can get as low as 1.0, which facilitates solubilization of iron. The apical surface of the absorptive duodenal cells contains a ferrireductase encoded by the

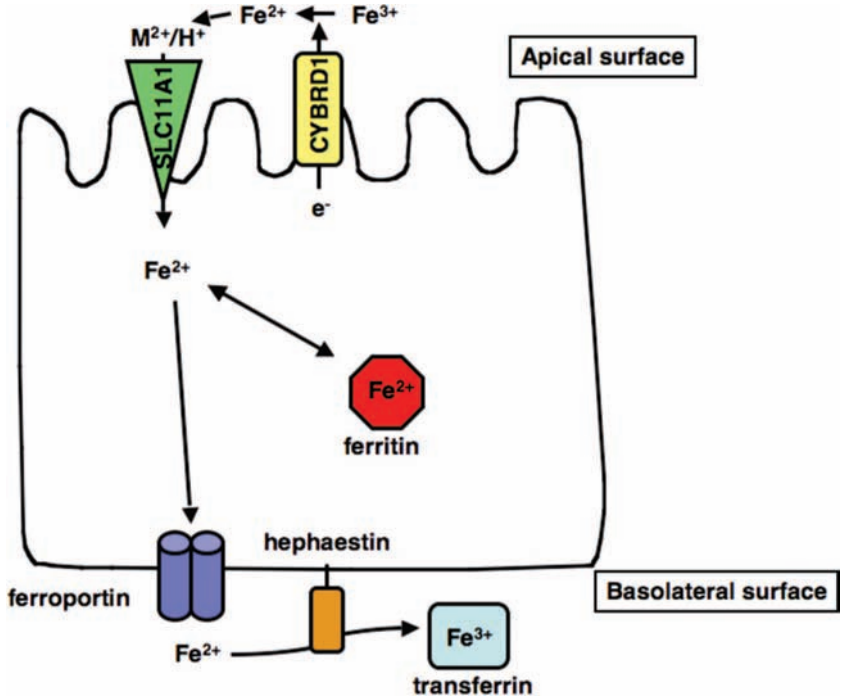

Figure 8. Iron transport in duodenal enterocytes. The apical surface of the duodenal enterocytes is exposed to low pH that helps to solubilize Fe^{3+}. A cell surface reductase CYBRD1/DCYTB converts Fe^{3+} to Fe^{2+}, which is then transported across the apical surface by the H^+/M^{2+} symporter SLC11A1/DMT1. Within the cytosol of the enterocytes, Fe^{2+} can either be stored in ferritin and or be exported across the basolateral membrane. Export is mediated by the iron transporter ferroportin (Fpn). Iron transport requires the activity of the multicopper oxidase hephaestin that converts Fe^{3+} to Fe^{2+}, which is then bound to the plasma glycoprotein transferrin.

Cybrd1 gene (protein product commonly termed DcytB), which can reduce Fe^{3+} to Fe^{2+}. While mice with a targeted gene deletion of *Cybrd1/Dcytb* showed little phenotype, there is extensive physiological data showing that a cell surface ferrireductase is required for iron transport. Intestinal ferrireductase activity is regulated in an iron-sensitive manner; activity is increased in iron deprivation and decreased when iron demands have been satisfied. The intestinal apical surface also contains a transmembrane permease, which can accept Fe^{2+} as a substrate. The apical iron transporter is encoded by *SLC11A1* (human; *Slc11a1* in the mouse nomenclature), commonly referred to as divalent metal transporter 1 (DMT1), which is a homologue of the yeast *SMF* family of transporters and is a H^+/M^{2+} symporter. Mutations in *Slc11a1/DMT1* in both mouse and rat lead to a marked inability to absorb iron from the diet.[106,107]

Expression of *Slc11a1/DMT1* is regulated both transcriptionally and translationally. *Slc11a1/DMT1* mRNA levels are altered in response to iron deficiency or sufficiency. Two recent studies determined that the transcriptional regulation of intestinal *Slc11a1/DMT1* depends on the oxygen-sensitive factor HIF-2α. Hypoxia-inducible factor (HIF) is a heterodimeric nuclear transcription factor consisting of two bHLH proteins, including an oxygen- and iron-sensitive regulatory subunit, HIFα. There are three different HIFα family members, HIF-1α, HIF-2α, and HIF-3α. HIF-1α is ubiquitously expressed, and HIF-2α shows cell-type specific expression. The expression pattern of HIF-3α remains to be clarified. The second subunit of the heterodimeric complex is another bHLH protein termed HIF-1β or aryl hydrocarbon receptor nuclear translocator (ARNT). This protein is constitutively expressed and, under basal conditions, is cytosolic. Under normal conditions of oxygen tension and iron sufficiency, HIFα proteins are rapidly degraded in the cytosol by ubiquitin-mediated proteosomal degradation. The signal for ubiquitinylation of HIF-α is the presence of hydroxylated prolines due to the action of prolyl hydroxylases (Figure 7). Prolyl hydroxylases are iron- and oxygen-dependent enzymes that utilize the metabolite oxoglutarate to hydroxylate specific prolines on HIF-α. Hydroxylated HIF-α is recognized by a specific subunit of the E3-ubiquitin ligase, termed the von Hippel-Lindau tumor suppressor factor (pVHL). This protein was named for a form of renal cancer that occurs when the protein is mutated or missing. In the absence of iron or oxygen, HIF-α is not prolyl-hydroxylated and, therefore, is not degraded. The level of HIF-α increases, permitting it to dimerize with HIF-1β. The heterodimeric complex then translocates to the nucleus, where it binds to specific DNA elements, activating transcription of genes involved in response to hypoxia, metabolic changes, and iron metabolism.

Shah et al. demonstrated that an intestinal specific *Hif1b* (*Hif-1β, Arnt*) knockout mouse showed increased expression of mRNA for *Cybrd1/Dcytb* and *Slc11a1/DMT1*.[108] Because an intestinal specific knockout of *Hif1a* did not induce increased expression of mRNA for *Cybrd1/Dcytb* and *Slc11a1/DMT1*, the authors surmised that HIF2A (HIF-2α) was responsible for the induction of mRNA involved in iron-transport activity. Mastrogiannaki et al. confirmed the role of HIF2A by comparing the effects of intestinal-specific deletion of *Hif1a* to that of *Hif2a*.[109] Deletion of *Hif1a* affected the level of the HIF1A (HIF-1α)-target genes (glucose transporters) but did not affect mRNA levels for *Cybrd1/Dcytb* and *Slc11a1/DMT1*. In contrast, deletion of *Hif2a* had no effect on the mRNA for glucose transporters but did result in reduced expression of *Cybrd1/Dcytb* and *Slc11a1/DMT1* mRNA. The promoter regions of *Cybrd1/Dcytb* and *Slc11a1/DMT1* contain binding sites for HIFA, and HIF2A (HIF-2α) was found bound to the *Slc11a1/DMT1* promoter in a cultured intestinal cell system in response to either iron chelators or hypoxic mimetics. Deletion of *Hif2a*

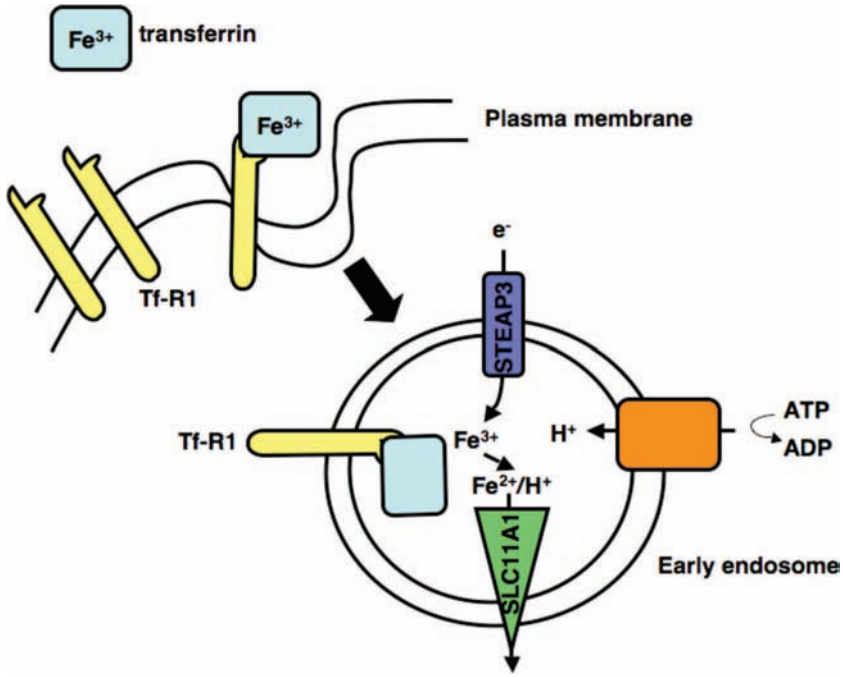

Figure 9. Transferrin-mediated iron delivery to cells. Plasma transferrin with two bound iron atoms ($Tf(Fe_2)$) binds to transferrin receptor 1 (Tf−R1) present on the surface of dividing cells and erythrocyte precursors. The $Tf(Fe_2)$−Tf−R1 complex is internalized into the early endosome, which is an acidic compartment. Within the early endosome, Fe^{3+} is released from Tf and is reduced to Fe^{2+} by the ferrireductase STEAP3. Fe^{2+} is transported across the endosomal membrane by SLC11A1/DMT1. apoTf remains bound to Tf-R1 within the endosome and is recycled back to the cell surface, where it is released into plasma.

resulted in decreased serum and liver iron levels, which demonstrated that control of *Slc11a1/DMT1* expression by HIF2A (HIF-2α) resulted in decreased iron acquisition. These studies indicate that a lack of dietary iron leads to decreased cytosolic iron in intestinal cells, resulting in decreased activity of the iron-containing prolyl hydroxylase. Decreased prolyl hydroxylase activity results in stabilization of HIF2A (HIF-2α) and increased transcription of *Cybrd1/Dcytb* and *Slc11a1/DMT1*. Increased expression of these genes would also occur in response to hypoxia, reflecting a need for increased red blood cells, as red blood cell heme synthesis is the largest consumer of iron.

The apical iron transporter SLC11A1/DMT1 in vertebrates, like the apical iron transporter IRT1 in plants, is not specific to iron. Both transporters can accumulate other transition metals such as Mn^{2+} and Zn^{2+}. The specificity for iron acquisition results from the combination of the transport specificities of the apical iron importer SLC11A1/DMT1 and the iron exporter ferroportin (Fpn, product of the *SLC40A1* gene, also referred to as Fpn1) at the baso-lateral surface of the intestinal cell. Fpn is the only identified vertebrate iron exporter.[110] Data suggests Fpn is a Fe^{2+} transporter that requires the activity of a multicopper oxidase to export iron from cells into plasma. Fpn-mediated iron transport is severely reduced in the absence of a companion multicopper oxidase. Mice with mutations in the intestinal multicopper oxidase hephaestin have impaired iron transport into plasma.[83] Fpn is found on other iron-exporting cells, and the absence of the multicopper oxidase ceruloplasmin results in an impaired iron transport in ferroportin-containing macrophages[111] and neural cells.[112] *Fpn* mRNA levels are increased in intestinal cells in response to systemic iron need and iron deprivation.[113] Mastrogiannaki et al. made the observation that deletion of intestinal *Hif2a* resulted in an increase in ferroportin mRNA but no HIFA binding sites were detected in the ferroportin promoter regions, suggesting that *Fpn* mRNA might be affected indirectly.[109]

Iron absorbed by the intestine enters the bloodstream, where it is distributed to other cell types that either utilize iron or store iron. Thus, iron acquisition by one cell type must be coordinated with iron utilization by other cell types. Iron within plasma is distributed to different tissues in response to their iron need. This is accomplished by the binding of two atoms of iron to the plasma protein transferrin. Transferrin (Tf) is considered a mammalian siderophore, as it binds two atoms of Fe^{3+} with extremely high affinity. The concentration of Tf in plasma is high ($3-5 \times 10^{-5}$ M), and under normal conditions only a fraction (30−50%) of transferrin molecules are iron-bound (for review, see ref 114). Tf−(Fe_2) is bound to cell-surface transferrin receptors (Tf−R1) (Figure 9). The $Tf(Fe_2)$−Tf−R1 complex is internalized into an acidic compartment, the early endosome. Within this compartment, Fe^{3+} is released from Tf and is reduced by a membrane-bound reductase STEAP3, and Fe^{2+} is transported across the endosomal membrane by SLC11A1/DMT1. There are two genes that encode Tf−R. Tf−R1 is involved in iron delivery to cells, while Tf−R2 is involved in systemic iron-homeostasis. Transcription of Tf−R1 is regulated in response to cell growth or, in developing red blood cells, in response to differentiation. The transcriptional regulation of erythroid Tf−R1 has not been defined, although an erythroid-specific deletion of the gene that encodes the STAT5 transcription factor results in a 50% decrease in transferrin receptor 1 mRNA.[115] The levels of Tf−R1 mRNA are regulated post-transcriptionally by intracellular iron through the action of the IRPs, which bind to the 3′-untranslated region of the transferrin receptor-1 mRNA (for review, see further discussion of IRP mechanism below). Binding of the IRP to the Tf−R1 mRNA stabilizes the

mRNA by protecting it from degradation.[116] Thus, the presence of Tf−R1 receptors on cell surfaces signals a need for iron.

In multicellular organisms, iron acquisition is coordinated with iron utilization and storage by other cell types and a mechanism must exist to regulate iron on a systemic level. In vertebrates, this occurs through the interaction of the peptide hormone hepcidin and the iron exporter Fpn. Hepcidin is synthesized by a number of cell types but primarily hepatocytes and is the product of the *HAMP* gene in humans (*Hamp* in mice). Hepcidin is a negative regulator of iron acquisition. Transcription of hepcidin is increased in response to iron sufficiency and is decreased under conditions of iron need such as hypoxia or anemia. Thus, hepcidin levels are decreased when there is demand for iron to increase red blood cell production. The reader is directed to recent reviews on the transcriptional regulation of hepcidin.[117−119] Hepcidin regulates iron entry into blood by binding to Fpn on the surface of iron-exporting cells such as duodenal enterocytes, macrophages, and syncytial trophoblasts. Hepcidin binding to Fpn induces the internalization and degradation of Fpn.[120] The loss of Fpn prevents iron export and leads to increased cellular iron retention through increased ferritin levels. The absorptive duodenal intestinal cell has a short life span of 3−5 days, depending on species. The cells are sloughed off into the intestine, and iron retained within cells is lost to the body. Thus, increased hepcidin prevents cells from exporting iron to plasma, while decreased hepcidin by maintaining cell surface Fpn promotes iron absorption.

1.6. Regulation of Iron Acquisition Occurs at Multiple Levels

Iron acquisition is critical for all eukaryotes and shows exquisite regulation at multiple levels of gene expression. As described above, in *S. cerevisiae*, decreased cytosolic iron reduces expression of the vacuolar iron transporter Ccc1p by decreasing transcriptional activation and increasing the degradation of *CCC1* mRNA. A parallel situation exists for the vertebrate intestinal transporter gene *SLC11A1/DMT1*. Activation of HIF-2α-induced transcription of *Slc11a1/DMT1* is specific for an *Slc11a1/DMT1* isoform that contains an iron-responsive element (IRE) in the 3-untranslated (UTR) region of its mRNA. The IRE-containing *Slc11a1/DMT1* isoform (*Slc11a1/DMT1-IRE*) is dramatically induced upon iron starvation. IREs are found in either the 5′-UTRs or 3′-UTRs of mRNAs, and are binding sites for iron regulatory proteins (IRP-1, IRP-2, referred to here as IRP). These proteins, by binding to 3′-IREs, regulate mRNA stability or, by binding to 5′-IREs, regulate protein translation (for review, see refs 87 and 121). In response to low cytosolic iron, increased HIF-2α activity induces transcription of *Slc11a1/DMT1-IRE*. It is hypothesized that low cytosolic iron promotes the binding of IRP to the 3′-UTR of *Slc11a1/DMT1-IRE*, stabilizing mRNA levels by preventing degradation. Increased expression of *Slc11a1/DMT1-IRE* leads to increased cytosolic iron, which subsequently reduces transcription of *Slc11a1/DMT1-IRE*. Further, decreased cytosolic iron would induce release of IRP from *Slc11a1/DMT1-IRE*, decreasing mRNA levels through increased mRNA degradation. Deletion of IRP-encoding genes *Aco1* (*Irp1*) and *Ireb1* (*Irp2*) in intestinal cells of mice affects *Slc11a1/DMT1-IRE* mRNA levels, suggesting that this isoform can by affected by post-transcriptional regulation.[122]

As discussed above, the binding of hepcidin to Fpn, which induces Fpn degradation, regulates iron homeostasis in vertebrates. Fpn synthesis is also regulated by IRP, being increased by intracellular iron, as iron promotes the loss of IRP from the 5′ IRE of *Fpn*. Thus, hepcidin, which decreases Fpn levels in the plasma membrane, will increase Fpn synthesis within cells. The balance between increased synthesis and increased degradation results in fine-tuning of iron acquisition. Similarly in yeast, iron-induced degradation of the Fet3p/Ftr1p iron transport system is balanced by transcriptional activation of that system by iron. When the cytosol is iron-sufficient, the loss of Fet3p/Ftr1p from the cell surface is not accompanied by their replacement, as transcription is no longer activated. We extrapolate from these observations in yeast and humans to suggest that iron acquisition in all eukaryotes will be highly regulated and the levels of transporters determined by both transcriptional and post-transcriptional modes of regulation.

2. Glossary

Gene and protein nomenclature notes for *S. cerevisiae*, gene name *FET3*, mutants in gene *fet3*, protein name Fet3p. Terminology for *S.pombe*, gene name *fio1*$^+$, mutants in gene *fio1*, protein Fio1. Terminology for mouse; gene *Slc11a1*, protein SLC11A1. Terminology for human; gene *SLC11A1*, protein SLC11A1.

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