Cytosolic iron chaperones: Proteins delivering iron cofactors in the cytosol of mammalian cells

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Caroline C. Philpott 1‡, Moon-Suhn Ryu 5‡, Avery Frey 6§, and Sarju Patel 1¶

From the 1 Genetics and Metabolism Section, Liver Diseases Branch, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, the 5 Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108, and 6 BioLegend, San Diego, California 92110

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Eukaryotic cells contain hundreds of metalloproteins that are supported by intracellular systems coordinating the uptake and distribution of metal cofactors. Iron cofactors include heme, iron–sulfur clusters, and simple iron ions. Poly(rC)-binding proteins are multifunctional adaptors that serve as iron chaperones in the cytosolic/nuclear compartment, binding iron at import and delivering it to enzymes, for storage (ferritin) and export (ferroportin). Ferritin iron is mobilized by autophagy through the cargo receptor, nuclear co-activator 4. The monothiol glutaredoxin Glrx3 and BolA2 function as a [2Fe-2S] chaperon complex. These proteins form a core system of cytosolic iron cofactor chaperones in mammalian cells.

High-throughput approaches to elucidating the roles of metals in biology are in their infancy, yet they have yielded some surprising results (1, 2). Based on the findings from three species of prokaryotes (3), metalloproteins make up one-third of the cellular proteome. Of these, only half have been previously identified as metalloproteins. Iron and zinc are the most abundant metals in eukaryotic cells, and new iron-containing proteins continue to be identified in mammalian cells (4–6).

Because of the extent and complexity of the metalloproteome, cells require a distribution system for metal cofactors. Iron cofactors in the form of simple ionic iron, iron–sulfur clusters, and heme need to be taken up, assembled, and synthesized, respectively, before delivery to their recipient apoproteins, which localize to nearly every compartment of the cell. Mitochondria are the ultimate sites of heme synthesis and the initial sites of iron–sulfur cluster assembly. Nevertheless, heme, iron–sulfur clusters, and iron ions are required cofactors in the cytosol and nucleus as well. This review will focus on recent studies examining the trafficking of cytosolic iron cofactors and the function of cytosolic chaperone proteins in mammalian cells.

Poly(rC)-binding proteins are iron chaperones for iron transporters

The term metallochaperone is used to describe a metal-binding protein that mediates the transfer of the bound metal to a recipient apoprotein via a metal-mediated protein-protein interaction (7). Metallochaperones with specificity for iron, copper, and possibly zinc have been identified in eukaryotes (8–11). Poly(rC)-binding proteins (PCBPs) are a family of four multifunctional adaptor proteins that bind iron, single-stranded nucleic acids, and proteins, altering the fates of each of these ligands (12–16). PCBP1 and PCBP2 were initially identified as RNA-binding proteins called HN-RNP E1 and E2 or αCP-1 and -2. In their RNA-binding capacity, they affect the splicing, polyadenylation, processing, translation, and stability of many RNA species with cells. PCBP1 was later identified as an iron chaperone for ferritin (11), the major iron storage protein found in most eukaryotes (17). PCBP1 and PCBP2 can bind ferrous iron in vitro in a 3:1 Fe/PCBP stoichiometric ratio with low micromolar affinity, similar to the concentrations of kinetically labile iron that have been measured in cells (18). PCBP1 and PCBP2 were subsequently found to deliver iron cofactors to two classes of non-heme iron enzymes localized to the cytosol (19, 20). Studies continue to indicate that PCBP1 and PCBP2 play integral roles in cellular iron trafficking.

Iron initially enters the cytosol of mammalian cells via transmembrane transporters with specificity for divalent metal cations. These include DMT1 (SLC11A2) (21) and the ZIP-family transporters ZIP8 (SLC39A8) (22) and ZIP14 (SLC39A14) (23). A recent report suggests that PCBP2 can directly interact with DMT1 and facilitate the transfer of iron from DMT1 to the cytosol (24). In a yeast two-hybrid screen for proteins interacting with the amino-terminal domain of DMT1, the investigators identified two clones coding for the second HN-RNP K-homology (KH) domain of PCBP2. The second KH domain of PCBP2 differs from that of PCBP1 by only five similar amino acids. Nevertheless, full-length PCBP2 bound DMT1 both in vitro and in HeP-2 cells, whereas PCBP1 did not. Similarly, depletion of PCBP2 in cells impaired uptake of iron, whereas depletion of PCBP1 did not. These studies suggest that PCBP2 has a unique chaperone function facilitating iron uptake in HeP-2 cells. In contrast, when PCBP2 is depleted in HEK293

1 To whom correspondence should be addressed: Genetics and Metabolism Section, Liver Diseases Branch, NIDDK, National Institutes of Health, Bldg. 10, Rm. 9B-16, 10 Center Dr., MSC 1800, Bethesda, MD 20892-1800. Tel.: 301-435-4018; Fax: 301-402-0491; E-mail: Carolinep@mail.nih.gov.
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cells (19) or erythroid progenitor cells (25), total iron accumulation is not impaired, suggesting that the relative contribution of PCBP2 to iron uptake may differ among cell types or that concomitant loss of efflux may compensate for a loss of import activity. A single transporter, ferroportin, accounts for iron efflux activity in mammalian cells (26–28). Yanatori et al. (29) also find that PCBP2 binds to ferroportin at its carboxyl terminus in an iron-dependent manner. Overexpression of ferroportin in iron-loaded HEp-2 cells is associated with loss of intracellular iron. In cells depleted of PCBP2, this loss of intracellular iron is attenuated, suggesting that the interaction of PCBP2 with ferroportin promotes the efflux of cytosolic iron. Further investigation will clarify the relative contributions of the interactions of PCBP2 with DMT1 or ferroportin on iron transport activities.

**PCBP1 and PCBP2 facilitate the mettallation of ferritin and non-heme iron enzymes**

PCBP1 was initially identified as an iron chaperone for ferritin in a forward genetic screen in which iron loading of human ferritin was reconstituted in the cytosol of baker’s yeast expressing cDNAs derived from human liver (11). Animal ferritins have a conserved and distinctive structure, which consists of 24 subunits that form a hollow sphere in which iron oxyhydroxides accumulate (30). Animals express three types of ferritins: H-chain, L-chain, and mitochondrial chains. Cytosolic ferritin is assembled from a mixture of H- and L-chains (31). H-ferritin has ferroxidase activity and an active site similar to other oxo-bridged diiron enzymes. The enzymatic active sites are located within the interior of the ferritin sphere. As PCBP1 can only interact with the exterior of the ferritin shell, it is likely to transfer its bound iron to sites located in the ferritin pores that funnel iron to the interior. Studies utilizing heterologous expression of ferritins and PCBPs in baker’s yeast indicate that PCBP2, which is 83% identical to PCBP1, and PCBP3, which exhibits lesser sequence identity, can also function as iron chaperones for ferritin (32). PCBP4 expression in yeast activates the ferroxidase activity of PCBP1 and PCBP3, which exhibits lesser sequence identity, can also function as iron chaperones for ferritin (33). PCBP4 expression in yeast activates the iron deficiency response, suggesting a potential role in iron metabolism, but it exhibits weaker genetic and physical interaction with ferritin. Of the PCBPs, only PCBP1 and PCBP2 are ubiquitously expressed at high levels in mammalian cells and can therefore be studied by in vivo depletion. Huh7 cells depleted of either PCBP1 or PCBP2 exhibit defects in ferritin iron loading, consistent with their activities in yeast. PCBP3 and PCBP4 are expressed at low levels in some tissues and at some phases of development. These low levels of expression may not be sufficient to mediate iron chaperone activity, as formation of iron and client protein complexes are kinetically favored only when expression levels of the chaperone are high.

The iron chaperone activities of PCBP1 and PCBP2 are not limited to ferritin. Cytosolic enzymes activated by simple mono-nuclear or di-nuclear iron centers are also clients of these chaperones. The study of chaperone activities in cells is challenging due to the tendency of iron enzymes to lose their cofactors when cells are lysed and exposed to air. Thus, enzyme activities that can be monitored in intact cells are the best candidates for study. The prolyl and asparagyl hydroxylases that trigger degradation of HIF1α are such candidates. They require coordination of a single iron ion in the active site to activate oxygen for the hydroxylation reaction. In cultured human cells depleted of PCBP1 or, to a lesser extent, PCBP2, HIF1α accumulates, and these enzymes exhibit reduced activity due to absence of the iron cofactor. The hydroxylases are detectable in complexes with PCBP1 after affinity capture, but the enzymes are not detected in stoichiometric amounts, suggesting that the interactions are transient (20).

Similarly, a recent study found that PCBP1 and PCBP2 were required for the mettallation of the di-nuclear iron enzyme deoxyhypusine hydroxylase (DOHH) (19). DOHH catalyzes the second step in the conversion of a lysine residue to hypusine in eukaryotic initiation factor 5a (33). Although hypusination occurs at only a single residue on a single protein, it is conserved among all eukaryotes and is essential for life. The first step of hypusine synthesis is the aminobutylation of lysine by deoxyhypusine synthase to form deoxyhypusine. In the second step, DOHH hydroxylates deoxyhypusine to form hypusine. In cells lacking PCBP1 or PCBP2, deoxyhypusine accumulates and hypusine levels fall, indicating a loss of DOHH activity. Two iron ions in the active site of DOHH form a peroxo bridge to activate oxygen for this reaction (34). The apo- and holo-forms of the enzyme can be distinguished by their distinct electrophoretic mobilities. The holo-form is specifically lost in cells depleted of PCBP1 or PCBP2, indicating absence of the iron cofactor. Again, DOHH and PCBP1 are detectable as a complex that is stabilized in cells supplemented with iron.

These mono- and di-nuclear iron enzymes are structurally distinct; they utilize different substrates and co-substrates, and their reaction mechanisms differ. Yet they exhibit similar iron chaperone requirements. PCBP chaperone activity exhibits the greatest contribution to enzyme activity in cells made mildly iron-deficient. Incubating cells in supplemental iron can restore both prolylhydroxylase and DOHH activities in cells lacking PCBPs. Although the details of the reaction mechanisms are not known for all enzymes of these classes, both coordinate Fe(II) in the active site and both require oxidation of the Fe(II) centers to Fe(III) or Fe(IV) as part of the reaction mechanism (35, 36). Thus, restoration of the Fe(II) oxidation state is required to maintain enzyme activity. Although soluble reductants (e.g. ascorbate for prolylhydroxylases) may aid in reduction of oxidized iron centers, replacement of lost iron cofactors through PCBP activity may support the enzymatic activity in cells.

**Erythropoietic cells depend on PCBP1-mediated iron flux through ferritin**

Previous studies on iron chaperones examined their role in general cultured cell models. Mammals and individual mammalian tissues, however, exhibit markedly different patterns of iron transport and utilization. For example, intestinal epithelial cells are specialized for dietary iron uptake and efflux to the systemic circulation. The liver exhibits high levels of both iron utilization and storage. But no tissue is more highly specialized for iron handling than the erythron. In humans, 25 mg of iron per day is routed to the bone marrow, which releases into the bloodstream more than 2 million reticulocytes every second.
(37). Iron is taken up by erythropoietic precursors and intracellularly routed to the mitochondria, where it is used to synthesize heme that is ultimately incorporated into hemoglobin. The processes of iron accumulation, heme synthesis, and globin synthesis are precisely controlled during red cell development, as excesses of any of these components are toxic to developing red cells (38). Red cells develop from hematopoietic precursors that differentiate into erythroid progenitors. Terminal differentiation of erythroid cells begins with a dramatic expansion of transferrin-bound iron uptake. Ex vivo studies in erythroid progenitors indicate that intracellular iron initially accumulates in ferritin, which is also dramatically up-regulated during differentiation (39). Iron is later transferred to mitochondria and incorporated into heme. This would suggest that ferritin storage plays an important role in intracellular iron flux. However, a mouse model of ferritin deficiency exhibits relatively normal red blood cell production, indicating that ferritin can be bypassed in the delivery of iron to mitochondria in these cells (40). However, these mice also exhibit alterations of multiple iron parameters that could compensate for the loss of erythroid ferritin.

A recent study clarifies the role of ferritin in erythroid development and highlights the requirement for PCBP1 iron chaperone activity (25). Using both in vitro and ex vivo models of red cell development, PCBP1 depletion led to defects in the delivery of intracellular iron to ferritin, which in turn resulted in defects in iron incorporation into heme. As these cells had no defects in transferrin–iron uptake, these data indicated that PCBP1-mediated delivery of iron to ferritin is an important step in the trafficking of erythroid iron. The observation that iron supplementation did not correct the heme synthesis defect of PCBP1-deficient cells supported this interpretation. PCBP1-ferritin complexes were abundantly isolated in these cells, supporting a model of direct transfer of iron from PCBP1 to ferritin. The PCBP1-mediated delivery of iron to ferritin was likely highest in the early stages of terminal differentiation, as the measured binding of PCBP1 to ferritin was high in early stages and diminished greatly in later stages of differentiation.

A surprising finding in these studies was the antagonistic role played by PCBP2. Whereas depletion of PCBP2 in general cell culture models has inhibitory effects on iron delivery to non-heme iron enzymes, in the red cell differentiation model, depletion of PCBP2 enhanced iron delivery to ferritin, PCBP1 binding to ferritin, and incorporation of iron into heme. The enhanced activities in PCBP2-depleted cells were dependent on the expression of PCBP1. Thus, in developing red cells, PCBP2 seems to function by diverting iron or iron-PCBP1 complexes away from ferritin. Perhaps PCBP2 is involved in ensuring the delivery of iron to other iron-dependent enzymes that continue to function in the red cell cytosol and nucleus.

These studies also employed a mouse model of inducible PCBP1 depletion (25). Post-natal PCBP1 deficiency resulted in heme deficiency in erythropoietic tissues and the development of microcytic anemia, with changes in red cell parameters that were similar to those seen in iron-deficiency anemia. The development of anemia in these mice was accompanied by compensatory changes in erythropoietic regulatory hormones, specifically erythropoietin, erythroferrone, and hepcidin. Thus, the PCBP1-mediated trafficking of iron through ferritin occurs in animal tissues and is physiologically important for hematopoiesis.

**Nuclear co-activator 4 directs ferritin to autophagosomes for degradation and iron release**

The iron chaperone activity of PCBP1 clearly facilitates the sequestration of iron in ferritin. Recent studies have also illuminated the machinery involved in ferritin iron release. Experiments monitoring the turnover of ferritin and the release of ferritin iron indicate that most iron release occurs with ferritin degradation (41, 42). Degradation largely occurs within the lysosome and involves the process of autophagy (43). The means to sort ferritin into the lysosome remained obscure until two groups independently identified nuclear co-activator 4 (NCOA4) as an autophagic cargo receptor for ferritin (44, 45). NCOA4 was initially identified as a coactivator for a variety of nuclear hormone receptors (46, 47). Proteomic approaches identified it in autophagosomes, however. Further investigation revealed that NCOA4 functioned as an autophagic cargo receptor, specifically recognizing ferritin and sorting it into autophagosomes destined for lysosomal fusion and degradation. The carboxyl-terminal domain of NCOA4 specifically interacted with H-ferritin subunits via conserved arginine 26, which is located on the surface of the 24-mer, and is not present on L-chain.

Deprivation of general nutrients or iron triggers the autophagic turnover of ferritin via NCOA4 in cultured cells. Iron appears to regulate this process in part by stimulating the turnover of NCOA4 itself (48). Cells deprived of iron accumulate NCOA4, leading to ferritin autophagy and iron liberation. Cells supplemented with iron degrade NCOA4, thereby impeding the autophagic degradation of ferritin. The iron-dependent turnover of NCOA4 may be mediated by direct binding of iron, as iron is detected in stoichiometric amounts in a purified, recombinant fragment of NCOA4.

The E3 ubiquitin ligase HERC2 partially accounts for the iron-dependent turnover of NCOA4 (48). HERC2 is detected in complexes with NCOA4, and removal of iron through chelation prevents HERC2 binding in cells and in vitro. Cells lacking HERC2 accumulate NCOA4, even in the presence of supplemental iron, although HERC2-independent turnover of NCOA4 is also detected. When cytosolic iron levels are high, NCOA4 binds iron and undergoes ubiquitination by HERC2, leading to degradation of NCOA4 and stabilization of ferritin. Thus, the release of iron from ferritin can be suppressed by the accumulation of iron in the cytosol. Other methods of regulating the autophagic turnover of ferritin are likely, as NCOA4 is transcriptionally induced under certain conditions of high ferritin turnover, such as erythroid cell development (25, 49).

The role of NCOA4 in ferritin turnover has been confirmed using knock-out mouse models (48, 50). In NCOA4-null mice, ferritin and iron accumulate in liver and spleen. Despite the abundance of tissue iron, these animals manifest a mild microcytic anemia, similar to that of animals lacking PCBP1 (50). These mice are hypersensitive to iron deficiency and rapidly develop severe anemia, profound microcytosis, and hypochromia when challenged with an iron-deficient diet. These studies

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did not determine whether the failure to mobilize iron stored in liver and spleen accounted for the anemia in the NCOA4-null animals or whether a direct role for NCOA4 in iron trafficking within the erythron was responsible for the phenotype. However, depletion of NCOA4 in human K562 cells interfered with hemoglobin formation (48). Furthermore, depletion of NCOA4 in murine erythroid cells triggered accumulation of iron in ferritin along with defects in iron incorporation into heme and hemoglobin, suggesting that the anemia could be accounted for by an intrinsic defect in erythroid iron trafficking (25).

The question of how ferritin iron that is released within the lumen of the lysosome is delivered to the cytosol or mitochondria for utilization is unresolved. Endosomal ferric reductase activities and iron transporters may exhibit dual localization in lysosomes or specific lysosomal systems may exist. These may facilitate the transfer of ferrous iron out of the lysosome. Erythroid development could rely on an alternative mechanism, however, as a direct mechanism of iron transfer from endosomes to mitochondria has been proposed for circulating reticulocytes (51), and a similar mechanism may operate to transfer iron from lysosomes to mitochondria in earlier stages of mitochondrial differentiation (Fig. 1).

Iron–sulfur cluster trafficking via glutaredoxin-BolA complexes

Trafficking of cytosolic ferrous ions to ferritin for storage or to non-heme iron enzymes for utilization occurs through the activities of PCBP-family iron chaperones. Iron ions are similarly needed for the de novo synthesis or repair of iron–sulfur clusters in the cytosol. Much progress has been made using genetic, proteomic, and biochemical approaches to identify components of the machinery that distributes [4Fe-4S] clusters in the cytosol (52). HEK293 cells lacking PCBP1 or PCBP2 exhibit loss of activity in cytosolic aconitase (19), an enzyme with a labile [4Fe-4S] cluster. PCBP1 or PCBP2 may be involved in repair of Fe-S cluster damage, but this activity has not been directly demonstrated. Details of early steps in cytosolic Fe-S biogenesis are less clear. Although some precursor for iron–sulfur cluster assembly originates in mitochondria and is exported to the cytosol; the chemical nature of this precursor and whether it contains only inorganic sulfur, sulfur and iron, or an iron–sulfur cluster has not been determined. Similarly, whether the iron ions in iron–sulfur clusters originate from the mitochondria or from the cytosol remains unclear. The simplest clusters in the cytosol are [2Fe-2S] clusters, and recent investigations in cells and in vitro have indicated that these Fe-S clusters in the cytosol are carried in complexes containing monothiol glutaredoxins (Grxs) and BolA-like proteins.

The capacity of Grxs to serve as [2Fe-2S] carriers in many species is well-documented (53), as is their importance in Fe-S cluster assembly and distribution in mitochondria (54–56). Cytosolic Grxs have important roles in iron homeostasis and in maintaining the activity of cytosolic iron enzymes in yeast (57, 58). The role of Grx in yeast is not precisely duplicated in mammals, which express a single cytosolic Grx, Glrx3. Glrx3 contains two tandem glutaredoxin domains, rather than the single domain found in yeast species, and both domains can independently coordinate a bridging [2Fe-2S] cluster as a homodimer in vitro (59, 60). Although Glrx3 may coordinate Fe-S clusters as a homodimer in vitro, this species does not accumulate to detectable levels in human cells. In HEK293 cells, Glrx homodimers are detected as a minor, unstable species; homodimers are preferentially formed in iron-deficient, rather than iron-replete, cells and do not require the amino acid residues that coordinate Fe-S clusters (61). Human cells lacking Glrx3 exhibit moderate deficiencies in cytosolic Fe-S enzyme activities, mildly altered iron homeostasis, and mild deficiencies in mitochondrial iron cofactors (62). The milder human cell phenotypes indicate Glrx3 has differing roles in yeast and human cells or that redundant systems can partially substitute for Glrx3 function in mammalian cells.

Both mitochondrial and cytosolic Grxs from many species may form complexes with BolA-like proteins (63). BolAs are conserved small proteins ubiquitously found in eukaryotes and prokaryotes (64). Separate loci encode BolA proteins that exclusively localize to either mitochondria or the cytosol. In yeast and humans, BolA1 and BolA3 localize to mitochondria,
where they function in Fe-S cluster metabolism (65–67). In budding (68–73) and fission (74–77) yeasts, cytosolic BolA (called Fra2) associates with cytosolic Grx and effects iron-dependent regulation of transcription factors through the transfer of \([2\text{Fe}-2\text{S}]\) clusters. As these transcription factors are not present in mammalian cells, the sole cytosolic BolA protein in mammals, BolA2, must have a different function.

Studies using recombinant Glrx3 and BolA2 demonstrate that these protomers form a heterotrimeric complex that consists of one Glrx3 and two BolA2 protomers that stably coordinate two bridging \([2\text{Fe}-2\text{S}]\) clusters (60, 78). As with Glrx3 homodimers, each cluster is coordinated by the active-site cysteine and a glutathione in the Grx domain and a conserved histidine residue in BolA2. A fourth ligand for the cluster has not been clearly identified. In vitro, Glrx3 homodimers with bound \([2\text{Fe}-2\text{S}]\) clusters undergo spontaneous rearrangement in the presence of recombinant BolA2 to form Glrx3-[2Fe-2S]_2-BolA2 complexes, suggesting that clusters preferentially form on the heterotrimeric complex.

Glrx3-BolA2 complexes function as \([2\text{Fe}-2\text{S}]\) chaperones in mammalian cells. In human cells, Glrx3 and BolA2 form a complex only when they coordinate bridging \([2\text{Fe}-2\text{S}]\) clusters (61); in cells supplemented with iron, the pool of clusters coordinated by Glrx3 and BolA2 rapidly expands to form a reservoir of cytosolic \([2\text{Fe}-2\text{S}]\) clusters. In vitro work demonstrates Glrx3-BolA2 complexes (and Glrx3 homodimers) can transfer \([2\text{Fe}-2\text{S}]\) clusters to the Fe-S protein ciapin1 (Cpn1) (59, 78). Human and yeast Cpn1 coordinates either a pair of \([2\text{Fe}-2\text{S}]\) clusters or a single \([2\text{Fe}-2\text{S}]\) and a \([4\text{Fe}-4\text{S}]\) cluster (79–83). It functions in the early steps of cytosolic \([4\text{Fe}-4\text{S}]\) cluster assembly in a complex with the flavoprotein Ndor1. The Ndor1-Cpn1 complex is thought to ferry electrons to the downstream components of the \([4\text{Fe}-4\text{S}]\) cluster assembly machinery; whether it also transfers Fe-S clusters is not known. The amino-terminal thioredoxin domain of Glrx3 mediates the initial interaction with Cpn1 in vitro and is required for cluster transfer (59).

Work in human cells supports the role of Glrx3-BolA2 complexes as \([2\text{Fe}-2\text{S}]\) cluster chaperones (61). In cells, a large fraction of Glrx3 is stably associated with Cpn1. However, a mutant Glrx3 that does not coordinate \([2\text{Fe}-2\text{S}]\) clusters also fails to complex with Cpn1, suggesting that, in addition to the thioredoxin domain, \([2\text{Fe}-2\text{S}]\) clusters contribute to initial complex formation. BolA2 is only weakly detected in Cpn1 complexes, suggesting that it is released when Glrx3 joins the Cpn1 complex. Using radioisotopic labeling of cells, iron, likely in the form of Fe-S clusters, is readily detected in Cpn1 complexes after affinity purification. This iron is lost in cells lacking Glrx3 or BolA2, suggesting that \([2\text{Fe}-2\text{S}]\) clusters from Glrx3-BolA2 complexes accounted for much of the iron bound by Cpn1. Actual transfer of the \([2\text{Fe}-2\text{S}]\) clusters from Glrx3-BolA2 to Cpn1 in cells occurs, as dissociation of Glrx3 from affinity-purified Cpn1 is accomplished without the loss of radioactively labeled Fe-S clusters. These studies all indicate that Glrx3-BolA2 complexes in mammalian cells function as \([2\text{Fe}-2\text{S}]\) chaperones in mammalian cells as \([2\text{Fe}-2\text{S}]\) cluster chaperones, storing and delivering \([2\text{Fe}-2\text{S}]\) clusters via direct protein-protein interactions (Fig. 2). Whether Glrx3 and BolA2 transfer \([2\text{Fe}-2\text{S}]\) clusters to proteins other than Cpn1 remains to be explored. The iron found in the Cpn1 complex did not completely disappear in cells lacking Glrx3 or BolA2; the source of this residual iron is not clear. It may represent a \([4\text{Fe}-4\text{S}]\) cluster that is not derived from Glrx3-BolA2 or iron from an alternative source of \([2\text{Fe}-2\text{S}]\) clusters in the cell. MitoNEET is a \([2\text{Fe}-2\text{S}]\) cluster protein localized to the outer membrane of mitochondria. In vitro, \([2\text{Fe}-2\text{S}]\) clusters on MitoNEET can be transferred to purified Cpn1 (84). In cells, depletion of MitoNEET is associated with mild to moderate loss of cytosolic
aconitase activity (85). Whether MitoNEET can directly transfer clusters to apoproteins in cells awaits further investigation.

Although metal researchers have made progress in understanding the cytoplasmic trafficking of simple iron ions and Fe-S cluster cofactors in mammalian cells, less is known about the machinery involved in heme cofactor distribution. Similarly, despite mitochondria being the major site of iron utilization in most cells, we do not understand iron delivery to this organelle. As these investigations are at a relatively early stage, the machinery identified to date may simply be the proverbial tip of the iceberg.

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