Published in final edited form as:

Curr Opin Chem Biol. 2011 April ; 15(2): 312–318. doi:10.1016/j.cbpa.2011.01.003.

Iron chaperones for mitochondrial Fe–S cluster biosynthesis and ferritin iron storage

Poorna Subramanian, Andria V Rodrigues, Sudipa Ghimire-Rijal, and Timothy L Stemmler

Department of Biochemistry and Molecular Biology, Wayne State University, 540 E. Canfield Ave., Detroit, MI 48201, USA

Abstract

Protein controlled iron homeostasis is essential for maintaining appropriate levels and availability of metal within cells. Recently, two iron chaperones have been discovered that direct metal within two unique pathways: (1) mitochondrial iron–sulfur (Fe–S) cluster assembly and (2) within the ferritin iron storage system. Although structural and functional details describing how these iron chaperones operate are emerging, both share similar iron binding affinities and metal–ligand site structures that enable them to bind and release Fe²⁺ to specific protein partners. Molecular details related to iron binding and delivery by these chaperones will be explored within this review.

Introduction

Iron is essential for life and its unique chemical characteristics are often exploited in nature to assist cells in performing inherently complex oxidation chemistry, promote oxygen transport/storage and drive electron transfer pathways [1]. Iron is typically absorbed into the body through diet [2] and on average, humans absorb 1–2 mg of iron from diet each day [3]. Although the amount of iron abstracted from the diet is low, tight regulation of absorbed iron is critical, as humans have no physiologic pathway to excrete metal. Once consumed, over 60% of the iron is incorporated into hemoglobin through developing erythroid precursors and finally into mature red blood cells. A second and third possible fate for Fe includes being directed towards the iron storage protein ferritin or being transferred throughout the body for incorporation into metalloprotein iron cofactor sites. As a cofactor, Fe is often found associated within iron–sulfur (Fe–S) clusters, found as mononuclear Fe–S, 2Fe–2S, 4Fe–4S and other higher nuclearity clusters common to nature [4]. Given the body's need to absorb and maintain appropriate iron levels, Fe reuse and recycling from degraded sources is another method the body uses to maintain iron homeostasis. A simple breakdown in any of these pathways can lead to conditions that are detrimental to human health.

When maintained at balanced levels, iron is essential for cellular and organism viability, however excess is toxic and deficiency leads to a variety of physiological and developmental irregularities [3]. Disorders related to a breakdown in iron homeostasis are among the most common human diseases. Diseases of iron overload are highly prevalent, with the hepatological disorder hereditary hemochromatosis being one of the most common [5]. Hemochromatosis patients absorb two to three times more iron than normal dietary iron levels. Excess iron is often deposited in the liver and redox chemistry performed by unregulated metal can kill the cell/organism. Neuronal iron accumulation is a phenotype of numerous neurodegenerative disorders (Parkinson's disease, Alzheimer's disease,

Friedreich's ataxia, etc.). In these disorders, unregulated but redox active metal is pooled within specific regions of the brain generating oxidative stress through iron produced reactive oxygen species (ROS) formation [6]. ROS can damage membrane phospholipids and generate reactive aldehydes that damage proteins, leading to the accumulation of misfolded protein aggregates. Although examples of the toxic effects of iron overload are prevalent, iron deficiency is an even larger human health issue affecting billions [7]. While iron deficiency leads to decreased cognitive development in children [8], continual long-term deficiency leads to numerous other health related issues [3]. It is clear that biological pathways controlling iron reactivity are essential, however it is equally clear that pathways ensuring metal delivery occurs are equally important for maintaining cell viability.

Many pathways that regulate cellular iron homeostasis are controlled at the genetic and at the protein level [9,10]. As outlined in the transferrin cycle, iron is imported through endocytosis of Fe³⁺ loaded transferrin (Tf) interacting with the transferrin receptor (Tf-R), in a tightly regulated feedback loop controlled at the genetic and protein level (Figure 1). In eukaryotes, iron can be diverted to the mitochondria where it can be utilized during heme production or during Fe-S cluster assembly (pathway #1 in Figure 1). During mitochondrial Fe-S cluster assembly, the ISC machinery proteins work together to form clusters that can be loaded onto apo-mitochondrial proteins or pumped out of the mitochondria in some manner (labeled X) to be used by the cytosolic Fe-S cluster assembly machinery (CIA). Cytosolic iron can also be directed for storage into ferritin in an inert form for future use (pathway #2 in Figure 1). Although there are obviously other fates for cellular iron, recently two iron chaperones have been discovered that control iron delivery in the pathways highlighted above. An overview of the structure and biophysical properties leading to iron binding by these two chaperones is the basis of this review.

Fe-S cluster biosynthesis iron chaperones

In eukaryotes, the mitochondrial ISC assembly is the major pathway for Fe-S cluster production. Key players in the yeast ISC machinery include the assembly scaffold protein (either Isu1 or 2, for clarity we will refer to only Isu1), the cysteine desulfurase (Nfs1) that provides sulfur to Isu1, the accessory protein (Isd11) that works in complex with Nfs1, a ferredoxin (Yah1) working with a ferredoxin reductase (Arh1) to provide electrons for the reaction, and the putative iron chaperone protein frataxin (Yfh1) [11]. Protein orthologs are highly conserved and found in all eukaryotes, while in prokaryotes most are found within the ISC pathway while variants of these proteins exist within the sulfur mobilization (SUF) and the nitrogen fixation (NIF) assembly pathways [12]. Isu1 is one of the most conserved proteins [13,14], containing three conserved cysteines critical for de novo Fe-S cluster assembly [15•,16•]. Sulfur transfer between Nfs1 and Isu1 occurs as a persulfide (–SSH) through a direct interaction between partners [17]; insight into the nature of this interaction has come from a recent structural characterization of bacterial orthologs [18•]. In *in vivo*, Yfh1 is essential for Fe-S cluster maturation [19•], and an interaction between Yfh1 and Isu1/Nfs1/Isd11 is critical for cluster biosynthesis [20]. In *in vitro*, frataxin was shown to deliver iron to the scaffold protein to stimulate Fe-S cluster assembly [21•]. On the basis of these and additional reports (see review [11]), frataxin was shown to play a direct role in mitochondrial Fe-S cluster assembly, possibly by serving as the iron chaperone [20,22], deficiency of which has direct consequences regarding cellular oxidative stress [23].

Insight into frataxin's function has come from investigating the structure and iron binding abilities of numerous orthologs [24•]. Frataxin is a member of the α-β sandwich motif family, with N-terminal and C-terminal helices constructing one molecular plane and the second constructed by at least five anti-parallel β-strands [25–30]. Key structural features for the frataxin orthologs include highly conserved surface exposed acidic residues lining

the helix-1/strand-1 junction, a short predominately unstructured N-terminal region in eukaryotic orthologs only, and a C-terminal region in the human and bacterial proteins that confer stability [31]. The iron binding ability of frataxin was first identified in the yeast ortholog associated with iron induced aggregation stabilized by low salt/oxygenated solution conditions [32]. Monomeric bacterial, yeast, fly and human frataxin have since been shown to bind iron with micromolar affinity [21••,28,33–36], and *in vivo* mutations preventing Yfh1 aggregation show no Fe–S cluster phenotypes under normal growth conditions [37], indicating while protein aggregation may be important under oxidative stress, monomeric frataxin is active during Fe–S cluster assembly. Experimental data verify conserved acidic residues on frataxin's helix-1/strand-1 junction are directly involved in iron binding [28,33,35]. Structural studies confirm monomeric frataxin binds high spin Fe²⁺ in a symmetric 6-coordinate ligand environment constructed of only oxygen and nitrogen ligands, in agreement with the conserved acidic residues identified by NMR as iron ligands [35,36,38]. These data suggest as a starting point for iron delivery, frataxin binds iron using carboxylate side chain oxygens from conserved Asp and Glu residues from the helix-1/strand-1 iron binding sites, most likely with assistance from water molecules. Binding in this manner would predispose iron for delivery to Isu1 (Figure 2) since the exposed metal would be positioned for delivery to the protein partner and the charged surface surrounding the metal would be amenable for forming an interaction with a complementary charged region of the protein partner.

Yfh1 undergoes an iron-stimulated interaction with Isu1/Nfs1 [20,39,40] in agreement with frataxin interacting directly with members of the ISC assembly machinery. *In vivo* mutational studies place frataxin's acidic helix-1/strand-1 ridge, with residues on the Yfh1 β -sheet surface, as interacting with Isu1 [39–43]. *In vitro* binding interactions show frataxin/scaffold binding is iron dependent and occurs at nM binding affinity [36,44,45]. Chemical shift mapping studies provide spectroscopic confirmation that Isu1 interacts with Yfh1 on frataxin's acidic helix-1/strand-1 ridge utilizing the protein's β -sheet surface, in close agreement with *in vivo* mutational analysis [45]. In eukaryotic systems, activity assays for Fe-S cluster production confirm iron loaded frataxin stimulates *in vitro* Fe–S cluster assembly, possibly by delivering the Fe²⁺ required for cluster production [21••,36,46]. Direct structural details of the frataxin/scaffold interaction are however lacking. Two recent structural papers shed light on the potential binding interaction between these protein partners. Crystallographic data on the *Aquifex aeolicus* IscU ortholog, with cluster bound, confirm the three conserved Cys residues implicated in assembly form the active site of IscU [47]. An even more exciting result comes from the structure of the bacterial cysteine desulfurase (IscS) in complex with IscU [18••]. This structure shows IscU binds to IscS such that the IscU Cys active site is in close contact with the IscS Cys residue implicated in forming the persulfide used for sulfur transfer. Binding of Isu1 to Nfs1 in this manner would prevent frataxin from interacting in this region, assuming sulfur delivery happens before iron delivery. Irrespective of this assumption, mounting evidence suggests all proteins in the ISC machinery form a complex that promotes the formation of a stable macromolecular complex used to accomplish cluster assembly, so the frataxin piece of this puzzle would need to fit in a unique location on Isu1. A likely candidate for where Yfh1 could bind to Isu1 is on the scaffold protein's C-terminal helix (Figure 2) [45]. The charge distribution from the electrostatic potential surface of Isu1 in this region matches nicely to the Yfh1 β -sheet surface. Conserved acidic/basic residues on Isu1's C-terminal helix could serve as the initial Fe²⁺ binding residues, metal could then translocate to the scaffold's active site [45]. Positional orientation of bacterial frataxin on the IscS/U from small angle X-ray scattering studies [48] suggests that such an orientation may be possible, although this orientation is likely to be altered between the prokaryotic and eukaryotic orthologs since the accessory protein Isd11 found only in eukaryotes is likely also positioned in this region. Following Fe delivery to Isu1 by Yfh1, the binding energetics are reduced, frataxin dissociates from its

partner and Isu1 is primed for delivery of the second Fe atom required to make a 2Fe–2S cluster.

Recently it has been suggested frataxin may participate in Fe-S cluster biosynthesis by regulating activity of the cysteine desulfurase. NMR mapping studies of bacterial frataxin (CyaY) onto the bacterial IscS suggest residues lining frataxin's β -sheet ridge tangential to the C-terminus of helix 1, with helix-1 residues, participate in IscS binding [49]. Mutagenesis and binding studies implicate a region on one IscS monomer, in proximity to the persulfide site in the second molecule in the dimer, as the CyaY binding surface on IscS [18••]. Recent studies suggest CyaY acts as an iron-dependent inhibitor of cluster formation by negatively regulating IscS activity [49]. In contrast, another recent report suggests human frataxin acts as an allosteric activator of Nfs1 [50]. Regardless, binding of frataxin to the ISC machinery is important during assembly and it will be interesting to see how this binding is finally correlated to iron delivery and assembly.

Ferritin iron chaperones

Cellular pathways directing iron storage are essential for preventing iron toxicity during overload and ensuring availability during deficiency. Storage is directed by the 4-helix bundle protein ferritin [51]. Mammalian cytoplasmic ferritins exist as a 24 subunits multimer of 2 ferritin subunits: H chain (21,000 kDa), which controls the ferroxidase chemistry coupled to iron storage, and the L chain (19,000 kDa). Apo-ferritin H and L chains selfassemble to form a spherical protein shell that stores up to 4500 iron atoms as ferroxhydryte within the central cavity. Iron storage involves two key steps: iron oxidation, leading to the formation of a μ -oxo(hydroxo)-bridged di-Fe(III) intermediate, and mineralization, depositing the intermediate as inert ferrihydrite mineral. To proceed, iron must first be delivered to ferritin, and ferrous iron delivery occurs at a ferritin oligomer hydrophilic threefold (iron entry) channel. H-chain surface exposed residues H118 [52] and C130 [53], with threefold channel funnellike forming residues Asp131 and Glu134 [54], are used during iron import. Binding of an iron chaperone must therefore proceed through direct contact with ferritin's threefold iron entry site (see Figure 3).

Recently, the human poly (rC)-binding protein (PCBP) was shown to function as a cytosolic iron chaperone that delivers Fe^{2+} to ferritin [55••]. The RNA-binding PCBP protein family was originally shown to be important in mRNA stabilization and translational activation/silencing [56]. PCBPs have 3 KH (hnRNP K homology) domains, with structures of the two N-terminal domains and the third domain of PCBP2 being characterized [57,58]. Expression of human PCBP1 recovered an iron loading deficiency for human ferritin expressed in yeast [55]. PCBP1 binds to ferritin *in vivo* and *in vitro* facilitates iron loading. PCBP1 binds 3 iron atoms at μM binding affinity comparable to frataxin's iron affinity [24•]. Metal binding sites on PCBP are unknown; however, recent iron structural data indicate PCBP1/2 bind ferrous iron in a 6-coordinate oxygen/nitrogen ligand environment (author's unpublished results). Although molecular details of PCBP as an iron chaperone are still emerging, it is interesting to note that thermodynamic and structural data regarding PCBP iron binding are so similar to that of the Fe–S assembly iron chaperone.

Conclusions

Evidence that frataxin and PCBP are iron chaperones is compelling, although clarification of their function at the genetic, molecular and atomic levels will be important for understanding how they operate. There are still many questions that remain regarding the identity of other chaperones, how iron is loaded onto a chaperone and what promotes chaperone metal specificity? A possible candidate for the first question is the Glutaredoxins

Grx3/4, members of the thioredoxin (Trx) fold family. Grx3/4 are central for cellular iron trafficking and a strong dependence of iron binding for Grx3/4 on core components in the ISC pathway indicate Grx3/4 bind iron as a Fe–S cluster [59]. The ability to form a bridging 2Fe–2S cluster with Fra1/2 for activation of Aft1 [60], coupled with the direct dependence of Grx3/4 on iron loading into heme, Fe–S clusters, into ribonucleotide reductase, etc. suggests iron activated by Grx3/4 may be utilized by other dedicated assembly factors [59]. Frataxin and PCBP are most likely the start of a soon to be recognized long list of iron chaperones.

Acknowledgments

We would like to thank Dennis Winge, Andy Dancis and Roland Lill for helpful discussions regarding the material. Work in the Stemmler laboratory is supported by the National Institute of Health (DK068139).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Crichton, R. Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences. edn 2. West Sussex, England: John Wiley & Sons, Ltd; 2001.
2. Andrews NC, Schmidt PJ. Iron homeostasis. *Annu Rev Physiol.* 2007; 69:69–85. [PubMed: 17014365]
3. Andrews NC. Disorders of iron metabolism. *N Engl J Med.* 1999; 341:1986–1995. [PubMed: 10607817]
4. Beinert H, Holm RH, Munck E. Iron–sulfur clusters: nature’s modular, multipurpose structures. *Science.* 1997; 277:653–659. [PubMed: 9235882]
5. Eisenstein RS. Interaction of the hemochromatosis gene product HFE with transferrin receptor modulates cellular iron metabolism. *Nutr Rev.* 1998; 56:356–358. [PubMed: 9884584]
6. Crichton RR, Dexter DT, Ward RJ. Brain iron metabolism and its perturbation in neurological diseases. *J Neural Transm.*
7. Andrews NC. Forging a field: the golden age of iron biology. *Blood.* 2008; 112:219–230. [PubMed: 18606887]
8. Pollitt E. Iron deficiency and cognitive function. *Annu Rev Nutr.* 1993; 13:521–537. [PubMed: 8369157]
9. Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. *Cell.* 2004; 117:285–297. [PubMed: 15109490]
10. Kaplan CD, Kaplan J. Iron acquisition and transcriptional regulation. *Chem Rev.* 2009; 109:4536–4552. [PubMed: 19705827]
11. Lill R. Function and biogenesis of iron–sulphur proteins. *Nature.* 2009; 460:831–838. [PubMed: 19675643]
12. Lill R, Muhlenhoff U. Maturation of iron–sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. *Annu Rev Biochem.* 2008; 77:669–700. [PubMed: 18366324]
13. Tokumoto U, Kitamura S, Fukuyama K, Takahashi Y. Interchangeability and distinct properties of bacterial Fe–S cluster assembly systems: functional replacement of the isc and suf operons in *Escherichia coli* with the nifSU-like operon from *Helicobacter pylori*. *J Biochem.* 2004; 136:199–209. [PubMed: 15496591]
14. Schilke B, Voisine C, Beinert H, Craig E. Evidence for a conserved system for iron metabolism in the mitochondria of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A.* 1999; 96:10206–10211. [PubMed: 10468587]

15. Yuvaniyama P, Agar JN, Cash VL, Johnson MK, Dean DR. NifS-directed assembly of a transient [2Fe–2S] cluster within the NifU protein. *Proc Natl Acad Sci U S A*. 2000; 97:599–604. [PubMed: 10639125] . This pioneering study showed the active site cysteines of the scaffold protein are essential for cluster assembly.
16. Frazzon J, Dean DR. Formation of iron–sulfur clusters in bacteria: an emerging field in bioinorganic chemistry. *Curr Opin Chem Biol*. 2003; 7:166–173. [PubMed: 12714048] . This paper led to the proposal that the N-terminal region of NifU serves as the scaffold protein in the NIF pathway that directs cluster assembly.
17. Zheng L, Cash VL, Flint DH, Dean DR. Assembly of iron–sulfur clusters, Identification of an iscSUA-hscBA-fdx gene cluster from *Azotobacter vinelandii*. *J Biol Chem*. 1998; 273:13264–13272. [PubMed: 9582371]
18. Shi R, Proteau A, Villarroja M, Moukadiri I, Zhang L, Trempe JF, Matte A, Armengod ME, Cygler M. Structural basis for Fe–S cluster assembly and tRNA thiolation mediated by IscS protein-protein interactions. *PLoS Biol*. 2010; 8:1–18.. This exciting paper provides structural details for understanding the interaction between the cysteine desulfurase and the scaffold protein. From this, the location of the scaffold assembly site is in close proximity to the cysteine desulfurase persulfide forming loop.
19. Muhlenhoff U, Richhardt N, Ristow M, Kispal G, Lill R. The yeast frataxin homolog Yfh1p plays a specific role in the maturation of cellular Fe/S proteins. *Hum Mol Genet*. 2002; 11:2025–2036. [PubMed: 12165564] . This is a pivotal paper implicating frataxin as an essential factor in mitochondrial Fe–S cluster assembly.
20. Gerber J, Muhlenhoff U, Lill R. An interaction between frataxin and Isu1/Nfs1 that is crucial for Fe/S cluster synthesis on Isu1. *EMBO Rep*. 2003; 4:906–911. [PubMed: 12947415]
21. Yoon T, Cowan JA. Iron–sulfur cluster biosynthesis, characterization of frataxin as an iron donor for assembly of [2Fe–2S] clusters in ISU-type proteins. *J. Am. Chem. Soc*. 2003; 125:6078–6084. [PubMed: 12785837] . This was the first paper to suggest frataxin acts as an iron chaperone during mitochondrial Fe–S cluster biosynthesis, based on *in vitro* activity assay data showing stimulation of cluster assembly on the scaffold when frataxin is present.
22. Layer G, Ollagnier-de Choudens S, Sanakis Y, Fontecave M. Iron–sulfur cluster biosynthesis: characterization of *Escherichia coli* CYaY as an iron donor for the assembly of [2Fe–2S] clusters in the scaffold IscU. *J Biol Chem*. 2006; 281:16256–16263. [PubMed: 16603772]
23. Moreno-Cermeno A, Obis E, Belli G, Cabisco E, Ros J, Tamarit J. Frataxin depletion in yeast triggers upregulation of iron transport systems before affecting iron–sulfur enzyme activities. *J Biol Chem*. E-published ahead of print.
24. Bencze KZ, Kondapalli KC, Cook JD, McMahon S, Millan-Pacheco C, Pastor N, Stemmler TL. The structure and function of frataxin. *Crit Rev Biochem Mol Biol*. 2006; 41:269–291. [PubMed: 16911956] . This is a comprehensive review of frataxin's structure and function.
25. Dhe-Paganon S, Shigeta R, Chi YI, Ristow M, Shoelson SE. Crystal structure of human frataxin. *J Biol Chem*. 2000; 275:30753–30756. [PubMed: 10900192]
26. Musco G, Stier G, Kolmerer B, Adinolfi S, Martin S, Frenkiel T, Gibson T, Pastore A. Towards a structural understanding of Friedreich's ataxia: the solution structure of frataxin. *Structure*. 2000; 8:695–707. [PubMed: 10903947]
27. Cho SJ, Lee MG, Yang JK, Lee JY, Song HK, Suh SW. Crystal structure of *Escherichia coli* CyaY protein reveals a previously unidentified fold for the evolutionarily conserved frataxin family. *Proc Natl Acad Sci U S A*. 2000; 97:8932–8937. [PubMed: 10908679]
28. He Y, Alam SL, Proteasa SV, Zhang Y, Lesuisse E, Dancis A, Stemmler TL. Yeast frataxin solution structure, iron binding, and ferroxidase interaction. *Biochemistry*. 2004; 43:16254–16262. [PubMed: 15610019]
29. Nair M, Adinolfi S, Pastore C, Kelly G, Temussi P, Pastore A. Solution structure of the bacterial frataxin ortholog, CyaY: mapping the iron binding sites. *Structure (Camb)*. 2004; 12:2037–2048. [PubMed: 15530368]
30. Karlberg T, Schagerlof U, Gakh O, Park S, Ryde U, Lindahl M, Leath K, Garman E, Isaya G, Al-Karadaghi S. The structures of frataxin oligomers reveal the mechanism for the delivery and detoxification of iron. *Structure*. 2006; 14:1535–1546. [PubMed: 17027502]

31. Adinolfi S, Nair M, Politou A, Bayer E, Martin S, Temussi P, Pastore A. The factors governing the thermal stability of frataxin orthologues: how to increase a protein's stability. *Biochemistry*. 2004; 43:6511–6518. [PubMed: 15157084]
32. Adamec J, Rusnak F, Owen WG, Naylor S, Benson LM, Gacy AM, Isaya G. Iron-dependent self-assembly of recombinant yeast frataxin: implications for *Friedreich ataxia*. *Am J Hum Genet*. 2000; 67:549–562. [PubMed: 10930361]
33. Bou-Abdallah F, Adinolfi S, Pastore A, Laue TM, Dennis Chasteen N. Iron binding and oxidation kinetics in frataxin CyaY of *Escherichia coli*. *J Mol Biol*. 2004; 341:605–615. [PubMed: 15276847]
34. O'Neill HA, Gakh O, Park S, Cui J, Mooney SM, Sampson M, Ferreira GC, Isaya G. Assembly of human frataxin is a mechanism for detoxifying redox-active iron. *Biochemistry*. 2005; 44:537–545. [PubMed: 15641778]
35. Cook JD, Bencze KZ, Jankovic AD, Crater AK, Busch CN, Bradley PB, Stemmler AJ, Spaller MR, Stemmler TL. Monomeric yeast frataxin is an iron-binding protein. *Biochemistry*. 2006; 45:7767–7777. [PubMed: 16784228]
36. Kondapalli KC, Kok NM, Dancis A, Stemmler TL. *Drosophila frataxin*: an iron chaperone during cellular Fe–S cluster bioassembly. *Biochemistry*. 2008; 47:6917–6927. [PubMed: 18540637]
37. Aloria K, Schilke B, Andrew A, Craig EA. Iron-induced oligomerization of yeast frataxin homologue Yfh1 is dispensable in vivo. *EMBO Rep*. 2004; 5:1096–1101. [PubMed: 15472712]
38. Bencze KZ, Yoon T, Millan-Pacheco C, Bradley PB, Pastor N, Cowan JA, Stemmler TL. Human frataxin: iron and ferroxidase binding surface. *Chem Commun (Camb)*. 2007:1798–1800. [PubMed: 17476391]
39. Ramazzotti A, Vanmansart V, Foury F. Mitochondrial functional interactions between frataxin and Isu1p, the iron–sulfur cluster scaffold protein, in *Saccharomyces cerevisiae*. *FEBS Lett*. 2004; 557:215–220. [PubMed: 14741370]
40. Wang T, Craig EA. Binding of yeast frataxin to the scaffold for Fe–S cluster biogenesis, Isu. *J Biol Chem*. 2008; 283:12674–12679. [PubMed: 18319250]
41. Correia AR, Wang T, Craig EA, Gomes CM. Iron binding activity in yeast frataxin entails a trade off with stability in the alpha1/beta1 acidic ridge region. *Biochem J*. 2009
42. Foury F, Pastore A, Trincal M. Acidic residues of yeast frataxin have an essential role in Fe–S cluster assembly. *EMBO Rep*. 2007; 8:194–199. [PubMed: 17186026]
43. Leidgens S, De Smet S, Foury F. Frataxin interacts with Isu1 through a conserved tryptophan in its beta-sheet. *Hum Mol Genet*. 2010; 19:276–286. [PubMed: 19884169]
44. Wu SP, Wu G, Surerus KK, Cowan JA. Iron–sulfur cluster biosynthesis. Kinetic analysis of [2Fe–2S] cluster transfer from holo ISU to apo Fd: role of redox chemistry and a conserved aspartate. *Biochemistry*. 2002; 41:8876–8885. [PubMed: 12102630]
45. Cook JD, Kondapalli KC, Rawat S, Childs WC, Murugesan Y, Dancis A, Stemmler TL. Molecular details of the yeast frataxin–Isu1 interaction during mitochondrial Fe–S cluster assembly. *Biochemistry*. 2010; 49:8756–8765. [PubMed: 20815377]
46. Li H, Gakh O, Smith DYt, Isaya G. Oligomeric yeast frataxin drives assembly of core machinery for mitochondrial iron–sulfur cluster synthesis. *J Biol Chem*. 2009; 284:21971–21980. [PubMed: 19491103]
47. Shimomura Y, Wada K, Fukuyama K, Takahashi Y. The asymmetric trimeric architecture of [2Fe–2S] IscU: implications for its scaffolding during iron–sulfur cluster biosynthesis. *J Mol Biol*. 2008; 383:133–143. [PubMed: 18723024]
48. Prischi F, Konarev PV, Iannuzzi C, Pastore C, Adinolfi S, Martin SR, Svergun DI, Pastore A. Structural bases for the interaction of frataxin with the central components of iron–sulphur cluster assembly. *Nat Commun*. 2010; 1:95. [PubMed: 20981023]
49. Adinolfi S, Iannuzzi C, Prischi F, Pastore C, Iametti S, Martin SR, Bonomi F, Pastore A. Bacterial frataxin CyaY is the gatekeeper of iron–sulfur cluster formation catalyzed by IscS. *Nat Struct Mol Biol*. 2009; 16:390–396. [PubMed: 19305405]
50. Tsai CL, Barondeau DP. Human frataxin is an allosteric switch that activates the Fe–S cluster biosynthetic complex. *Biochemistry*. 2010; 49:9132–9139. [PubMed: 20873749]

51. Liu X, Theil EC. Ferritin: dynamic management of biological iron and oxygen chemistry. *Acc Chem Res.* 2005; 38:167–175. [PubMed: 15766235]
52. Granier T, Langlois d'Estaintot B, Gallois B, Chevalier JM, Precigoux G, Santambrogio P, Arosio P. Structural description of the active sites of mouse L-chain ferritin at 1.2 Å resolution. *J Biol Inorg Chem.* 2003; 8:105–111. [PubMed: 12459904]
53. Takeda S, Yamaki M, Ebina S, Nagayama K. Site-specific reactivities of cysteine residues in horse L-apoferritin. *J Biochem.* 1995; 117:267–270. [PubMed: 7608110]
54. Levi S, Santambrogio P, Corsi B, Cozzi A, Arosio P. Evidence that residues exposed on the three-fold channels have active roles in the mechanism of ferritin iron incorporation. *Biochem J.* 1996; 317:467–473. [PubMed: 8713073]
55. Shi H, Bencze KZ, Stemmler TL, Philpott CC. A cytosolic iron chaperone that delivers iron to ferritin. *Science.* 2008; 320:1207–1210. [PubMed: 18511687] . This paper provided *in vivo* and *in vitro* data that implicate PCBP as an iron chaperone within the ferritin cellular iron storage system.
56. Makeyev AV, Liebhaber SA. The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms. *RNA.* 2002; 8:265–278. [PubMed: 12003487]
57. Du Z, Fenn S, Tjhen R, James TL. Structure of a construct of a human poly(C)-binding protein containing the first and second KH domains reveals insights into its regulatory mechanisms. *J Biol Chem.* 2008; 283:28757–28766. [PubMed: 18701464]
58. Fenn S, Du Z, Lee JK, Tjhen R, Stroud RM, James TL. Crystal structure of the third KH domain of human poly(C)-binding protein-2 in complex with a C-rich strand of human telomeric DNA at 1.6 Å resolution. *Nucleic Acids Res.* 2007; 35:2651–2660. [PubMed: 17426136]
59. Muhlenhoff U, Molik S, Godoy JR, Uzarska MA, Richter N, Seubert A, Zhang Y, Stubbe J, Pierrel F, Herrero E, et al. Cytosolic monothiol glutaredoxins function in intracellular iron sensing and trafficking via their bound iron–sulfur cluster. *Cell Metab.* 2010; 12:373–385. [PubMed: 20889129]
60. Li H, Mapolelo DT, Dingra NN, Naik SG, Lees NS, Hoffman BM, Riggs-Gelasco PJ, Huynh BH, Johnson MK, Outten CE. The yeast iron regulatory proteins Grx3/4 and Fra2 form heterodimeric complexes containing a [2FeFe–2S] cluster with cysteinyl and histidyl ligation. *Biochemistry.* 2009; 48:9569–9581. [PubMed: 19715344]

Figure 1.

Major pathways for iron incorporation/utilization within a cell. Two pathways for Fe^{2+} utilization highlighted within the review include: (1) frataxin and its role in mitochondrial Fe-S cluster assembly via the ISC-machinery (* including the cysteine desulfurase/Isd11 complex that provides sulfur); (2) PCBP and its role in ferritin associated iron storage.

Figure 2. Molecular details of the 2 Fe^{2+} binding sites on Yfh1 (PDB #2GA5), and the Cys rich active site on Isu1 (modeled structure) coupled with possible Isu1 Fe^{2+} binding sites on the protein's C-terminus. Ferrous iron (blue dots) shown with arrow denoting Fe transfer events between the two proteins.

Figure 3.

Structural details of PCBP's as a ferritin iron chaperone. Top right: PCBP2 total projected structure based on domains 1 and 2 (PDB #2JZX) and domain 3 (PDB #2P2R) structure linked together for visual clarity associated with 3 bound iron atoms (blue dots). Top left: structure of the human ferritin 24-mer (PDB #3AJ0) with iron loaded (blue dot) trimeric symmetry center. Bottom left: expansion of the ferritin trimeric interface with residues from three subunits involved in Fe^{2+} loading (H118 and C130). In addition, residues (D131 and E134) on the inner side of the iron incorporation gate that support metal exchange are also given.