Therapeutics

COMPREHENSIVE INVITED REVIEW

Brain Iron Homeostasis: From Molecular Mechanisms To Clinical Significance and Therapeutic Opportunities

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Abstract

Iron has emerged as a significant cause of neurotoxicity in several neurodegenerative conditions, including Alzheimer's disease (AD), Parkinson's disease (PD), sporadic Creutzfeldt-Jakob disease (sCJD), and others. In some cases, the underlying cause of iron mis-metabolism is known, while in others, our understanding is, at best, incomplete. Recent evidence implicating key proteins involved in the pathogenesis of AD, PD, and sCJD in cellular iron metabolism suggests that imbalance of brain iron homeostasis associated with these disorders is a direct consequence of disease pathogenesis. A complete understanding of the molecular events leading to this phenotype is lacking partly because of the complex regulation of iron homeostasis within the brain. Since systemic organs and the brain share several iron regulatory mechanisms and iron-modulating proteins, dysfunction of a specific pathway or selective absence of iron-modulating protein(s) in systemic organs has provided important insights into the maintenance of iron homeostasis within the brain. Here, we review recent information on the regulation of iron uptake and utilization in systemic organs and within the complex environment of the brain, with particular emphasis on the underlying mechanisms leading to brain iron mis-metabolism in specific neurodegenerative conditions. Mouse models that have been instrumental in understanding systemic and brain disorders associated with iron mis-metabolism are also described, followed by current therapeutic strategies which are aimed at restoring brain iron homeostasis in different neurodegenerative conditions. We conclude by highlighting important gaps in our understanding of brain iron metabolism and mis-metabolism, particularly in the context of neurodegenerative disorders. *Antioxid. Redox Signal.* 20, 1324–1363.

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I. Overview of Brain Iron Homeostasis and Scope of the Review

R EDOX-ACTIVE METALS such as iron and copper are necessary for normal cellular function because of their ability to participate in electron-transfer reactions. Iron, in particular, participates in several vital biochemical reactions, including synthesis of DNA, RNA, and proteins; serves as a co-factor for several enzymatic reactions, including heme synthesis; and aids in the synthesis of myelin that is necessary for brain function (197, 451). At the same time, free iron is cytotoxic due to its ability to react with molecular oxygen that is usually abundant in metabolically active cells to generate hydroxyl radicals and hydroxyl anions by the Fenton chemistry (186) (Fig. 1). These cause lipid peroxidation, DNA strand breaks, and protein modifications that ultimately lead to cell death (251). Metabolism of iron is, therefore, tightly regulated by a set of iron uptake, transport, and storage proteins that are themselves regulated by iron at the cellular and systemic levels.

Regulation of iron homeostasis begins at the gastro-intestinal tract where duodenal cells are primed to absorb iron based on systemic iron needs. Once in circulation, most of the iron is bound tightly to serum transferrin (Tf). Small amounts associate with albumin, citrate, amino acids, and other un-identified negatively charged ligands. Most parenchymal cells take up iron from Tf for their metabolic needs by the Tf-transferrin receptor 1 (Tf-TfR) pathway. Non-transferrin bound iron (NTBI) is taken up by the divalent metal trans-

porter 1 (DMT1) as well as by Zip 14/Slc39a14 (197, 275). Within the cell, iron is used for essential metabolic processes, and any excess is stored in a relatively inert form in cytosolic ferritin (451). The brain acquires iron from the systemic circulation through a tightly controlled mechanism at the blood brain barrier (BBB). Within the brain, iron serves essential functions that are best exemplified by brain disorders which arise from iron deficiency, excess, or mis-metabolism (97, 224, 312, 413). Table 1 provides a partial list of human disorders that result from dysfunction or absence of one or more proteins involved in brain iron metabolism, and representative animal models.

Next, we discuss recent information on the regulation of iron in the systemic circulation, across the BBB, within the complex milieu of the brain, and consequences of brain iron mis-metabolism and associated brain disorders. The main emphasis is on brain disorders associated with dysfunction of iron-modulating proteins ferritin and ceruloplasmin (Cp) that result in hereditary ferritinopathies and aceruloplasminemia, respectively, and neurodegenerative conditions which are associated with brain iron mis-metabolism such as Alzheimer's disease (AD), Parkinson's disease (PD), and sporadic Creutzfeldt-Jakob disease (sCJD). Recent information on the regulation of iron transport to the mitochondria is discussed in the context of Friedreich's ataxia (FA) and sideroblastic anemias to highlight the importance of iron mis-metabolism at this site on brain function. Current information on the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) as models of PD, FA, and Huntington's disease (HD) is discussed to emphasize its usefulness in understanding the complexity underlying these conditions. Finally, therapeutic strategies aimed at reducing iron-induced oxidative stress, including chelation, are discussed.

$$Fe^{3+} + O_2^{\cdot-} \longleftrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\cdot} + OH^-$$
(Fenton reaction)

$$O_2^{\cdot-} + H_2O_2 \longleftrightarrow O_2 + HO^{\cdot} + OH^-$$
(Haber-Weiss reaction)
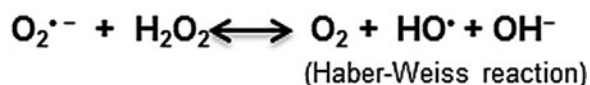
FIG. 1. Fenton and Haber Weiss reactions resulting in the generation of hydroxyl radicals in the presence of iron.

II. Molecular Regulation of Iron Metabolism

The only route of iron acquisition by the body is through duodenal epithelial cells that absorb heme and non-heme iron from ingested food in response to body iron stores. Absorbed iron is loaded to serum Tf for utilization by systemic organs and for hematopoiesis. Daily absorption of iron in a healthy individual rarely exceeds 1–2 mg, and a similar amount is lost through intestinal epithelial shedding, desquamation, or menstruation. Most of the body iron (80%) is in hemoglobin in

TABLE 1. MOUSE MODELS AND CORRESPONDING HUMAN DISORDERS OF IRON METABOLISM

Gene	Mouse model	Iron phenotype	Human disease parallel	Reference
Abcb7	Abcb7$^{-/-}$ Abcb7^{E433K}	Siderocytosis	X-linked sideroblastic anemia	(153)
Aco1	Irp1$^{-/-}$	NA	Cardiac problems	(163)
Alas2	Alas2$^{-/-}$	Increased iron in primitive erythroid cells	X-linked sideroblastic anemia	(153)
Beta-2 microglobulin	B2m$^{-/-}$	Parenchymal iron deposition; decreased hepcidin	ND	(382)
Bmp6	Bmp6$^{-/-}$	Increased body iron; decreased hepcidin	ND	(16, 304)
Ceruloplasmin	Cp$^{-/-}$	Iron accumulation in hepatocytes and macrophages	Aceruloplasminemia	(189, 211, 341)
DMT1 (G185R)	mk (missense) Belgrade rat	Systemic iron deficiency; impaired iron uptake from the duodenum and by erythroid precursors	ND	(52, 151, 320)
Duodenal cytochrome B	Cybrd1$^{-/-}$	Little impact	ND	(153)
Feline leukemia virus receptor	Flvcr$^{-/-}$	Macrocytic anemia	ND	(153)
Ferrochelatase	FECH^{m1Pas}	Microcytic hypochromic anemia & severe porphyria	ND	(279)
Ferrochelatase	FECH$^{-/-}$	Homozygous state is embryonic lethal; Heterozygotes have decreased FECH activity and mild porphyria	ND	(284)
Ferroportin	Slc40a1 (missense)	Iron loading of Kupffer cells, high serum ferritin, low transferrin saturation	ND	(153)
	Fpn$^{-/-}$	Increased iron absorption and overload	Hfe4 hemochromatosis	(124)
Frataxin	Fxn$^{-/-}$ with human mutant Fxn YAC constructs	Mitochondrial iron deposits; neurodegeneration; cardiomyopathy; embryonic lethal	Friedreich's ataxia	(10, 153)
	Fxn$^{-/-}$	Embryonic lethal	NA	(153)
H-ferritin[a]	Fth$^{+/-}$	Elevated tissue and serum L-ferritin	ND	(147, 428)
Haptoglobin	Hp$^{-/-}$	Increased export of iron from duodenum to plasma	ND	(270, 293)
Heme oxygenase 1	Hmox1$^{-/-}$	Anemia; low serum iron; tissue iron deposition	Hmox 1 deficiency	(354)
Hemojuvelin	Hjv$^{-/-}$	Increased body iron, decreased hepcidin	ND	(153)
Hemopxin	Hx$^{-/-}$	Increased CNS iron	ND	(153)
Hepcidin	HAMP (transgene)	Iron deficiency; Anemia	NA	(261)
Hepcidin[b]	Usf2$^{-/-}$	Hepatocellular iron accumulation; decreased macrophage iron; increased Tf saturation	Juvenile hemochromatosis	(321)

(continued)

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TABLE 1. (CONTINUED)

Gene	Mouse model	Iron phenotype	Human disease parallel	Reference
Hepcidin/HFE	Hfe$^{-/-}$ Hfe$^{C282Y/C282}$ HFE$^{H63D/H63D}$	Amelioration of hepatic iron loading relative to Hfe$^{-/-}$ mice; increased body iron; decreased hepcidin	NA	(321)
Hephaestin	sla (deletion)	Microcytic hypochromic anemia; impaired iron transfer from the duodenum	ND	(447)
Hfe	Hfe$^{-/-}$ Hfe$^{C282Y/C282Y^C}$	Hepatocellular iron accumulation; decreased macrophage iron; increased Tf saturation	Hfe hemochromatosis	(484)
Irp2	Irp2$^{-/-}$	Microcytic anemia; duodenal and hepatic iron loading	Pulmonary disease	(89, 163)
Mitoferrin 1	Mfrn1$^{-/-}$	Severe anemia	ND	(153)
Sec1511	Hbd	Deficiency in Tf cycling	ND	(464)
Slc25a37	Mfrn1$^{-/-}$	Embryonic lethal with profound anemia	Tumors or cancer of the prostate	(81)
Smad4	Smad4$^{-/-}$ (liver specific)	Increased body iron; decreased hepcidin	ND	(452)
Steap3	Steap3$^{-/-}$ Nm1054 (deletion)	Congenital, hypochromic, microcytic anemia; elevated red cell zinc protoporphyrin; functional erythroid iron deficiency; Impaired ferriereductase activity	ND	(153, 279, 327)
Tmprss6 (matriptase-2)	Tmprss6$^{-/-}$ Tmprss6$^{msk/msk}$	Microcytic anemia; low iron stores; low serum iron; increased hepcidin	ND	(128)
Transferrin	Hpx	Microcytic hypochromic anemia; tissue iron deposition	Atransferrinemia	(434)
Transferrin receptor-1^d	TfR$^{+/-}$	Microcytic hypochromic erythrocytes; decreased iron stores; embryonic death	ND	(264)
Transferrin receptor-2	TfRr2$^{246x/246}$ TfR2$^{-/-}$	Hepatocellular iron accumulation; decreased macrophage iron; increased Tf saturation	TfR2 hemochromatosis	(125, 152, 222)
Transferrin	Tf$^{hpx/hpx}$	Severe anemia, iron overload	Congenital hypotransferrinemia	(153)

^{a}Fth$^{-/-}$ mice: early embryonic lethality.
^{b}Includes deletion of gene for upstream stimulatory factor-2.
^{c}Mutation is in mouse codon 294.
^{d}Tf$^{-/-}$ mice: embryonic lethal by E 12.5.
ND, not described; NA, not applicable; BMP, bone morphogenetic protein; Cp, ceruloplasmin; DMT1, divalent metal transporter 1; FECH, ferrochelatase; Fxn, frataxin; HFE, hereditary hemochromatosis gene product; Hp, hephaestin; Hpx, hemopexin; Irp1/Irp2, iron regulatory proteins 1 and 2; Tf, transferrin; TfR, transferrin receptor.

the erythroid compartment, and 10%–15% is in myoglobin in muscle fibers. Other tissues contain variable amounts of iron that is used for metabolic purposes. The liver is the main site of iron storage where excess iron is stored in ferritin within the parenchymal cells and reticuloendothelial macrophages (Fig. 2). Iron from senescent red blood cells is recycled by the spleen and bone marrow macrophages that degrade hemoglobin and release associated iron for reloading to serum Tf for re-utilization (Fig. 3) (197, 451).

Duodenal epithelial cells form a tight monolayer that separates intestinal luminal contents from the blood stream while allowing access to selected molecules. Heme and elemental iron, the main sources of iron in ingested food, are absorbed through distinct proteins on the apical membrane of epithelial cells. Heme is taken up by the heme carrier protein and broken down by heme-oxygenase-1 (HO-1) to release the associated iron moiety. Elemental or non-heme iron in ingested food is mainly in the relatively stable ferric (Fe^{3+}) form, and, unless associated with chelatable compounds, is reduced to ferrous (Fe^{2+}) iron by the ferrireductase (FR) duodenal cytochrome b (Dcytb) on the apical plasma membrane before uptake by DMT1. In the cytosol, iron contributes to the labile iron pool and is utilized for metabolic reactions, or is transported to the basolateral membrane for efflux to the blood stream by the coupled action of the metal transporter ferroportin (Fpn) and the ferroxidase hephaestin (Hp) that oxidizes released Fe^{2+} to the Fe^{3+} form for loading to serum Tf (Fig. 4). Excess Fe^{2+} is converted to Fe^{3+} by the ferroxidase activity of ferritin H-chain (Ft-H) and stored in cytosolic ferritin (451, 462).
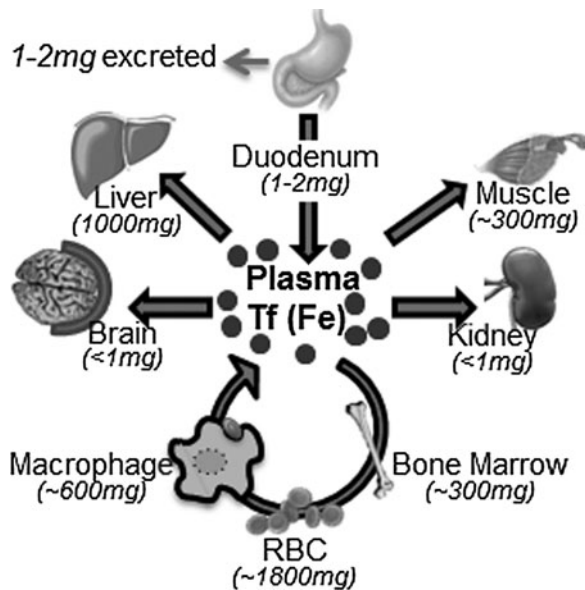

FIG. 3. **Iron recycling by macrophages.** Almost 20–30 mg of Iron is recycled by the macrophages and reused for erythropoiesis daily. Macrophages phagocytize senescent red blood cells, and the heme-associated iron released by HO-1 activity is exported out by Fpn for loading to Tf. Macrophages also acquire heme by endocytosis of haptoglobin and hemopexin. In addition, these cells possess the capability of acquiring iron by the Tf/TfR pathway and exporting heme through the FLVCR receptor. CD173 (marker of early hematopoiesis) and CD91 (low density lipoprotein receptor 1 or α-2-macroglobulin receptor). Cp, ceruloplasmin; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; FLVCR, feline leukemia virus subgroup C receptor; Fpn, ferroportin; HO-1, heme-oxygenase-1; Hp, hephaestin; TfR, transferrin receptor.

FIG. 2. **Systemic iron cycle.** Duodenal enterocytes absorb 1–2 mg of dietary iron daily and transport to plasma Tf. Most of the absorbed iron is utilized by precursor red blood cells in the bone marrow for Hb synthesis. Iron from senescent red blood cells is recovered by macrophages and released to the plasma for re-utilization. Excess iron is stored in the liver and macrophages. Other tissues take up various amounts of iron from plasma Tf. Only 1–2 mg of iron is excreted daily. Numbers in parentheses indicate the amount of iron in each tissue. Tf, transferrin.
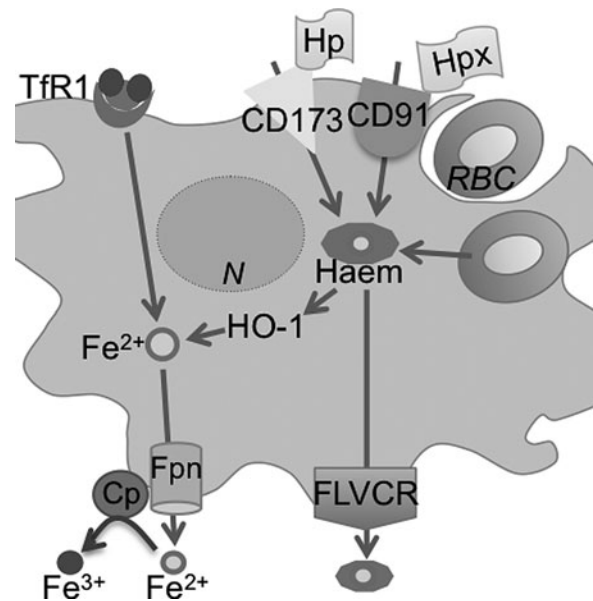
The uptake and release of iron from the duodenal epithelial cells to the blood stream is regulated by hepcidin, a peptide hormone that is released by hepatocytes in response to iron saturation of Tf. When serum iron is adequate or high, hepcidin binds to the extracellular region of Fpn between transmembrane domains 7 and 8. Binding causes Jak-2 mediated tyrosine phosphorylation at residues 302 and 303 in the cytosolic loop of Fpn, which is internalized, dephosphorylated, ubiquitinated, and, ultimately, degraded in the late endosome/lysosome compartment, thus blocking transport of iron from the basolateral membrane to the blood stream. When iron saturation of Tf is low, expression of hepcidin is down-regulated, sparing Fpn and allowing iron transport (313) (Fig. 5). Non-transported iron is lost by shedding of epithelial cells into the intestinal lumen.

Most cells are equipped for taking up Tf-bound and NTBI from the serum, but with varying efficiency. Cells that express TfR1 use the Tf-TfR pathway, a process modulated by the hemochromatosis gene product (HFE) (164). The Tf-TfR complex is internalized in clathrin-coated pits, and Tf-bound Fe^{3+} is released in the acidic environment of the endosomes while the Tf-TfR complex is recycled back to the plasma membrane. Fe^{3+} released in endosomes is reduced to Fe^{2+} by membrane-bound FR proteins such as the Steap family of proteins (Steap 2–4, depending on the organ), and transported to the cytosol through DMT1 where it contributes to the labile

FIG. 4. Iron transport across the enterocyte. Oxidized Fe^{3+} iron in the food is reduced by the ferrireductase Dcytb on the AP plasma membrane to the Fe^{2+} form for transport by DMT1 to the cytosol. Heme is taken up *via* heme carrier protein 1 and is degraded by HO-1 to release Fe^{2+} iron. A receptor for ferritin has also been described on the AP plasma membrane. Iron from these sources enters the common labile iron pool, and is either stored in ferritin or exported from the baso-lateral membrane by the Fpn/Hp complex that oxidizes exported Fe^{2+} iron to the Fe^{3+} form for loading to plasma Tf. Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1.
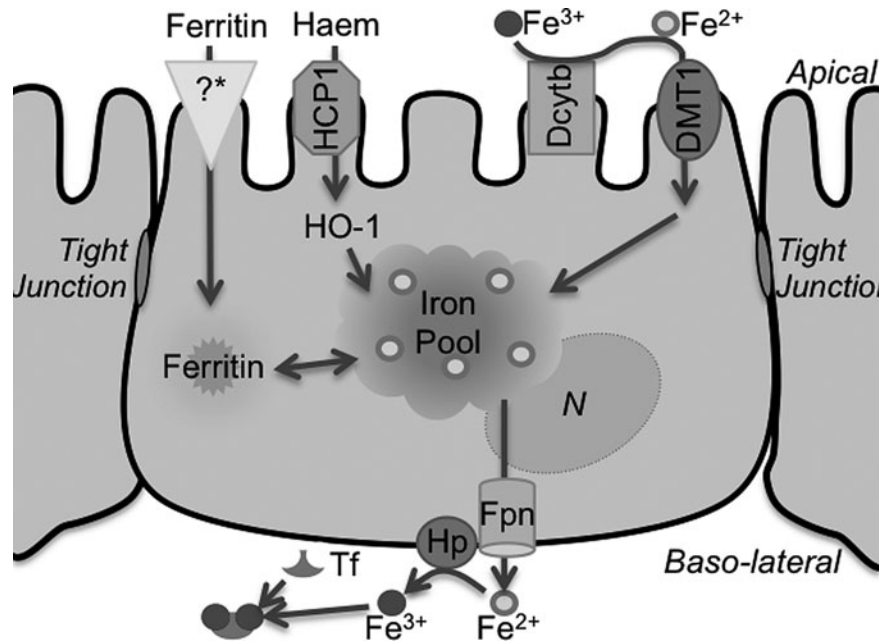
iron pool. Excess Fe^{2+} is oxidized and stored in the ferritin shell in the cytosol. Uptake of NTBI is mediated by DMT1 after reduction to Fe^{2+} by FR proteins at the plasma membrane, and joins the cellular labile iron pool or is stored within ferritin. Iron homeostasis within the cells is maintained by the coordinated regulation of Tf, TfR1, and ferritin at the transcriptional and translational level by iron regulatory proteins 1 and 2 (IRP1/IRP2). Based on cellular iron levels, IRPs interact with iron-responsive elements (IREs) in the mRNA of specific proteins, modulating their stability (and translation) or degradation to meet cellular iron demands (196, 197, 218) (Fig. 6). Specific regulation of these proteins is discussed next with relevance to brain disorders that are associated with iron mis-metabolism.

III. Iron Homeostasis Within the Brain and Regulation at the BBB

Brain is rich in non-heme iron, reflecting its high metabolic rate. Most of the iron is concentrated in the *substantia nigra pars compacta* (SN) and basal ganglia, the latter reaching levels equivalent to the liver (126, 177, 183). The BBB protects the brain from fluctuations in systemic iron levels, and disturbances of iron homeostasis in peripheral organs have a minimal effect on brain iron metabolism. Thus, levels of iron and iron-modulating proteins in the serum and cerebrospinal fluid (CSF) that bathe the brain differ significantly (311).

The BBB is formed by a monolayer of polarized capillary endothelial cells with tight junctions that regulate the

FIG. 5. Role of hepcidin in systemic iron homeostasis. Systemic iron homeostasis is regulated by hepcidin, a peptide hormone that is released by hepatocytes in response to iron saturation of serum Tf. Excess iron (*left*) triggers the release of hepcidin that binds to Fpn. This initiates the binding of Jak2 to the cytosolic portion of Fpn. Subsequent autophosphorylation of Jak2 leads to phosphorylation of Fpn, followed by internalization and degradation of the Fpn-hepcidin complex. In an iron deficiency (*right*), hepcidin expression is inhibited, sparing Fpn that restores normal iron transport. Figure adapted form Hentze *et al.* (197). HFE, hereditary hemochromatosis gene product.
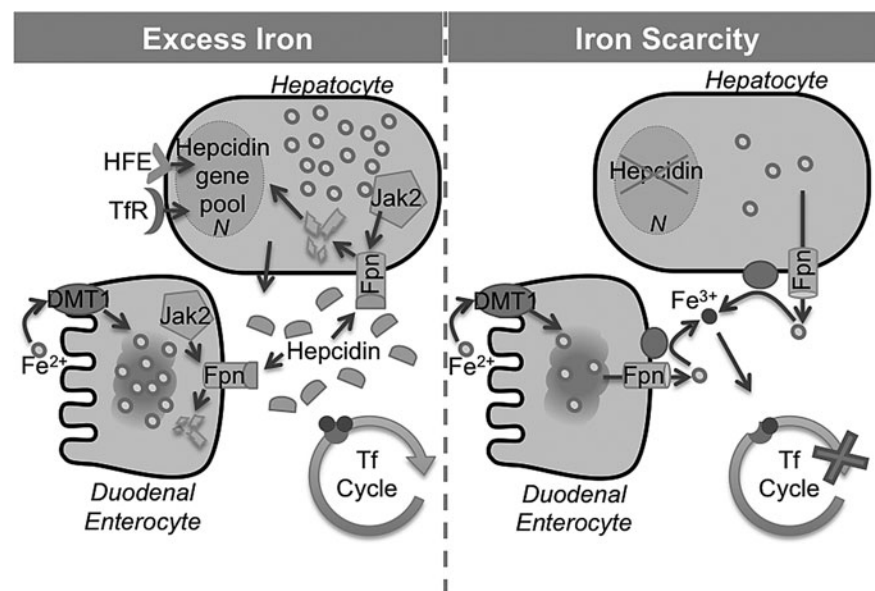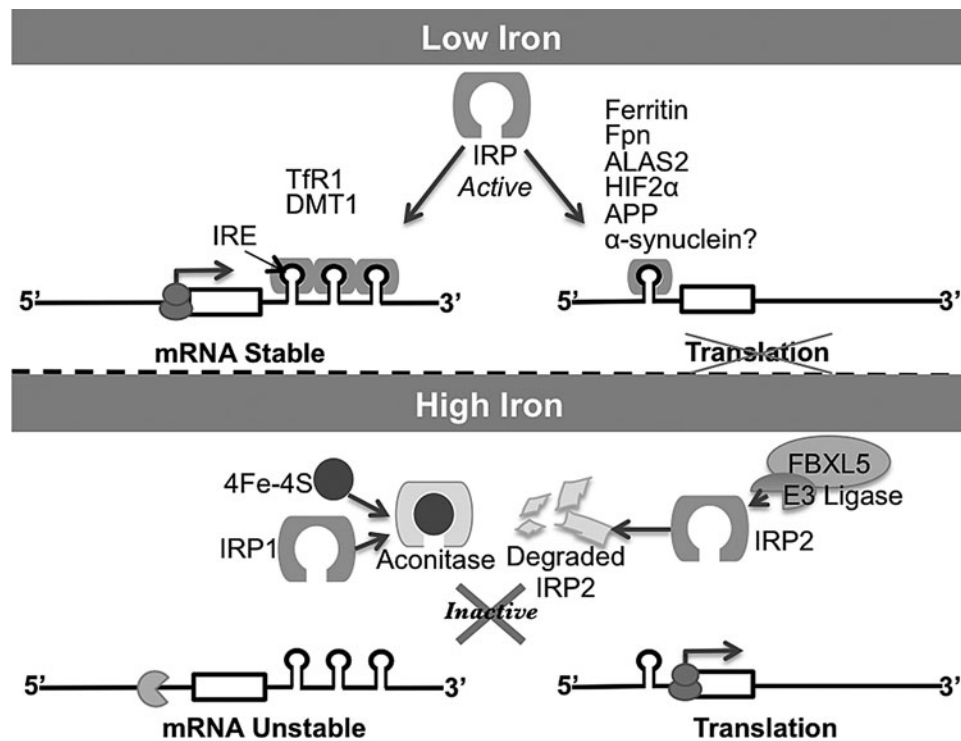
FIG. 6. **Iron regulatory proteins**. IRP-IRE system regulates iron uptake and storage by modulating the expression of mRNAs coding for iron uptake, storage, and export proteins. When iron levels are low (*top*), IRP binds to 3′ IREs of target mRNAs (TfR1, DMT1), stabilizing the transcript to enable translation and increase uptake of iron. Concomitant binding to 5′ IRE of target mRNAs (ferritin, Fpn, ALAS2, HIF-2α, APP, and, possibly, α-synuclein) prevents binding of the 43S pre-initiation complex, thus inhibiting translation and reducing iron storage and efflux. In the presence of excess iron (*bottom*), IRP1 incorporates ISC to acquire aconitase activity while IRP2 is degraded. IRPs, therefore, lose their affinity for IREs, resulting in degradation of mRNAs with 3′ IRE sequences coding for iron uptake proteins and translation of mRNAs with 5′ IREs encoding iron storage and efflux proteins. Figure adapted from Crichton *et al.* (97). ALAS2, δ-aminolevulinate synthase 2; APP, amyloid precursor protein; HIF-2α, hypoxia-inducible factor-2α; ISC, iron-sulfur cluster; IREs, iron-responsive elements; IRP, iron regulatory protein.

transport of cargo from blood at the luminal or apical surface to the abluminal or basolateral surface bathed by the CSF and brain interstitial fluid (Fig. 7) (110, 376). Transport of iron across this barrier is mediated by the Tf-TfR and DMT1-Fpn pathways as in systemic organs. Fe^{2+} released by Fpn at the basolateral surface of endothelial cells is oxidized to Fe^{3+} by Cp expressed on astrocytic foot processes lining the endothelial cells (210), and captured by circulating Tf in the brain interstitial fluid and CSF. A significant amount of transported iron circulates in association with citrate, ascorbate, or ATP (224, 286). Brain Tf is secreted by cells of the choroid plexus and oligodendrocytes, though participation of the latter is controversial. Serum Tf does not gain access to the brain, and brain Tf does not leak into the serum except in certain pathological conditions (376). Other pathways of iron transport across the BBB involve uptake of ferritin by a specific though unidentified receptor on the apical surface of endothelial cells (Fig. 8) (53, 150, 429).

Different cell types in the brain acquire iron by distinct pathways, and iron homeostasis within the organ is maintained by a complex set of interacting mechanisms. Neurons express abundant TfR and acquire most of their iron from Tf. Astrocytes express DMT1 and reduce Fe^{3+} in the interstitial fluid to the Fe^{2+} form before uptake (253). Oligodendrocyte progenitors acquire most of their iron from Tf by the TfR1

pathway, and from ferritin through the Tim-2 receptor as they mature (429). Most brain cells express cytosolic ferritin for storing excess iron, but the abundance of ferritin varies based on functional requirements for iron in different cell types. Neurons contain the least, and microglia contain the most amount of cytosolic ferritin. In addition, the abundance of ferritin subunits varies between cell types. Neuronal ferritin has a higher proportion of Ft-H, oligodendrocyte ferritin has equivalent amounts of Ft-H and ferritin light chain (Ft-L), and microglial ferritin is comprised mostly of Ft-L chains. However, the relative abundance of Ft-H is higher than Ft-L in total brain homogenates compared with liver and spleen tissue. Excess iron is exported from cells by the combined action of Fpn and Hp, similar to other cell types (281, 312).

It is notable that the iron saturation of CSF Tf is ∼100%, while that of serum Tf is ∼30% (53, 312). Several factors are likely to contribute to this observation, including low concentrations of Tf in the CSF relative to serum, high concentrations of ascorbic acid, and low concentrations of Cp that are necessary for oxidizing available Fe^{2+} to the Fe^{3+} form for binding to Tf. Serum Tf, therefore, has a much higher buffering capacity under conditions of iron overload relative to CSF Tf, increasing the vulnerability of neuronal cells to iron-induced toxicity. Thus, redundant pathways of iron import, export, and storage exist to protect the neurons from iron
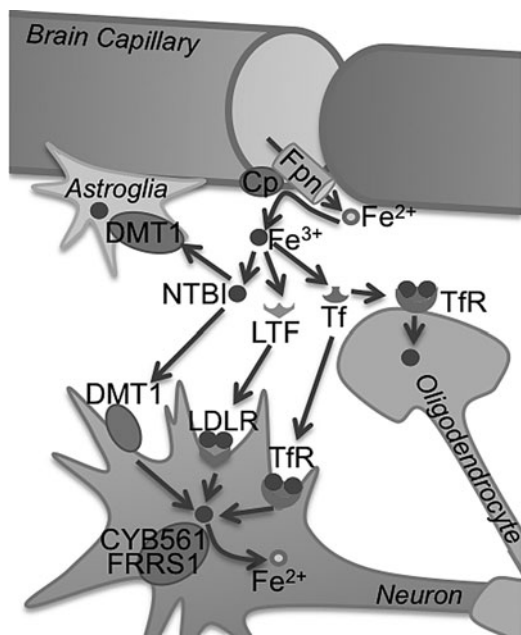
FIG. 7. **Transport of iron to the brain.** Iron is exported from endothelial cells of the BBB to brain interstitial fluid through Fpn, and is oxidized to Fe^{3+} by Cp. Within the brain, iron is bound to Tf or exists as NTBI. Tf bound iron is taken up by TfRs on the neuronal and oligodendroglial plasma membrane. NTBI enters the neuron and astroglia by the DMT1 pathway. Figure adapted from Ke and Qian (224). BBB, blood brain barrier; LDLR, low density lipoprotein receptor; LTF, lactotransferrin; NTBI, non-transferrin bound iron.

imbalance. This is exemplified by mouse models of hypotransferrinemia and loss of function mutations of Fpn that show minimal change in brain iron levels (38, 124). These observations also indicate that mechanisms regulating brain iron homeostasis are not fully known. Although mouse models carrying a deletion of one or more genes involved in iron regulation or representing specific brain disorders have been helpful (Table 1), continued research in this area is necessary to understand the pathogenesis of brain disorders that are associated with iron metabolism.

IV. Brain Iron Mis-Metabolism and Associated Disorders

Functional mutation(s) in iron-modulating proteins disrupt iron homeostasis in systemic organs and the brain to a varying extent, and, in some cases, result in specific human disorders (61, 226, 283). Ferritinopathies and aceruloplasminemia are clear examples where dysfunction of ferritin or absence of Cp alter brain iron homeostasis and induce neurotoxicity. The correlation between brain iron metabolism and neurotoxicity associated with neurodegenerative conditions such as AD, PD, HD, and sCJD remains controversial (13). Some studies suggest non-specific co-precipitation of iron and other metals with aggregated proteins, while others associate brain iron directly with disease pathogenesis (129, 372). Current information on the role of iron in the pathogenesis of some of these disorders is reviewed next.

A. Ferritin and ferritinopathies

Ferritin performs the essential function of storing excess iron within its shell, and is considered a pro- as well as an anti-
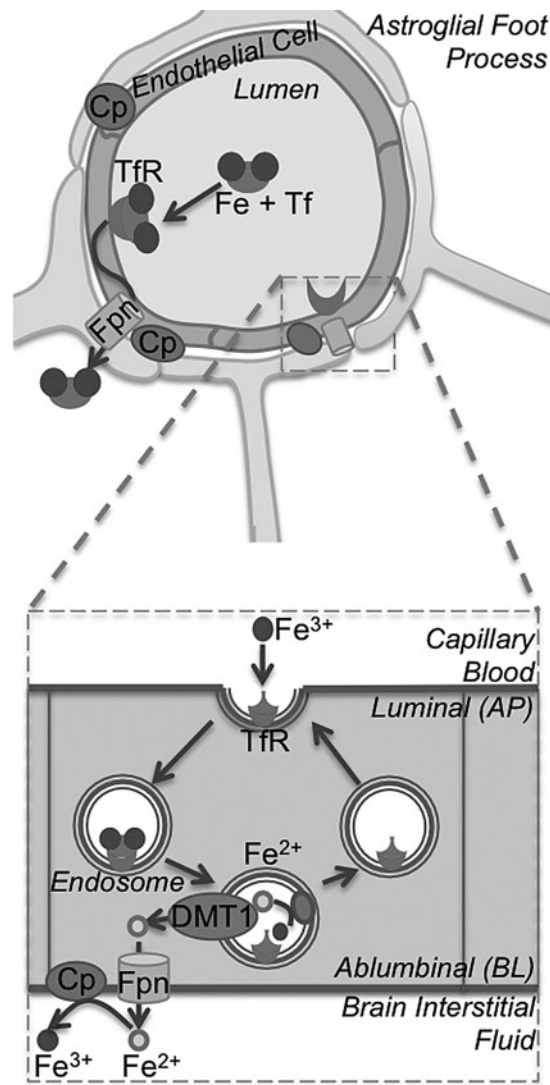

FIG. 8. **Iron transport across the BBB.** The BBB (*top*) is formed by a tight monolayer of endothelial cells surrounded by astroglial foot processes on the baso-lateral surface. Tf-bound Fe^{3+} iron is captured by TfRs on the luminal or apical membrane of endothelial cells and released in the acidic environment of the endosomes (*below*). Released Fe^{3+} iron is reduced to the Fe^{2+} form before transport across DMT1 to the cytosol. Subsequently, iron is transported across the abluminal or basolateral membrane through Fpn to the brain interstitial fluid where it is oxidized back to Fe^{3+} by Cp or Hp for loading to Tf. The TfR is recycled back to the apical membrane. Figure adapted from Crichton *et al.* (97) and Ke and Qian (224).

oxidant. In addition to its function in iron storage, ferritin is involved in several physiological and pathological processes. We will focus on the role of ferritin in brain iron metabolism and disorders that result from its dysfunction (20, 281, 451).

Ferritin is a ubiquitous, mainly cytosolic, globular protein of ~ 450 kDa comprising 24 subunits of Ft-H and Ft-L chains (20, 21, 87, 216). Ft-H possesses an active ferroxidase center that catalyzes the oxidation of Fe^{2+} to the Fe^{3+} form, while Ft-L mediates its nucleation within the protein shell for storage. Together, these chains form a nano-cage that can store approximately 4500 Fe^{3+} ions in a non-toxic, bioavailable form

as the mineral ferrihydrite (258, 306, 425). High Ft-H:Ft-L ratios are found in tissues with high rates of oxidative respiration to maximize iron turnover, while high Ft-L:Ft-H ratios are observed in tissues specialized for iron storage, such as the liver (20, 21, 64). Ferritin also circulates in the CSF, although at 10-fold lower concentrations than in the plasma. Hemosiderin is a degradation product of ferritin and is often observed in areas of hemorrhage and massive cell death. Iron within hemosiderin is insoluble, bio-unavailable, and unlikely to react with free radicals.

The precise mechanism of binding, storage, and release of iron from ferritin requires more clarity. Available information suggests that a cytosolic iron chaperone Poly (rC)-binding protein 1 binds cytosolic iron for delivery to ferritin (398). Poorly characterized ferritin-binding proteins, amino acids, and small molecules regulate the release of iron from ferritin, supporting the gated pore model (273, 274, 427). Stored iron is released in response to low intracellular iron, thus maintaining equilibrium between ferritin iron and the labile iron pool. Expression of ferritin is regulated post-transcriptionally by IRPs, and degradation is induced by certain iron chelators and lysosomal activity (108, 227). Free radicals, high levels of ascorbic acid, and acidic milieu also trigger release of iron from ferritin, leading to oxidative burst and cytotoxicity. The cell protects itself against such events by up-regulating ferritin approximately 80-fold through the antioxidant-response element, in part by binding to the transcription factor NF-E2-related factor 2 (200, 348). The resultant increase in ferritin sequesters iron and minimizes the generation of reactive oxygen species (ROS) (200, 346, 431). Other regulators of ferritin expression include inflammatory cytokines, tumor necrosis factor alpha, and interleukins, IL-1 and IL-6 (431). Inflammation up-regulates the expression of Ft-H, which sequesters iron as a protection against oxidative stress (139, 432, 457), resulting in the anemia of chronic inflammation (461). This is compounded further by the release of hepcidin that blocks export of iron from the macrophage/reticuloendothelial system, resulting in intracellular iron overload (313, 460). Thus, the role of ferritin extends beyond that of iron storage.

Hereditary ferritinopathy or neurodegeneration with brain iron accumulation (NBIA) type 3 is an autosomal dominant condition that affects motor and cognitive function (94, 316). The underlying cause is duplication of nucleotides in exon 4 of Ft-L gene on chromosome 19 that alters the C-terminus of the translated product (28). Mutant Ft-L (Phe167SerfsX26) accumulates intracellularly in inclusion bodies that include wild-type Ft-L and Ft-H polypeptides (28, 59). Crystal structure of mutant Ft-L at 2.85 Å resolution shows insertion of structures that are similar to wild-type Ft-L between residues Ile-5 and Arg-154, resulting in multiple polypeptide conformations instead of the normal E-helices, and disruption of the fourfold (~ 2 Å) or threefold (3.4 Å) axis pores in the ferritin nano-cage (27). This structural disruption manifests as loss of iron storage ability and accumulation of ferritin aggregates in association with iron (25, 27). The accompanying increase in brain iron levels triggers translation of additional ferritin by the IRE/IRP system, accentuating ferritin aggregation and toxicity (28, 29, 442, 444). The inclusions stain positive for iron, ferritin, and ubiquitin (101), and are prominent in the nuclei of neurons, oligodendrocytes, and microglial cells as well as the extracellular space, increasing brain iron levels. Similar inclusions are also noted in muscles, peripheral nerves, and the skin of affected individuals (389, 442, 444). So far, six such insertions, 442InsC (292), 442Ins 4nt (246), 458InsA (116), 460InsA (101), 469–484dup16nt (329), and 498InsTC (443), have been reported that cause frame shifts (59, 101). In addition, a missense mutation A96T that results in a similar disease phenotype has been identified (280). The neurological symptoms manifest in the fourth to sixth decade, and include choreoathetosis, dystonia, spasticity, and rigidity. Cognitive decline is rare or subtle in the early stages, but deteriorates with disease progression (59, 98, 471).

More than 30 additional mutations have been reported in the Ft-L chain, mostly in the regulatory 5′ IRE stem-loop region, that cause hereditary hyperferritinemia cataract syndrome with increased serum ferritin, and early-onset bilateral cataracts. Serum iron and Tf saturation are normal to low in most cases (168, 303). Mutations in this region reduce the binding affinity of IRE to the IRPs, up-regulating Ft-L expression and altering the ratio of Ft-H to Ft-L. This affects the ability of lens ferritin to store iron, and micro-crystalline aggregates of Ft-L result in cataracts. The degree of hyperferritinemia and severity of cataracts are characteristic of the individual mutation. Affected individuals do not show any change in systemic or brain iron homeostasis (171, 317).

Mutations in the coding region of Ft-L are rare. The heterozygous missense mutation Thr30Ile in the N-terminus of Ft-L causes genetic hyperferritinemia without iron overload or clinical symptoms. The unusually high glycosylation of mutant Ft-L is probably due to increased hydrophobicity of the N-terminal α-helix (217). In addition, a His133Pro mutation with low Ft-L levels and mild chronic anemia in a case of PD (154) and a heterozygous mutation of the first nucleotide of Ft-L start codon have been reported. The latter mutation did not alter synthesis of the functional protein (95). Mutations in the Ft-H chain are infrequent. Of the three reported mutations in the 5′ IRE of Ft-H chain mRNA, only A49U identified in a Japanese subject is associated with iron overload; the others (C20G and G34T) are silent (96, 219). It is likely that genetic alterations in Ft-H are lethal, and, hence, detected infrequently.

Pathologically, the brain tissue of hereditary ferritinopathy cases shows over-expression of neuroglobin, deposits of cytoglobin, and p53-mediated apoptosis of neurons and glia in specific regions (355). The presence of lipid peroxidation and abnormal nitration of proteins suggests contribution of Fe^{2+}-mediated oxidative stress in the pathophysiology of these disorders (292). Imbalance of iron metabolism in affected regions also causes mitochondrial abnormalities as evidenced by changes in the biochemical and histochemical characteristics and accumulation of oxidatively damaged DNA in the mitochondria of a mouse model of hereditary ferritinopathy (114). Interestingly, mutant Ft-L itself is targeted by ROS, resulting in its cleavage and disruption of the ferritin shell. The free radical trap 5,5-dimethyl-1-pyrroline N-oxide effectively rescues mutant Ft-L from cleavage, confirming the role of free radicals in the process (26). It is likely that co-aggregation of wild-type Ft chains is initiated by the free radicals generated by mutant Ft-L, creating iron imbalance in the affected brains (25). Accumulation of iron-rich, aggregated ferritin has also been observed in sCJD brains, suggesting the presence of common pathogenic events initiated by brain iron imbalance and free radicals (407, 410).

B. Cp and aceruloplasminemia

Cp is a copper-containing, acute-phase α2-glycoprotein that functions as a multi-copper ferroxidase to regulate body iron homeostasis (331). The holoprotein contains six copper atoms that confer ferroxidase activity which is responsible for the oxidation of Fe^{2+} iron released from the intestinal epithelial, capillary endothelial, and reticuloendothelial cells to Fe^{3+}, thereby modulating iron transport at multiple sites. Cp exists as two isoforms; a soluble form in the plasma synthesized mainly by hepatocytes, and a glycosylphosphatidyl inositol-(GPI)-linked form synthesized by astrocytes. The GPI-linked isoform is generated by alternate splicing of exons 19 and 20 and replacement of the C-terminal 5 amino acids of the secretory form by a 30-amino acid signal peptide for GPI anchor addition (340). Within the brain, Cp is expressed mainly on glial cells in the cerebellum, SN, microvasculature, (235), and the inner nuclear layer of the retina (236).

Although Cp requires copper for its function, absence of Cp causes imbalance of iron metabolism rather than disturbance of copper homeostasis (308). This is exemplified by the human disease aceruloplasminemia, a rare autosomal recessive disorder resulting from mutations in the Cp gene, and characterized by progressive accumulation of iron in the liver, pancreas, basal ganglia, SN, and the retina. Typical clinical presentation includes ataxia, involuntary movements, parkinsonism, cognitive dysfunction, retinal degeneration, and diabetes mellitus (283, 309). More than 30 mutations in the Cp gene segregate with aceruloplasminemia. Most are truncation mutations due to a premature stop-codon, resulting in the generation of a protein product that is either not transported to the plasma membrane or lacks one or more copper binding sites, rendering it non-functional (199, 283). Absence of Cp causes iron overload mainly by down-regulating Fpn, the iron export protein that is stabilized by the ferroxidase activity of Cp (107). Hp, another copper containing ferroxidase, is unable to substitute for the complete lack of Cp, highlighting important differences between the two ferroxidases (78, 79, 189).

Aceruloplasminemia is best exemplified by transgenic mouse models with a targeted deletion of the Cp gene. These mice exhibit age-dependent accumulation of non-heme iron in the brain, especially in the brainstem and cerebellar regions. The spinal cord and retina also show a significant increase in iron (341). Most of the accumulated iron is within astrocytes; oligodendrocytes, Purkinje neurons, and large neurons of the deep nuclei do not accumulate iron. Instead, Purkinje neurons upregulate DMT1 expression and show evidence of iron deprivation, probably because of sequestration in astrocytes (Fig. 9). Iron-loaded astrocytes are a source of ROS, and neuronal death ensues through free radical injury and lack of trophic support by astrocytes that eventually succumb to iron-mediated injury (211). It is interesting to note that ROS down-regulates Cp through an mRNA decay mechanism, creating a destructive positive-feedback loop (423). Since ROS is implicated in the neurotoxicity associated with several neurodegenerative conditions, it is likely that ROS-mediated down-regulation of Cp and perhaps Fpn contributes to the neurotoxicity associated with diverse neurodegenerative conditions.

C. Alzheimer's disease

AD is the most common dementia of individuals older than the age of 65, accounting for 50%–80% of all dementias. An
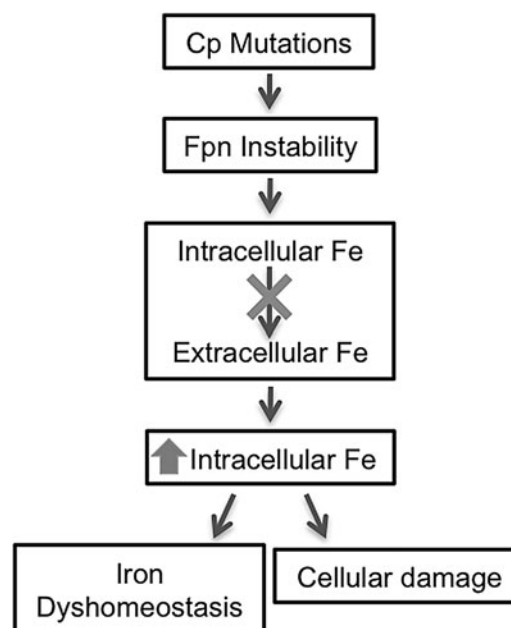

FIG. 9. Aceruloplasminemia: absence or dysfunction of Cp destabilizes Fpn, resulting in intracellular accumulation of iron.

estimated 5 million Americans currently suffer from AD, and the number is likely to reach 11–16 million by the year 2050. The majority of AD cases are sporadic in origin and do not show symptoms till advanced age. A small number (5%–10%) are inherited as an autosomal dominant trait and develop the disease at a much younger age. Affected individuals present with short-term memory deficits that gradually progress to loss of cognition, aphasia, apraxia, agnosia, and general loss of executive functions. The hallmark lesion of AD brains is the extracellular presence of amyloid plaques comprising mainly of amyloid β (Aβ), and intra-neuronal accumulation of neurofibrillary tangles (NFT) composed of hyperphosphorylated tau. Aβ arises from sequential proteolytic processing of amyloid precursor protein (APP), a transmembrane protein that is expressed abundantly on the neuronal plasma membrane. Mutations in APP and presenilin genes 1 and 2 increase Aβ generation and are associated with the dominantly inherited form of AD, implicating Aβ as the principal cause of neurotoxicity (184). However, Aβ plaques and NFT do not account for all aspects of AD, which is now believed to involve additional pathways that converge to produce the typical pathology and clinical symptoms. Prominent among these are brain metal dyshomeostasis, mitochondrial dysfunction, impaired glial cell function, inflammation, and oxidative stress (61, 446). These mechanisms are not mutually exclusive, and are likely to work through intersecting biochemical pathways.

Although brain iron increases with aging, this process is enhanced and localized to certain regions in AD such as the parietal cortex, motor cortex, and hippocampus (45, 480). Most of the iron is associated with Aβ plaques or sequestered within ferritin in the surrounding glial cells (60, 277, 414). It is believed that iron accumulation precedes aggregation of tau and formation of NFTs, and is a major cause of protein and DNA oxidation, lipid peroxidation, and accumulation of advanced glycation end products, carbonyls, malondialdehyde,

and peroxynitrite in AD brains. A concomitant decrease in anti-oxidant enzymes is also noted, potentiating the oxidative stress. Neurons are particularly vulnerable to this change because of their high metabolic rate and dependence on oxidative metabolism (415).

Accumulation of iron in AD brains is attributed to two main causes: (i) relatively high affinity of $A\beta$ for metals such as iron, copper, and zinc (203), and (ii) impaired ferroxidase activity of APP at the neuronal plasma membrane (129). Although $A\beta$ is generated under normal physiological conditions and is neurotrophic, increased production and aggregation into insoluble fibrils renders it pathogenic. A possible cause of $A\beta$ toxicity is the binding of iron and copper to histidine residues 6, 13, and 14 in its hydrophilic N-terminal domain, and the reduction of these metals by methionine at residue 35 to release hydrogen peroxide and hydroxyl radicals through Fenton chemistry. This causes oxidation, cross-linking, and aggregation of $A\beta$, sequestering associated iron and copper that confer pro-oxidant activity to $A\beta$. Aggregation of additional $A\beta$ peptides then proceeds exponentially (63). Insoluble $A\beta$ aggregates activate surrounding microglia, contributing to oxidative stress (Fig. 10). NFTs also bind iron and copper, complementing the actions of $A\beta$ to create an environment of metal imbalance and oxidative stress. Zinc, on the other hand, allosterically inhibits the iron and copper-binding sites on $A\beta$, and, therefore, may play a protective role. However, the role of zinc in AD pathogenesis is complex, and it is premature to assign a clear protective or toxic role to this metal (Fig. 10) (366).

An important consideration in discussing the role of iron in AD pathogenesis is that APP functions as a major ferroxidase on the neuronal plasma membrane, and is regulated by cellular iron levels (129). A functional "type-II" IRE has been identified in the 5′ UTR of APP mRNA, including it in the family of proteins that are regulated by IRPs (373). An increase in intracellular iron increases APP expression, while exposure to iron chelators down-regulates its expression in neuroblastoma cells. In neuronal cells, APP functions as a ferroxidase partner for Fpn-coupled export of iron from cells, an activity that is inhibited by zinc released from $A\beta$ plaques (129, 366). Chelation of zinc restores the ferroxidase activity of APP to control levels, implicating APP and zinc as pro-oxidants in AD pathogenesis (129). Although Cp is an equally effective ferroxidase and facilitates the export of iron from cells, it is mainly expressed on astrocytes. Thus, the absence of APP increases intracellular iron in neocortical and hippocampal neurons of $APP^{-/-}$ mice, while deletion of Cp causes iron accumulation in the astrocytes of $Cp^{-/-}$ mice (129, 211). APP is also involved in the efflux of copper from cells by functioning as a copper chaperone, though the details of this pathway are not clear (31). A recent study explains neuronal iron accumulation in tauopathies, AD, and PD due to a common defect in APP trafficking, a process that is usually facilitated by tau. Consequent loss of ferroxidase activity of APP links the three neurodegenerative disorders to a common pathway of intracellular iron accumulation and oxidative stress (259).

Neurons and glial cells protect themselves against oxidative stress with the help of inducible and constitutive forms of heme-oxygenase enzymes HO-1 and HO-2, respectively, and biliverdin reductase (BVR). However, both isoforms of heme-oxygenase are induced by oxidative stress and free heme (66, 134) and catalyze the degradation of heme to generate equimolar amounts of Fe^{2+}, carbon monoxide, and biliverdin (229, 470). Fe^{2+} participates in the generation of ROS, while biliverdin is further reduced by BVR to the lipophilic antioxidant bilirubin (285, 420). Although increased activity of these enzymes in AD brains is believed to be neuroprotective (290), recent studies suggest that Fe^{2+} released by this reaction causes aggregation and phosphorylation of tau that aggravates the toxicity by interfering with APP transport and other cellular processes (205, 259). Other studies suggest that heme-oxygenase and biliverdin-reductase undergo post-translational modifications in subjects with mild cognitive impairment and AD, reducing their ability to generate antioxidant molecules including bilirubin (33–35), thereby potentiating the oxidative damage by Fe^{2+} (35, 121). These findings provide a new perspective on the imbalance between oxidant species such as iron and anti-oxidant enzymes in AD pathogenesis.

D. Parkinson's disease

PD is the second most common age-related neurodegenerative disorder affecting 1%–2% of the population older than the age of 65 (254, 295, 323). Clinically, patients present with motor dysfunction due to loss of dopamine-producing neurons in the SN, the primary neurotransmitter in the extrapyramidal system. Typical symptoms of PD include dyskinesia, rigidity, and tremor, eventually followed by cognitive decline and depression (204, 310). Pathologically, PD is characterized by loss of neuromelanin in the SN and the appearance of proteinaceous inclusions or Lewy bodies in the surviving neurons (142, 276).
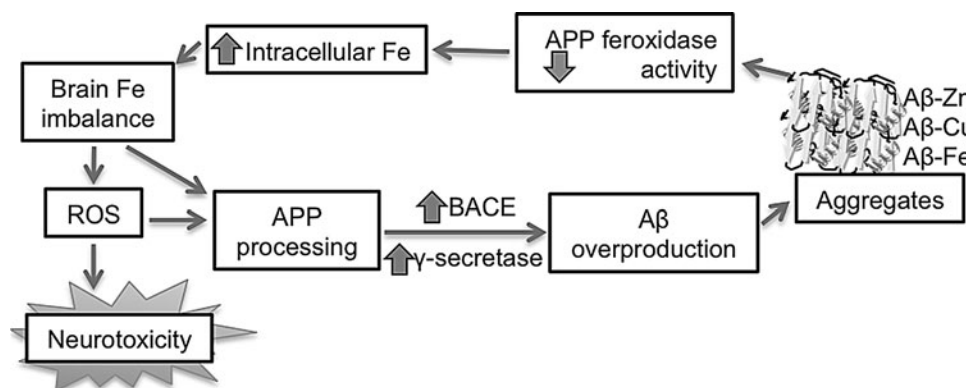

FIG. 10. Over-production of $A\beta$ by ROS. Amyloidogenic processing of APP to generate $A\beta$ is increased by ROS. Accumulated $A\beta$ binds redox-active metals, acquires pro-oxidant characteristics, and creates a vicious feedback loop that generates additional ROS. $A\beta$, amyloid β; ROS, reactive oxygen species.

Most cases of PD ($\sim$90%) are sporadic and attributed to diverse causes, including exposure to certain toxins (106, 206, 384). A small percentage ($\sim$10%) are familial in nature, and segregate with mutations in α-synuclein, parkin, PTEN-induced putative kinase 1 (pink1), DJ-1, leucine rich repeat kinase-2 (LRRK2), ubiquitin carboxy-terminal hydrolase L1, and ATP13A2 (49, 140, 233, 352, 438). The majority of familial cases are associated with mutations in α-synuclein, a protein involved in synaptic vesicle formation in addition to other partially characterized roles (1, 479). The normal function of other proteins is still emerging. It is believed that Parkin and ubiquitin carboxy-terminal hydrolase L1 play a role in ubiquitination and de-ubiquitination of proteins targeted for proteasomal degradation (482). DJ-1 is an antioxidant and a redox-sensitive chaperone (49), and PINK-1 is a mitochondrial protein kinase (438). LRRK2 is associated with mitochondria, binds to parkin, and is involved in membrane and protein trafficking (140).

Mutations in α-synuclein and LRRK2 are autosomal dominant, while those in DJ-1, parkin, pink1, and ATP13A2 segregate with autosomal recessive forms of PD. Notably, over-expression of α-synuclein is sufficient to cause PD in humans and animal models (111, 188, 193, 324, 365), suggesting a central role in PD pathogenesis. Pathogenic events common to most sporadic and familial forms of PD include accumulation of misfolded α-synuclein, mitochondrial dysfunction, oxidative stress, impaired autophagy, and neuronal iron accumulation (137, 296, 403, 433). Some of these defects are partially reversed by over-expression of parkin, pink1, DJ1, or the mitochondrial chaperone TRAP1 that requires phosphorylation by pink1 for functional activity (62, 349), suggesting a prominent role of autophagy in this process (445). Impaired autophagy due to mutations in parkin or pink1 impacts mitochondrial function by inhibiting mitophagy (257), and mitochondrial dysfunction directly inhibits autophagy by interfering with microtubule-dependent transport of autophagosomes, ultimately increasing intracellular levels of ROS and associated lysosomal leakage (17, 112). Lysosomal deficiency and α-synuclein rich Lewy bodies reactive for the autophagosomal marker LC3 have been reported in the SN of PD brains and mouse models of PD, supporting this sequence of events (445). Conditional inhibition of autophagy and mitochondrial complex 1 activity in experimental mouse models causes age-dependent loss of dopaminergic (DA) neurons (9, 86), further supporting the observations cited earlier.

Accumulation of iron in the SN of PD cases has been described by several groups, and is attributed to the chain of events triggered by mitochondrial dysfunction (301) and death of the relatively iron-rich DA neurons. However, the presence of iron in PD brains is not simply an outcome of α-synuclein aggregation, mitochondrial dysfunction, and DA cell death. An active process of iron import is suggested by the up-regulation of DMT1 ($+$IRE), TfR1, and transferrin receptor 2 (TfR2) (278, 298, 380), and down-regulation of Fpn (6) in DA neurons of PD cases and mouse models of PD, while loss-of-function mutations in DMT1 abolish this effect (380). Mutations in parkin increase DMT1 levels by inhibiting its degradation by the proteasomal pathway (374), providing the principal pathway of iron accumulation in DA neurons of the SN (380). Other brain regions such as the cerebellum, caudate nucleus, putamen, and cerebral cortex show minimal change

(117, 118). Exposure of a DA cell line MES23.5 to the active metabolite of PD-inducing toxin MPTP (1-methyl-4-phenylpyridinium or MPP$+$) induces up-regulation of DMT1 and increased uptake of Fe^{2+} from the extracellular milieu, supporting these observations (481). The presence of a mitochondrial-targeting signal in TfR2 and alteration of its function by rotenone, another toxin used for inducing experimental PD, further suggests that alteration of iron metabolism in the SN is an active process (298, 325).

Accumulation of iron in DA neurons through DMT1 is further compounded by impaired activity of Cp, APP, and Hp, principal brain ferroxidases that are responsible for the export of excess iron from cells (30, 201, 259, 330, 367). Thus, mutations in Cp co-segregate with PD, and treatment of mouse models of PD exposed to 6 hydroxydopamine (6-OHDA) causes down-regulation of Fpn and Hp (271, 418, 450). Likewise, mouse models with deletion of tau develop PD due to impaired transport of APP to the plasma membrane, suggesting that a combination of increased iron uptake by DMT1 and decreased efflux due to compromised activity of APP-Fpn complex is responsible for the accumulation of iron in DA neurons of the SN (259). On similar lines, aggregates of α-synuclein cross-seed intracellular aggregation of tau in cell lines, inhibiting the transport of APP and accumulation of intracellular iron. This scenario provides a plausible explanation for the co-existence of AD and PD pathology in certain cases (455).

Similar to APP, α-synuclein has an IRE element in the 5' UTR of its mRNA at exon 1–2 splice junction. The functionality of this element, however, has not been demonstrated unequivocally (159). Apart from this, α-synuclein functions as a FR when expressed in neuroblastoma cells, suggesting a role in cellular iron uptake and transport (104). When exposed to free radicals or excess iron, α-synuclein aggregates and induces the aggregation of additional molecules due to the redox-active nature of these aggregates, creating an environment of ongoing oxidative stress (149, 332). In transfected cell lines, co-expression of DMT1 with wild-type or mutant α-synuclein enhances toxicity of the latter (84), and oral administration of excess iron to neonatal or early post-natal mice predisposes these animals to MPTP and paraquat induced PD (220, 343, 367). Iron-fed mice show significantly more DA toxicity relative to controls, indicating that excess iron increases the vulnerability of DA neurons to toxic insults (220). Furthermore, mutations in genes involved in cellular iron homeostasis increase susceptibility to PD (50, 178) while chelation of iron is protective (221), underscoring the role of iron and brain iron mis-metabolism in PD pathogenesis.

Paradoxically, L-DOPA, the mainstay of PD treatment, triggers oxidative stress and contributes to the already existing redox imbalance in PD cases. Catabolism of dopamine by monoamine oxidase results in the generation of free radicals under physiological conditions, and rats treated with L-DOPA show biomarkers of oxidative and nitrosative stress and activation of the heat shock pathway in the SN and striatum (65). Additional factors that contribute to an environment of oxidative stress in DA neurons include (i) pro-oxidant characteristics of iron-rich neuromelanin that releases Fe^{2+} because of weak affinity; (ii) release of Fe^{3+} from ferritin in the presence of superoxide; (iii) inefficient activity of superoxide dismutase (SOD) that prevents the conversion of superoxide radical to hydrogen peroxide; (iv) decrease in

astrocytes expressing glutathione peroxidase which reduces hydrogen peroxide to water and prevents the generation of hydroxyl radicals; and (v) dysfunction of the ubiquitin-proteasome system (9, 41, 118, 154, 172, 310, 417, 482). Together, the observations cited earlier suggest a significant role of iron-induced oxidative stress in the pathogenesis of PD.

E. Sporadic Creutzfeldt-Jakob disease

Prion diseases are a group of neurodegenerative conditions of humans and animals that are sporadic, familial, and infectious in origin. Common disorders include sCJD in humans, scrapie in sheep and goats, and chronic wasting disease in the deer and elk population (359, 360). Approximately 80% of CJD cases are sporadic in origin with no known underlying cause, and the remaining cases segregate with point mutations in the prion protein gene. Only 1%–2% are acquired from an external source through contaminated meat or iatrogenically following a medical procedure (8). Although relatively rare in comparison to the aforementioned neurodegenerative conditions, the infectious nature of these disorders has prompted intense investigations in this area. It is now clear that the main event underlying sCJD and other prion disorders is a conformational change of the prion protein (PrP^C), a ubiquitously expressed glycoprotein that is most abundant on neuronal cells, from α-helical to a β-sheet rich isoform termed PrP-scrapie (PrP^{Sc}) (8, 359). Deposits of PrP^{Sc} in the brain parenchyma are the principal cause of infectivity and neurotoxicity in all prion disorders, though the mechanisms underlying these processes are not clear (32). Amplification of PrP^{Sc} from recombinant PrP^C in vitro by several laboratories leaves little doubt that PrP^{Sc} arises from PrP^C, and is sufficient to initiate infection and cause disease when inoculated intracerebrally into recipient animals (113, 449). However, the mechanisms by which PrP^{Sc} induces neurotoxicity are not as clear, and search for the toxic molecule continues. It is clear that expression of host-encoded PrP^C on the neuronal plasma membrane is necessary for transmitting the toxic signal of PrP^{Sc}, but the nature of this signal and the pathways involved remain ambiguous (83, 288, 362).

As noted for AD and PD, markers of oxidative stress are prominent in prion disease affected brains, and have been attributed to the loss of anti-oxidant activity of PrP^C combined with gain of pro-oxidant activity of PrP^{Sc} in diseased brains. Though plausible, direct evidence for either of these processes is lacking. Regarding the loss of normal function of PrP^C, several studies have demonstrated a protective role of PrP^C in the face of oxidative stress and other insults, and possible mechanisms for this activity have been proposed (85). In addition, controlled alteration of certain parameters in $PrP^{-/-}$ mice and cell lines lacking PrP^C expression has revealed several putative functions of PrP^C (7). However, lack of an overt phenotype in transgenic mice carrying a deletion of the PrP gene ($PrP^{-/-}$) suggests that either the normal function of PrP^C is not significant, or is essential for life and is compensated for by other proteins in its absence. Further studies are necessary to resolve this question. In this review, we will focus on the antioxidant function of PrP^C and its role in iron uptake and transport, and possible mechanisms leading to the pro-oxidant nature of PrP^{Sc}-aggregates.

Several studies have indicated that absence of PrP^C increases the vulnerability of $PrP^{-/-}$ mice and neuronal cell lines to superoxide, hydrogen peroxide, and copper ions, an observation attributed to the function of PrP^C as an SOD (234, 474). A similar susceptibility to oxidative stress is noted in cell lines propagating PrP^{Sc} in culture (463), though it is unclear whether the decrease in PrP^C levels due to conversion to PrP^{Sc} or the pro-oxidant activity of PrP^{Sc} aggregates is responsible for this phenotype. Although both processes could be involved to varying degrees, it is likely that PrP^{Sc} aggregates play a dominant role, as cells propagating PrP^{Sc} in culture show alteration of iron-modulating proteins, cellular iron mis-metabolism, and markers of oxidative stress (144, 145). Likewise, scrapie-infected mouse brains show increased amounts of Fe^{2+} and Fe^{3+} in the cerebral cortex, striatum, and brain stem. Deposition of iron is also noted around amyloid plaques in certain human prion disorders. (207, 230, 345).

It is unclear whether the pro-oxidant characteristics of PrP^{Sc} are due to non-specific co-precipitation with iron or other metals as observed for $A\beta$, or results from a specific physiological or pathological process. Evidence from cell and animal models suggests that PrP^C binds copper in the micromolar range in a pH-sensitive manner. The C-terminal residues 90–126 and N-terminal histidine-rich octa-peptide repeat region serve as copper-binding sites (19, 57, 214, 215). Externally added copper stimulates the endocytosis of PrP^C in cell models, suggesting that PrP^C captures copper ions from the extracellular milieu for delivery to intracellular compartments (342). In addition, PrP^C reduces Cu^{2+} to Cu^{1+} prior to transport via copper-specific intracellular trafficking proteins, suggesting a dual function in copper uptake and reduction to facilitate transport across the endosomal membrane (307). The interaction of PrP^C with other metals such as manganese, iron, zinc, and nickel, and the physiological implications of this interaction are poorly understood (85, 198). The majority of these metals induce aggregation of purified or recombinant PrP^C to a form resembling PrP^{Sc} under certain conditions, but the contribution of these metals to prion disease pathogenesis in vivo is unclear.

Although PrP^C does not appear to bind iron under in vivo conditions (412), recent studies on cell and mouse models indicate that PrP^C promotes iron uptake and transport in neuroblastoma cells in culture, and uptake by hematopoietic precursors and parenchymal cells of major organs in mouse models (408, 409). When over-expressed in neuroblastoma cells, PrP^C increases the labile iron pool and iron saturation of ferritin. Deletion of the octa-peptide repeat region in the N-terminus of PrP^C abolishes iron uptake, implicating this region in iron uptake in addition to its role in copper uptake (409). The differential iron content of cells expressing PrP^C and the mutant PrP isoform lacking the octa-peptide repeat region is maintained in the presence of excess extracellular iron, suggesting a dominant role of PrP^C in iron uptake and transport (409). Consistent with these observations, $PrP^{-/-}$ mice display a phenotype of systemic iron deficiency relative to matched wild-type controls (408). Absence of PrP^C in $PrP^{-/-}$ mice affects iron transport at several sites. When introduced orally, radioactive iron is transferred to peripheral red blood cells of $PrP^{-/-}$ mice much before wild-type controls, but unlike wild-type mice in which the mature red cells continue to accumulate radioactive iron from recycled hemoglobin for several days, the amount of radioactive iron in red cells of $PrP^{-/-}$ mice does not increase significantly with time (408). These results are explained by a recent report in

which bone marrow macrophages of $PrP^{-/-}$ mice show less stainable iron and do not incorporate radioactive iron from the medium effectively when cultured *in vitro*. These findings have been attributed to the FR function of PrP^C, thereby facilitating the transport of NTBI through DMT1 (406). The iron-deficient phenotype of $PrP^{-/-}$ mice is reversed by expressing wild-type PrP^C on the $PrP^{-/-}$ background, reinforcing the functional role of PrP^C in iron transport (408).

Consistent with the role of PrP^C in iron uptake, conversion to the PrP^{Sc} form induces a phenotype of apparent iron deficiency in sCJD-affected human and scrapie infected mouse brains (409). However, this phenotype is not merely due to loss of function of PrP^C in iron uptake, as diseased brains show minimal change in total iron and a significant increase in Fe^{2+} iron. Evaluation of scrapie-infected animal brains shows progressive increase in iron deficiency that correlates directly with PrP^{Sc} levels, implicating sequestration of iron in PrP^{Sc}-protein complexes in a biologically unavailable form (407). Isolation of PrP^{Sc} using harsh conditions co-purifies iron rich ferritin, suggesting co-aggregation of PrP^{Sc} with ferritin in a detergent insoluble complex. Likewise, ferritin isolated from sCJD brain homogenates shows significant alterations in its biochemical characteristics. Unlike normal brain ferritin, ferritin isolated from prion disease-affected brains is insoluble, partitions with denatured ferritin when purified using conventional methods, and retains associated iron even after boiling in the presence of SDS (410). The PrP^{Sc}-ferritin aggregates are rich in iron and induce the aggregation of additional PrP^C to the PrP^{Sc} form due to their pro-oxidant characteristics (36). It is likely that PrP^{Sc} and ferritin co-aggregate in lysosomal structures where the two proteins turnover and are likely to encounter low pH conditions which favor denaturation. Co-localization of PrP^{Sc} and ferritin has been observed in scrapie infected cell lines, supporting this hypothesis (36, 408). Notably, PrP^{Sc} and ferritin resist harsh biochemical procedures and co-purify from sCJD and scrapie-infected brain homogenates, suggesting the formation of these complexes during disease progression (408). Sequestration of iron in such PrP^{Sc}-ferritin complexes *in vivo* is likely to induce an iron-deficient phenotype in diseased brains and confer pro-oxidant characteristics to PrP^{Sc}. Analysis of CSF from sCJD cases indicates that iron deficiency occurs relatively early in the disease process and is reflected in the CSF as a significant increase in ferroxidase activity and decrease in Tf levels (185). When used in combination, these biomarkers detect CJD with an accuracy of 88.9%, providing a disease specific pre-mortem diagnostic test (185, 405). These observations also suggest mis-regulation of signaling between brain parenchymal cells and the blood-brain and brain-CSF barriers that are responsible for regulating iron uptake into the brain. Some of these observations have been reproduced using a systems biology approach, highlighting the significance of brain iron imbalance in prion disease pathogenesis (185, 208, 225).

F. Neurodegeneration with brain iron accumulation

NBIA is a heterogeneous group of inherited neurologic disorders that is characterized by excessive deposition of iron in the basal ganglia, globus pallidus, SN, striatum, and the cerebellar dentate nuclei. Age of onset is variable, and most cases present with a broad spectrum of overlapping clinical presentations, including progressive extrapyramidal signs with various combinations of movement disorders, seizures, visual disturbances, followed ultimately by cognitive decline (175, 248, 388).

NBIA disorders comprise two main syndromes: pantothenate kinase-associated neurodegeneration (PKAN) or NBIA type 1 comprising ∼50% of all reported cases, and phospholipase A2-associated neurodegeneration (PLAN) or NBIA-type 2 (101, 190, 194). Other relatively rare forms of NBIA include fatty acid hydroxylase-associated neurodegeneration (FAHN), mitochondrial membrane protein associated neurodegeneration (MPAN) (192), Kufor–Rakeb disease associated with a mutation in ATP13A2, static encephalopathy of childhood with neurodegeneration in adulthood syndrome (SENDA) (388), and aceruloplasminemia and neuroferritinopathy described above.

Multiple metabolic pathways are believed to contribute to the pathogenesis of NBIA, in particular the genes involved in iron and phospholipid metabolism (133). PKAN is associated with mutations in the PANK2 gene located on chromosome 20p, and expressed mainly in neurons of the cortex, globus pallidus, nucleus basalis of Meynert, and the pontine nuclei. Several mutations in the PANK2 gene segregate with PKAN, including missense mutations, deletions, duplications, and splice-site mutations (191). The two most common mutations G to A in codon 1231, and C to T in codon 1253 are detected in about a third of all cases. Several other mutations have also been detected in individual cases, resulting in unique phenotypes (174). Although the pathophysiology of PKAN is not entirely clear, PANK2 protein is believed to play a regulatory role in coenzyme A synthesis by catalyzing the phosphorylation of pantothenate (239). Since PANK2 is mainly targeted to the mitochondria, it is believed that dysfunction caused by mutations interferes with vital metabolic processes, compromising neuronal viability (239). A role for PANK2 in lipid metabolism has also been suggested, though the details are not clear. The correlation between PANK2 mutations and accumulation of iron in certain brain regions, especially the globus pallidus interna, remains unclear, though some studies suggest dys-regulation of Fpn as the underlying cause (350).

NBIA type 2, or the PLAN group of disorders are associated with missense, insertion, deletion, and splice site mutations in PLA2G6 (175). This gene is located on chromosome 22q and encodes iPLA2 β, a calcium-independent phospholipase A2 that hydrolyzes phospholipids to free fatty acids and lysophospholipids. Consequently, iPLA2 β plays a significant role in membrane remodeling, and is, therefore, implicated in vital cellular processes such as signal transduction, cell proliferation, and apoptosis. Dysfunction of iPLA2β alters membrane lipid composition, thus compromising the functional activity of proteins regulating vesicle transport within axons and dendrites. This causes accumulation of membranes in distal axons, resulting in neuronal dysfunction (287). Interestingly, certain human cases of NBIA type 2 show evidence of α-synuclein-positive Lewy bodies and hyperphosphorylated tau and NFT, suggesting an overlap with pathogenic mechanisms underlying PD (335).

Several other rare forms of NBIA have been recently recognized, and they include FA2H-associated neurodegeneration (FAHN) (244), Kufor–Rakeb Disease (PARK9) (365), MPAN, and SENDA (243). FA2H plays a critical role in the maintenance of normal myelin integrity, and mutations in this gene segregate with childhood NBIA characterized by altered

brain iron homeostasis, lipid signal transduction, cyclin-dependent kinase inhibitor expression, and intracellular ceramide pool composition (244, 248). Kufor–Rakeb disease is a rare type of NBIA caused by mutations in ATP13A2 (245), and MPAN segregates with mutations in the open reading frame 12 (c19orf12) of chromosome 19 that encodes an orphan mitochondrial protein (119, 192). SENDA has also been associated with mutations in WD-repeat domain 45 (WDR45), which might lead to a defect in autophagy (377).

Current diagnosis of NBIA disorders is limited to clinical assessment, neuroimaging, and, in some cases, molecular genetic testing. Treatment options are limited at this time, and focus mainly on palliation therapy. A better understanding of the pathogenic mechanisms underlying these disorders is essential for developing viable therapeutic strategies.

V. Mitochondrial Iron Homeostasis and Associated Disorders

The brain consumes ~20% of resting total body oxygen despite comprising only 2% of total body weight, and utilizes a substantial amount of glucose to meet its high metabolic demands. Both oxygen and glucose metabolism occur in the mitochondria and are highly dependent on iron, which serves as an essential cofactor (326). Mitochondria are the site of critical reactions in the pathway, leading to heme synthesis that uses iron as a prosthetic group. Heme is required for the functioning of hemoglobin, is a component of cytochromes abundant in neurons, and paradoxically, can also function as a pro-oxidant (22). Mitochondria are also major generators of iron-sulfur clusters (ISCs) that are essential for the electron transport ability of various proteins (368). Thus, maintenance of iron homeostasis in the mitochondrion is essential for its function and overall cell viability.

A. Iron uptake and utilization in the mitochondria

Iron uptake and utilization by the mitochondria is a tightly regulated because of the ready availability of oxygen that can react with Fe^{2+} to generate hydroxyl radicals (135, 182, 272). Several different, though not mutually exclusive, hypotheses of iron transport to the mitochondrion have been proposed. The prevailing hypothesis suggests that the labile iron pool in the cytosol contributes to the mitochondrial iron content (357, 368). Other studies suggest that Fe^{2+}, once transported out of the endosome, is bound by hydrophobic pockets of chaperone proteins for transport to the mitochondria (369). One such protein is Poly (rC)-binding protein 1 (PCBP1) that transports iron to cytosolic ferritin (398). Additional cytosolic protein chaperones are believed to transport iron to mitochondria in a chelator-impermeable, endosome-independent manner (402). Depending on the cell lineage, however, the specific transport mechanism may vary (396). A third hypothesis proposes the "kiss and run" mechanism of iron delivery to the mitochondria, especially in developing reticulocytes (397). According to this model, endosomes form a close contact with mitochondria to accomplish the transport of iron, bypassing the cytosol. It is likely that Sec15l1, a component of the mammalian exocyst complex involved in TfR-endosome recycling modulates the docking of endosomes to the mitochondria for the transfer of iron (Fig. 11) (269). It is unclear whether this 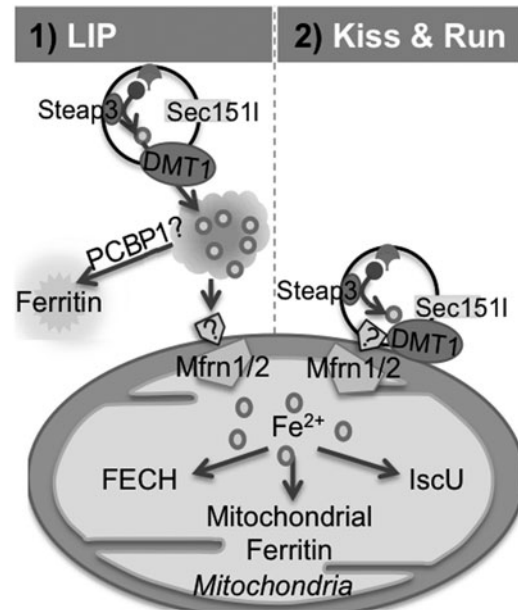mechanism is limited to cells of the erythroid lineage or operates in other cells. As mentioned earlier, iron transport to

FIG. 11. Transport of iron for mitochondrial uptake. Transport of iron to mitochondria occurs by two potential mechanisms: [1] Fe^{2+} from the cytosolic labile iron pool is transported by low-molecular-weight chaperones for delivery to mitochondria, and [2] Tf-iron containing endosomes transiently fuse with the mitochondria for iron delivery. Within the mitochondria, iron is used for heme or ISC synthesis or stored in mitochondrial ferritin. FECH, ferrochelatase; IscU, ISC assembly protein U.

DA neurons of the SN is mediated by an additional mechanism involving TfR2 (298) that shares 45% homology with the extracellular domain of TfR1, but unlike TfR1, does not contain an IRE (223, 436). TfR2 contains an N-terminal mitochondrial targeting sequence and facilitates the delivery of Tf bound iron to this organelle (298).

To access the respiratory chain in the mitochondrial matrix, iron needs to be ferried across the outer and inner mitochondrial membranes (368, 395). The mechanisms underlying iron transport across the outer mitochondrial membrane are not well understood. Transport across the inner membrane is mediated by mitoferrin 1 and mitoferrin 2, the conserved vertebrate homologs of yeast mitochondrial carrier genes Mrs3 and Mrs4 (158, 160, 315). Silencing of mitoferrin 1 and mitoferrin 2 in mammalian cells reduces mitochondrial iron transport and decreases heme and ISC synthesis (338). Mitoferrin 1 functions in erythroid tissues with high iron demands, while mitoferrin 2 is expressed ubiquitously in tissues with lower iron demands (395). Abcb10 is a mitochondrial inner membrane ATP-binding cassette transporter that stabilizes mitoferrin 1 and enhances iron transport to the mitochondria (81). The Abcb10 protein physically interacts with mitoferrin 1 but not with mitoferrin 2 (80, 81). It was additionally shown that ferrochelatase (FECH), the final enzyme in the heme synthesis pathway, can form an oligomeric complex with mitoferrin 1 and Abcb10, thereby bypassing the release of iron in the mitochondrial matrix in favor of a highly efficient direct delivery to heme synthesis enzymes (Fig. 12) (80).
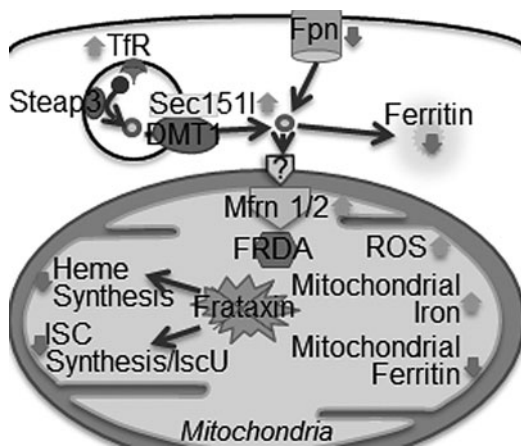
FIG. 12. **Uptake of iron by mitochondria.** Mfrn1 and Mfrn2 are located on the inner mitochondrial membrane transport Fe^{2+} from the inter-membrane space to the matrix, where it is used for heme or ISC synthesis, or stored in mitochondrial ferritin. Fxn participates in heme and ISC-synthesis pathways. Fxn knock-out mice show up-regulation of cellular iron uptake proteins, down-regulation of storage and export proteins, and increased uptake of iron by mitochondria. Heme and ISC synthesis are decreased, as in FA patients. FA, Friedreich's ataxia; Fxn, frataxin.

Within the mitochondrial matrix, iron is used as a catalyst for oxidative metabolism and for the synthesis of heme and ISC proteins (263). Since free iron in the mitochondrial matrix produces ROS, mechanisms are in place to limit the exposure of mitochondrial matrix and inter-membrane space to iron (272). These include targeted delivery of iron to the heme and ISC synthesis complexes by protein chaperones, and storage of excess iron in mitochondrial ferritin (262, 387, 478). Human mitochondrial ferritin is a H-type ferritin encoded by an intron-less gene on chromosome 5q23.1, and translates a 30 kDa precursor protein that matures to a 22 kDa product after cleavage of the N-terminal mitochondrial targeting signal which is rich in positively charged arginine residues (90, 127, 262). Unlike cytosolic ferritin that is rich in organs with iron storage functions such as the liver and spleen, mitochondrial ferritin is highly expressed in the brain, spinal cord, and certain other organs that have high metabolic demands (381). Mitochondrial ferritin forms a homopolymer of 24 subunits that shares a high degree (79%) of sequence homology with cytoplasmic H-ferritin, assembles into shells that bind iron, and demonstrates ferroxidase activity, though the activity is lower than cytosolic H-ferritin chain (51, 90, 262). Over-expression of mitochondrial ferritin results in cytosolic iron deficiency, up-regulation of iron uptake proteins, increased iron import into the cell, and sequestration of iron within mitochondrial ferritin (322). High levels of mitochondrial ferritin in the SN have been reported in cases of Restless-leg syndrome, and could account for the brain iron deficiency reported in these cases (416). Thus, levels of mitochondrial ferritin influence iron metabolism throughout the cell. Unlike cytosolic ferritin, no IRE has been identified in mitochondrial ferritin, and little is known about its regulation (21, 262).

Mitochondria are the key site for the biogenesis of ISCs that function as cofactors for proteins of cellular respiration, DNA replication and transcription, FECH, and regulatory proteins such as IRP1(476). Mitochondrial ISC assembly is a complex pathway in which more than 20 components participate. In brief, ISCs are composed of iron and sulfide ions that form either 2Fe-2S or 4Fe-4S clusters. The human cysteine desulfurase nitrogen fixation 1 homologue (Nfs1) along with an accessory protein Isd11 serves as sulfur donors for the assembly of ISCs (44, 252, 336). The frataxin (Fxn) protein has been implicated in ISC synthesis in addition to other proposed functions (383, 421). ISCs are assembled on the human scaffold protein ISC assembly protein U (IscU), and interactions between IscU, Nfs1, and Isd11 form the core ISC complex, which then interacts with Fxn (387).

The precise mechanism of ISC export from the mitochondria to the cytoplasm has not been established, except that the ATP-binding cassette (ABC) protein ABCB7 is involved (40). ABCB7 shares a high degree of sequence identity with the yeast inner mitochondrial membrane protein Atm1p (400). Deletion of Atm1p in yeast has been shown to result in mitochondrial iron overload (393). In humans, mutations in ABCB7 cause mitochondrial iron overload and X-linked sideroblastic anemia with ataxia (XLSA/A) (11). ABCB7 and Atm1p are also required for the biogenesis of mature cytosolic ISC proteins in humans and yeast, respectively (40, 232, 353). Interestingly, ABCB7 has also been shown to interact with FECH (422). Further research is needed to fully elucidate the details of this interaction.

B. Regulation of mitochondrial iron homeostasis

Irp1 is an ISC-containing protein that functions as a cytosolic aconitase in the presence of an intact ISC (131), and it undergoes a conformational shift which facilitates IRE binding in its absence (448). When iron levels and ISC synthesis are low, transcripts with 5′ IREs such as δ-aminolevulinate synthase 2 (ALAS2), Fpn, and ferritin are translationally repressed; while those with 3′ IREs such as TfR1 and DMT1 are stabilized, allowing for the import of iron to the cytosol and mitochondria (72, 92, 180, 299) (Fig. 6). Irp2 is regulated by the F-box and leucine-rich repeat protein 5 (FBXL5), an oxygen and iron-sensing protein that is a component of the E3 ubiquitin ligase complex. When iron and oxygen levels are low, FBXL5 is targeted for degradation, blocking the formation of E3 ubiquitin ligase complex and thereby causing Irp2 levels to remain high and allow binding to IREs (379, 439). Ablation of Irp2 function in mice causes severe iron dysregulation and a progressive neurodegenerative disease with an average onset of 6 months (255). Mitochondrial dysfunction in these mice is mediated by decreased levels of ISC-containing proteins, which, in turn, results in decreased respiratory chain complex I and II activity (209). Studies have shown that Irp1 function may partially compensate for the lack of Irp2 activity, as mice with a homozygous ablation of Irp2 and heterozygous for Irp1 show a more severe phenotype of neurodegeneration and axonopathy, and activation of Irp1 IRE-binding activity in the Irp2 homozygous mutant mice restores activity of the respiratory chain complex 1 (209).

Export of iron from mitochondria is another important aspect of mitochondrial iron homeostasis. Decreased export of iron as heme or ISCs contributes to mitochondrial iron overload as noted in the Fxn-knockout mouse model (202). Only a small portion of heme and ISCs are used for mitochondrial

metabolism. The rest are exported by unknown transporters for use as cofactors in numerous proteins important for cell physiology. ABC transporters Abcb7 and Abcb10 are expressed on the inner mitochondrial membrane in higher eukaryotes and are essential for iron transfer and homeostasis as well as export of mitochondrial ISC intermediates (40, 319, 353, 401). Targeted knockout of Abcb7 is lethal, implying its essential role in various tissues (353). Deletions of ATM1, the yeast ABCB7 homolog causes a thirty-fold increase in mitochondrial iron accumulation along with reduced activity of cytosolic but not mitochondrial ISC-containing enzymes (231). ATM1 is also required for maturation of cytosolic ISC-containing proteins that could be complemented with Abcb7 in ATM-deficient cells (40, 100, 353). These findings strongly suggest that Abcb7 is a mitochondrial transporter for efflux of ISC intermediates that are important for use in the cytosol (40). Abcb10 participates in heme synthesis, but its precise role in this process is not clear.

Absence or dysfunction of one or more of the proteins involved in mitochondrial iron homeostasis causes systemic and brain disorders, some with serious consequences. Recent advances in our understanding of two such disorders, FA and sideroblastic anemias, are reviewed next.

C. Brain disorders of mitochondrial iron dyshomeostasis

1. **Fxn and Friedriech's ataxia.** Mutations in Fxn result in FA, a disorder that is characterized by severe neurodegeneration and cardiac disease accompanied by iron-sulfur protein deficiency and altered mitochondrial iron metabolism (54, 337, 361). It is a progressive disease with mean age of loss of ambulation at 25 years, causing 95% of patients to be wheelchair bound by the fourth decade (187). Symptoms of FA typically begin to manifest in puberty, but onset of the disease can range from infancy to as late as the fifth decade in atypical presentations (42). Consistent diagnostic findings are limb and truncal ataxia, absent tendon reflexes in the legs, pyramidal tract dysfunction, loss of joint proprioception and vibration sense, and eventual dysarthria (187). Other findings include impaired glucose tolerance or overt diabetes mellitus, as well as cardiac hypertrophy followed by cardiomyopathy, which is a leading cause of death in FA patients (132, 370). In 96% of patients, the underlying cause is expansion of GAA repeats in the first intron of the Fxn gene, resulting in markedly reduced levels of Fxn mRNA (70, 148). This reduction is caused by an unusual structure termed sticky DNA, which sequesters RNA polymerases (43, 70, 378). In addition, gene silencing due to repeat expansions has also been implicated in FA (328). These patients have an average of between 200 to more than 1000 intronic GAA repeat units compared with the normal number of 7–22 units (70, 148). Other cases are heterozygous with GAA expansions in one allele and point mutations in the other allele that result in weak binding to the Nfs1, Isd11, and IscU complex and loss of ISC assembly activities (56, 91, 138).

The human Fxn gene is encoded on chromosome 9q13, and translates a 210 amino acid protein that is targeted to the mitochondria where its transit sequence is cleaved to yield a 17 kDa mature form (69, 240). Fxn is highly conserved from bacteria to humans (166). Crystal structure-based studies of bacterial, yeast, and human Fxn reveal a conserved, nega-

tively charged patch of glutamic and aspartic acid residues that are thought to be involved in cation binding (3, 120). The conserved C-terminal beta sheet structure is important for mediating partner interactions (120, 260, 318). Fxn lacks any canonical structural motif that would provide a membrane anchor, and is associated with the membrane by forming a complex with mitochondrial proteins like FECH (478).

The function of Fxn and consequences of decreased Fxn levels on the pathophysiology of FA have been well studied in animal and cell models. Of these, the function of Fxn as an iron chaperone for ISC and heme synthesis is well supported. Initial evidence for this hypothesis came from findings of decreased ISC-dependent protein activities in FA patients and in yeast and animal models of the disease (54, 314, 361, 375). Subsequent studies have demonstrated interactions between Fxn and the ISC assembly complex (421). Results with the bacterial Fxn ortholog CyaY demonstrate interactions between CyaY and IscS, the bacterial cysteine desulfurase involved in ISC assembly (256, 358, 399). Yeast Fxn homologue Yfh1 is stable as an iron-loaded monomer under physiologic conditions and binds iron at micromolar binding affinity, consistent with the proposed function of Yfh1 as an iron donor (88). Yfh1 also interacts with the mitochondrial scaffold protein Isu1, and can form iron-dependent complexes with Nfs1, and Isu1, a finding reproduced with human Fxn (165, 364, 387). In addition, holo-Fxn but not apo-Fxn forms complexes with IscU, and it stimulates ISC assembly (477). Fxn also interacts with heme-synthesis pathways (195, 478). In the presence of iron, holo-Fxn binds with a higher affinity to FECH than to IscU, indicating a possible regulatory role in allocating iron toward heme or ISC synthesis. This is supported by studies showing down-regulation of Fxn in erythroid cells (39, 478). Yfh1p appears to bind, retain, and transfer Fe^{2+} iron to FECH through protein–protein interactions (339). Fxn has also been shown to transfer Fe^{2+} iron to mitochondrial aconitase, but whether it functions more ubiquitously as an iron donor remains to be characterized (58).

Diminished levels of Fxn lead to impaired ISC biogenesis, decreased respiratory chain function, altered cellular iron homeostasis, and increased oxidative stress (54, 202, 361, 375, 391). At the cellular level, degeneration is detected in the dorsal root ganglia with large sensory neurons (238). Eventual degeneration of sensory axons is seen in peripheral nerves, posterior columns of the spinal cord, corticospinal tracts, and dentate nucleus of the cerebellum ensues (237, 337). Immunocytochemical studies show dysregulation of ferritin, suggesting that depletion of Fpn occurs in the neuropil of the dentate nucleus (237).

The precise mechanism and role of iron in cell dysfunction and death in FA is unknown. Mouse models with complete loss of Fxn expression are embryonic lethal and exhibit no mitochondrial iron accumulation (91). In a conditional FA mouse model, deficits in respiratory chain complexes I–III precede intra-mitochondrial iron deposition in the cardiac tissue (91, 361). In yeast models carrying a deletion of Yfh1, mitochondria show iron accumulation and impaired mitochondrial function (24, 157, 363). Expression of mitochondrial ferritin in models of FA attenuates the pathological phenotype, providing evidence for the causative role of iron in FA pathology (67, 68). In addition, iron chelation therapy shows improvement of neurologic symptoms in some cases of FA, limits cardiac hypertrophy in mouse models, and rescues

human FA fibroblasts from oxidant stress, further suggesting the involvement of iron in the pathogenic process (48, 467, 473). It is believed that accumulation of iron in the mitochondria is due to the deficiency of ISCs rather than absence of Frx *per se*. Sequestration of iron in the mitochondria results in cytosolic iron depletion, further decreasing the transcription of Fxn and creating a negative feed-back loop (266). Thus, therapeutic strategies for FA need to be directed at increasing transport of accumulated iron from the mitochondria to the cytosol and restore iron homeostasis in affected neurons.

2. Sideroblastic anemias. Inherited sideroblastic anemias are a heterogeneous group of diseases that are characterized by iron-laden mitochondria clustered around the nucleus of red cell precursors. These disorders have provided valuable insights into the regulation of iron metabolism in the mitochondria and interactions between the heme and ISC synthesis pathways. A number of mutations responsible for congenital sideroblastic anemias have been characterized, including mutations in Abcb7, ALAS2, the proposed glycine/5-ALA exchanger SCL25A38, and GLRX5, a mitochondrial thiol-disulfide oxidoreductase involved in ISC biogenesis (213).

Mutations in ABCB7 have been described in patients with XLSA/A, a condition of mild sideroblastic anemia with early onset cerebellar ataxia (11, 40, 353). Deletion of ATM1, the yeast ABCB7 homolog, results in reduced activity of cytosolic ISC-containing proteins and increased mitochondrial iron. The activity of mitochondrial ISC-containing proteins, however, is not affected (231). Decreased levels of cytosolic ISC may affect the Irp system, explaining the anemia exhibited in XLSA/A patients. Anemia may also be caused by dysfunction of FECH (102, 334), as evidenced by elevated levels of free protoporphyrin in XLSA/A patients, or by the proposed interactions between ABCB7 and FECH in erythroid cells (422). The pathogenesis of cerebellar ataxia has not been well characterized for this disease, but the ubiquitous role for ABCB7 in iron metabolism may provide an important link to this question.

X-linked sideroblastic anemia (XLSA) is due to a mutation in the heme synthesis mitochondrial enzyme ALAS2 (46, 93). The phenotype of anemia in XSLA is likely due to the restricted expression of ALAS2 in erythroid cells (241). Studies have revealed increased expression of mitochondrial ferritin in the erythroblasts of patients with XLSA (74, 262), which may be either the cause or consequence of cellular iron overloading. Previous experiments have shown that elevated levels of mitochondrial ferritin sequester iron away from cytosolic ferritin and result in up-regulation of TfR levels (90). Expression of mitochondrial ferritin in yeast models of FA, and in fibroblasts from FA patients attenuates the pathological phenotype, supporting this hypothesis (67, 68). Mitochondrial ferritin is not expressed at high levels in normal erythroid cells, and its regulation is not well understood (262). Future studies of XLSA and associated changes in mitochondrial ferritin will elucidate the mechanisms underlying the pathobiology of sideroblastic anemias.

VI. Yeast as Models of Neurodegenerative Conditions

Many cellular and biochemical processes are highly conserved from unicellular yeast to humans. The ease of genetic manipulations in the budding yeast *S. cerevisiae* provide a simple and experimentally manipulable model for understanding complex cellular and biochemical processes that are difficult to model in higher eukaryotes (179, 250, 472). The model yeast *S. cerevisiae* has provided important information on the biochemical and genetic basis of several human disorders, including AD, PD, prion disorders, and HD (297, 305, 435, 468, 469). Several factors make yeast a convenient model for investigations pertaining to brain iron homeostasis and dyshomeostasis: (i) pathways regulating iron metabolism are highly conserved between yeast and humans; (ii) iron transport pathways are well characterized in *S. cerevisiae*, and the ease of genetic manipulation provides an opportunity for identifying downstream targets that are unapproachable in higher eukaryotes; and (iii) yeast growth conditions are well defined and can be altered to monitor the effect of metal ion concentrations. However, several limitations need to be considered while interpreting data from yeast models. *S. cerevisiae* is a unicellular organism and is, therefore, unable to recapitulate the cross-talk among cells as in multicellular organisms. Proteins involved in many of the mammalian disorders such as prion and Alzheimers' disorders are membrane associated, and unlike neurons, the plasma membrane *S. cerevisiae* is surrounded by a cell wall. Thus, biology at membranes could differ in *S. cerevisiae* and neuronal cells. Unlike neurons, yeast replicate and it is believed that asymmetric distribution during cytokinesis sequesters potentially toxic protein species in mother cells, generating healthier daughter cells. Thus, the protective mechanisms in yeast could differ from neuronal cells (5). In addition, yeast lack many of the components of protein quality control machinery present in mammalian cells such as the Hsc70 partner Carboxyl terminus of Hsp70-interacting protein (CHIP) that bridges Hsc70 to the proteasomal machinery. Thus, results obtained using yeast models need to be validated in higher eukaryotic models such as neuronal cells and animal models. Despite these limitations, the fundamental biological processes are highly conserved between yeast and mammalian cells, providing a convenient tool to rapidly screen components of the cellular machinery as well as chemical compounds to identify targets against several human disorders, including AD, PD, HD, and CJD (Fig. 13).

A. Iron metabolism in S. cerevisiae

As for most organisms, iron is an essential element for yeast growth, and exists primarily in the Fe^{3+} form to prevent interaction with available oxygen. For cellular utilization, the more soluble Fe^{2+} iron is preferred. Thus, yeast cells have two distinct reductive and non-reductive pathways for iron transport. The reductive pathway is facilitated by transcriptionally regulated FRs Fre1p and Fre2p present on cellular membranes that reduce Fe^{3+} to Fe^{2+} for further uptake by a low or high affinity pathway (15, 103). The low-affinity pathway is utilized when iron is abundant in the growth media. Due to its poor specificity, the low-affinity pathway also mediates the transport of other metals such as copper. The low-affinity pathway for direct uptake of Fe^{2+} is mediated by *FET4* or *SMF1* gene products (82, 123, 454). Smf1 mediated iron uptake is proton dependent. The high-affinity pathway is negatively regulated by iron abundance and is specific for iron uptake. The high-affinity iron uptake is induced on iron deprivation in growth media and is mediated
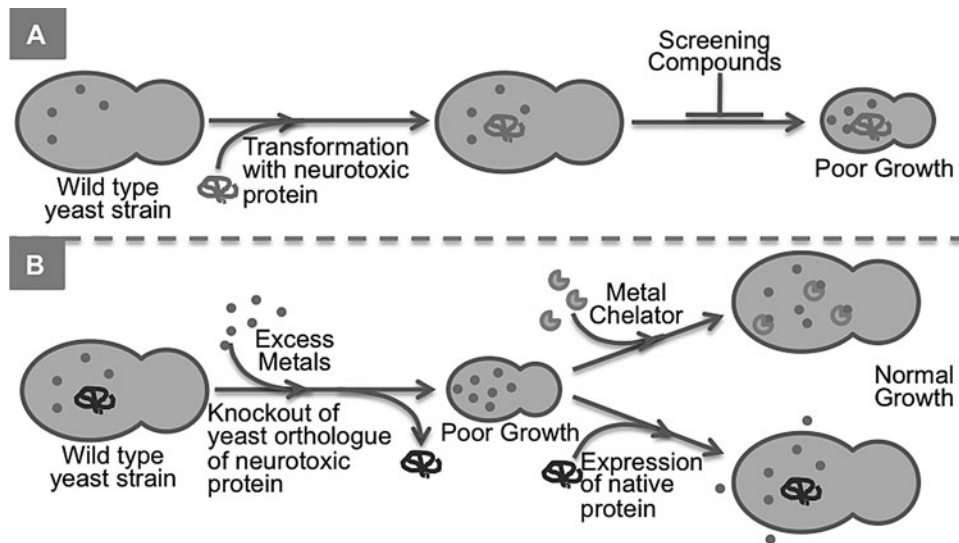
FIG. 13. Schematic describing yeast as model organisms for the study of metal dyshomeostasis in human neurodegenerative disorders. (A) To construct a yeast model of a specific neurodegenerative disease, the disease-associated human gene is overexpressed in yeast and examined for its pathological effects. If expression is toxic to yeast growth, a suppressor screen using chemically synthesized compounds is carried out. Obtained suppressors are further analyzed to understand the underlying mechanism. **(B)** The yeast orthologue of disease-associated protein is knocked out, and the growth medium is supplemented in various metal ions to assess the effect on growth. Normal growth is restored on incubation with specific rescuing compounds or by restoration of the native protein expression in the knockout strain. *PARK9* metal specificity was explored using a similar strategy. Figure adapted from Froschauer *et al.* (160).

by the ferroxidase Fet3p and permease Ftr1p (109, 419). The ferroxidase Fet3p catalyzes the oxidation of Fe^{2+} to Fe^{3+}, which is then further transported into the cell by Ftr1p permease. Both Fet3p and Ftr1 are transcriptionally regulated by the transcription factor Aft1p and localize to cellular membranes only when both proteins are present in the cell (71).

The non-reductive pathway involves internalization of the Fe^{3+}-siderophore complex. Siderophores are low-molecular-weight organic compounds with high affinity for iron and are secreted by many microorganisms. Due to their ability to chelate iron, siderophores solubilize otherwise insoluble Fe^{3+}, facilitating transport across the plasma membrane. *S. cerevisiae* itself does not synthesize siderophores, but has the ability to use siderophores secreted by other microbes. Plasma membrane permeases belonging to the major facilitator superfamily mediate iron-siderophore uptake. The four distinct facilitators in *S. cerevisiae* are Arn1p, Arn2p (Taf1p), Arn3p (Sit1p), and Arn4p (Enb1p) (47).

B. S. cerevisiae *as a model of brain disorders of iron dyshomeostasis*

1. *Parkinson's disease.* Yeast models of PD-associated α-synuclein toxicity are among the most established models of neurodegenerative disease. Though a clear homolog of α-synuclein has not been identified in yeast, heterologous overexpression of human α-synuclein causes its aggregation in *S. cerevisiae* and suppresses growth, recapitulating familial forms of PD associated with duplication and sometimes triplication of the gene encoding wild-type α-synuclein (333). Likewise, mutant forms of α-synuclein (E46K and A53T) cause increased toxicity in yeast as in cell models, reproducing certain aspects of familial PD. Important neurotoxic processes

of PD are recapitulated by yeast models such as defects in mitochondrial biogenesis, protein homeostasis, vesicular trafficking, and increased sensitivity to oxidative stress (333, 394). Though limited in scope at the present time, these models have helped in the identification of genetic suppressors of α-synuclein toxicity, belonging mainly to the ER-to-Golgi transport proteins.

Yeast models have also been successful in understanding the correlation between environmental risk factors and PD pathogenesis. Regulated expression of α-synuclein-GFP chimeras under the control of galactose-inducible promoter has been instrumental in identifying modifiers of α-synuclein toxicity (333). Thus, metals such as iron, zinc, and manganese induce aggregation of α-synuclein and cytotoxicity, presumably due to oxidative stress (176). Yeast cells grown in the presence of excess Fe^{3+} induce aggregation and aggravate the toxicity of wild-type and pathogenic mutations of α-synuclein, while iron chelators and ROS-quenching agents are protective, providing a model that mimics mammalian cell culture models, and yet is manipulable at the genetic level to enable identification of gene products involved in toxicity and the rescue of this phenotype. A suppressor screen of ∼10,000 compounds yielded two compounds that reduced $FeCl_3$-mediated toxicity. Interestingly, these compounds were flavonoids that are known to possess anti-oxidant and metal-chelating properties (176).

A clear example of the versatility of this model system is the functional characterization of PARK9 as a manganese transporter. The human gene PARK9 encoding a P-type transmembrane cation-transporting ATPase (ATP13A2) is linked to early onset-Parkinsonism. This hereditary form of PD is caused by homozygous mutations in PARK9 (122, 365). The role of wild-type ATP13A2 and its disease causing mutants

has primarily been deduced from yeast models using the *PARK9* ortholog *YPK9*, a transmembrane cationic metal transporter in *S. cerevisiae* (169). The substrate specificity of Ypk9 was explored by growing wild-type and ypk9Δ yeast strains in the presence of a wide range of metal ions, including cadmium, calcium, cobalt, Fe^{3+}, zinc, and copper, and metal chelators EDTA, EGTA. The *ypk9Δ* strain was more sensitive to manganese, and the sensitivity was reversed on over-expression of wild-type Ypk9 but not mutant Ypk9 carrying the disease-associated mutation. It is likely that mis-metabolism of manganese leads to downstream events leading to early onset PD.

2. Prion disorders and HD. As discussed earlier, mammalian prion disorders result from the conformational transition of PrP^C to PrP^{Sc}. Since *S. cerevisiae* does not encode a PrP orthologue, attempts have been made to establish yeast models by expressing mammalian PrP in yeast. However, until now, there are no well-established yeast models of PrP that recapitulate PrP associated toxicity as seen in mammalian cells. Similarly, yeast models expressing PrP have been unable to recapitulate the role of PrP in iron or copper metabolism as seen in higher eukaryotic models (265), and iron metabolism has not been thoroughly investigated.

Yeast models of HDs have been constructed by expressing huntingtin protein fragment containing polyQ stretch of varying lengths (130, 302). As observed in neurons, length of the glutamine expansion determines toxicity in yeast. Significant toxicity is generally seen when the polyQ stretch exceeds 72 glutamines. Though not extensively studied, compounds analogous to clioquinol (CQ), a weak metal chelator of the hydroxyquinoline family, show protection against polyglutamine toxicity, suggesting a role of metals, primarily Fe^{3+}, Cu^{2+}, and Zn^{2+} in HD pathogenesis (424).

3. Fxn toxicity. *S. cerevisiae* encodes the Fxn homolog Yfh1p, which, similar to Fxn, is involved in ISC biosynthesis (12, 58, 339, 392). Deletion of Yfh1 causes iron accumulation in mitochondria, reduced cell growth, and increased sensitivity to H_2O_2. Reconstitution of Yfh1 expression restores cell growth on non-fermentable carbon source, clarifying the role of Yfh1 in iron metabolism (73). Moreover, wild-type Fxn complements yfh1 deletion while FA-associated mutations fail to do so, suggesting that Fxn is involved in mitochondrial iron homeostasis, and disease-associated mutations cause accumulation of iron in mitochondria and oxidative stress. Observations on Fxn mouse models support these findings (466).

VII. Therapeutic Options

The potential for iron to cause oxidative stress and protein aggregation is well established, and appropriate chelation provides an opportunity to alleviate this component of the pathogenic process (162). When considering chelation for clinical application, factors such as efficacy *versus* toxicity, route of administration, and transport across the BBB need to be considered. An ideal iron chelator, for instance, should bind iron in a nontoxic metal complex, have appreciable lipid solubility to allow for penetration of the BBB and inner mitochondrial membrane, and be uncharged and of small molecular size (<500 KDa) to facilitate membrane diffusion

(161). Some studies suggest that an iron chelator should have good oral bioavailability and have even smaller size (<300 Da); however, the main delivery methods with proven effectiveness so far have been through the intra-venous route (483). It would also be preferable for iron chelators to selectively bind Fe^{3+}, as chelators that prefer Fe^{2+} can also bind other divalent metals such as copper and zinc and inhibit important metalloenzyme activity (465).

Generally, iron chelators can be classified into four categories: desferrioxamine (DFO), 8-hydroxyquinolone (8HQ) analogs, prochelators, and aroylhydrazones. DFO is a hexadentate ligand with a higher binding affinity for Fe^{3+} than Fe^{2+}. It has been traditionally used to treat β-thalassemia to minimize iron overload from chronic blood transfusions. Some studies have shown that DFO also prolongs life in AD mouse models (105). In AD, DFO is believed to reduce Aβ levels by disrupting the iron-mediated up-regulation of APP through its 5'- UTR, and by stabilizing the transcriptional activator hypoxia-inducible factor-1α (HIF-1α) (458). The stability of HIF-1α is under the control of a class of iron-dependent and oxygen-sensing enzymes, HIF prolyl-4-hydroxylases that target it for degradation. Prolyl-4-hydroxylases contain non-heme Fe^{2+} in their catalytic center, and are able to extract iron from the intracellular chelatable labile iron pool as a cofactor (458). Iron chelation can inhibit the action of prolyl-4-hydroxylases and stabilize HIF-1α, increasing expression of target genes that include Tf, TfR1, and other survival genes (23). Iron chelation may also benefit PD patients (344). Thus, DFO pretreatment in PD mouse models of 6-OHDA, MPTP, or proteasome inhibitor show beneficial results (458). However, DFO is highly hydrophilic, making its oral bioavailability and BBB penetration extremely low and necessitating parenteral administration. Due to its half life of 12 min, patients on DFO therapy should undergo subcutaneous infusions from 8–12 h approximately 7 times a week (351, 430, 437). A recent study, however, highlights the possibility of intranasal DFO administration, which may improve patient compliance (181). Deferiprone and deferasirox are two drugs with iron-chelation activity that are designed to overcome the hydrophilic and poor BBB penetration of DFO. They have been approved by the FDA for treatment of secondary iron overload and have been demonstrated to reduce ataxia and neuropathy in FA, but are difficult to manage in FA because some of the patients develop anemia (48). However, current clinical trials and studies show promising results for AD and PD (115, 156, 440).

CQ is an analog of 8HQ, and confers metal chelation properties by forming stable 5-membered chelate rings with Fe^{3+} (347). CQ is small, lipophilic, and can freely traverse the BBB. However, large quantities can induce neurotoxicity as evidenced by the development of sub-acute myelo-opticoneuropathy in a group of Japanese patients in the 1960s (136). It is also able to form complexes with copper and zinc that play a significant role in plaque and amyloid formation in AD (453, 465). Recent studies have demonstrated reduction in the number and size of zinc-containing plaques in the brains of AD mouse models (APP/presenilin 1 double transgenic mice), as well as decreased expression of APP, BACE1, and presenilin-1 after CQ treatment (453). Thus, CQ has the potential to reduce neuritic plaque formation and oxidative stress in AD and other neurodegenerative disorders of metal imbalance. The drawback of possible CQ-induced neurotoxicity has led to the development of other CQ analogs that

include VK28, HLA-20, and M30. VK28 is an antioxidant-iron chelating agent with the 8HQ moiety that protects against MPTP- and 6-OHDA-induced lesions in rat brains (14, 267). HLA-20 and M30 are hybrid drugs that combine the N-propargyl moiety found in rasagiline and selegiline (MAO-B inhibitors used to treat PD) with an 8HQ moiety (458). It is of note that MAO inhibitors inhibit oxidation of DA by MAO-A and MAO-B, thus preventing the formation of hydrogen peroxide generation and hydroxyl radicals and associated neuronal damage. Compared with CQ, HLA-20 and M30 have a higher binding affinity for iron than copper and may have less cross-metalloenzyme effects (344). The multimodal functions of HLA-20 and M30 make them more effective than previous "silver-bullet" iron chelators. M30, for instance, has been shown to up-regulate HIF-1α expression, attenuate tau phosphorylation, protect cultured cortical neurons from Aβ toxicity, and prevent lipid peroxidation (23, 247) (Table 2).

Prochelators are molecules that can be converted into chelators under certain circumstances such as oxidative stress. The advantage of this model compared with other iron chelators is the ability to concentrate its activity at sites of damage (77). Two prominent prochelators, SIH-B and BSIH, as well as a few other derivatives have recently come to light. The parent molecule, salicylaldehyde isonicotynoyl hydrazine (SIH), is a lipophilic tridentate chelator (282). SIH-B does not bind iron or copper strongly, but is converted to SIH after exposure to hydrogen peroxide, which can then bind iron and copper. Similarly, BSIH interacts weakly with iron unless hydrogen peroxide removes the boron-ester-protecting group to reveal SIH (282). Thus, SIH-B and BSIH prevent the generation of harmful hydroxyl radicals from hydrogen peroxide and the formation of ROS from iron (76). Another derivative BHAPI (based on metal chelator HAPI, which possesses an isonicotynoyl backbone) has been shown to bind divalent and trivalent metal ions when converted to its chelator form (228). A prochelator termed SWH is enzymatically activated by β-secretase, which is up-regulated in AD, to produce a high affinity copper chelator that sequesters copper from Aβ, preventing copper-induced Aβ aggregation, and decreasing copper-promoted ROS formation (155). Thus, prochelators can be targeted to multiple pathways of disease pathogenesis.

The first aroylhydrazones were derived from pyridoxal isonicotinoyl hydrazone, highly effective and lipophilic molecules that prevent iron uptake while mobilizing intracellular iron (268). Subsequently, new iron chelators known as the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogs were developed. Initial studies revealed that isonicotinoyl hydrazone was not a selective iron chelator and instead, formed a more stable complex with copper (268, 344). This led to subsequent derivatives, of which 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH) was the most effective (465). Experiments conducted on FA fibroblasts treated with PCTH revealed significant improvement in cell viability (70%) when compared with cells treated with DFO (1%–5%) (268). The effectiveness of PCTH in rescuing cells from hydrogen peroxide-induced cytotoxicity could be attributed to its more efficient rate of penetration. In FA, iron accumulation occurs in the mitochondria, and chelating agents should cross both the outer and inner mitochondrial membrane to have noticeable effects. With its highly lipophilic nature and rapid penetration ability, PCTH is a promising candidate for FA treatment (Table 2).

Plant polyphenols are a class of naturally occurring, non-toxic compounds that exhibit metal chelating, anti-inflammatory, radical scavenging, and neuroprotective effects. Green tea is high in catechin, a subclass of polyphenols, with epigallocatechin gallate (EGCG) comprising more than two-thirds of the catechin content (459). EGCG has been shown to protect against hydrogen-peroxide-induced toxicity in astroglial cells and to alleviate mitochondrial oxidative stress (2, 386, 390). There are conflicting opinions on the effect of polyphenols on transcription factor NF-E2-related factor 2 activation that up-regulates ferritin (386, 390). High consumption of green tea, as shown by a cross-sectional Japanese study, has a neuroprotective function and slows the progression of AD (249, 459). Several epidemiological studies have confirmed the moderate risk reduction in tea drinkers compared with non-tea drinkers (411, 459). The neuroprotective effects of tea are believed to stem from the flavanol class-related catechins and their derivatives, which possess antioxidant, radical scavenging, and transcription activating capabilities. EGCG has been shown to rescue DA depletion and SN neuronal loss in MPTP-induced PD mouse brains, possibly through its catechol-like structure that can act as a potent anti-oxidant and iron chelator (411). Its effect in AD is still unclear, but in vitro studies have demonstrated that EGCG destabilizes Aβ aggregates, prevents elongation of fibrils, and prevents Aβ-induced neurotoxicity. In addition, EGCG promotes the non-amyloidogenic pathway through up-regulation of α-secretase, suggesting a promising therapeutic option for AD (459). Interestingly, however, some studies have found that tea catechins function as pro-oxidants at high concentrations or in the presence of copper ions, necessitating further research to fully characterize this compound. While EGCG is the main focus of plant polyphenol research, other compounds such as ethyl ferulate have been shown to induce heme oxygenase-1 and antioxidant activity (385).

Other naturally occurring compounds include curcumin, rosmarinic acid, and the wine polyphenols resveratrol, myricetin, quercetin, and kaemferol. Curcumin is a major compound found in the Indian spice turmeric and has been shown to decrease the concentration of free Fe^{3+} in thalassemia plasma. The beta-diketo moiety of curcumin is likely its iron-binding motif. Studies in pheochromocytoma PC12 cells have shown that curcumin increases cell viability against MPTP induction (212). Similarly, rats injected with 6-OHDA benefit from curcumin treatment. However, other reports disagree with this conclusion, as 1–4 g/day of curcumin for 6 months was unable to improve cognitive performance or reduce biomarkers of inflammation in AD patients (37). In addition, since curcumin interferes with drug metabolism, it should be used with caution (289, 291). Wine polyphenols, especially resveratrol, have come under scrutiny of late due to its proposed neuroprotective effects. Resveratrol is a natural polyphenol found in many plants and, in particular, the skin of red grapes. While several studies have highlighted the role of resveratrol as anti-amyloidogenic and fibril-destabilizing, there is still dispute on whether these properties can fully account for its neuroprotective effects (173). There is wide consensus, however, that resveratrol possesses antioxidant activity, scavenges free radicals, and prevents toxicity-mediated through Aβ and Aβ-metal complexes (173). Diketopiperazine is another compound that is believed to be

TABLE 2. MAJOR CLASSES OF CHELATORS

Chelator	Properties	Mechanism of action	Clinical studies
DFO and analogs			
DFO	Hexadentate chelator hydrophilic, poor BBB penetration, short half-life (8–12 min)	Up-regulates HIF-1α by inhibiting PHDs (458), down-regulates APP *via* 5'-UTR (373, 441, 458), down-regulates α-synuclein *via* 5'-UTR (143)	DFO treatment may slow the rate of decline in AD patients by as much as twofold (300).
DFP	Bidentate chelator, lipophilic, high BBB penetration, iron-DFP complex is hydrophilic	Reduces Aβ40, Aβ42, and BACE1 levels in the brain (356), decreases phosphorylation of tau but not ROS formation or brain iron levels (356).	Approved by FDA for treatment of secondary iron overload, phase II trials for treatment of PD undergoing (115, 156), open-label single-arm study for treatment of FA using combination therapy of idebenone and DFP (440).Treatment of FA adolescents with DFP reduced neuropathy and ataxic gait (48).
DFX	Tridentate chelator, high bioavailability, long half-life (8–16h)	Chelates iron in 2:1 DFX:iron ratio	Approved by FDA for treatment of secondary iron overload
8-HQ analogs			
CQ	Lipophilic, high BBB penetration, may induce SMON (136)	8HQ moiety forms 5-member chelate rings with Fe^{3+}, forms complexes with copper and zinc: reduces plaque formation in AD (75, 453, 474), decreases levels of interstitial Aβ (4), down-regulates APP, BACE1, and presenilin 1 (453, 474)	Pilot phase II trial shows significant decrease in AD plasma Aβ42 after CQ treatment (371).
PBT2	Lipophilic, high BBB penetration	Inhibits redox activity *via* metal chelation (146), neutralizes Aβ toxicity by promoting clearance from existing deposits (99)	Phase IIa trial shows significant decrease in AD CSF Aβ42 following PBT2 treatment (141)
VK28	Contains 8HQ moiety	Protects against MPTP and 6-OHDA induced lesions in rat brains (14)	
HLA-20 and M30	Contains N-propargyl moiety, contains 8HQ moiety	Prevents ROS generation by inhibiting MAO (458), up-regulates HIF-1α (23), and attenuates tau phosphorylation (23, 247).	
Prochelators			
SIH-B BSIH	Parent molecule SIH, lipophilic, tridentate chelator	SIH-B is converted to SIH after exposure to hydrogen peroxide, which can then bind iron and copper (77, 282), BSIH is converted to SIH after removal of boron ester by hydrogen peroxide (76, 77, 282)	
BHAPI	Isonicotynoyl backbone, binds trivalent and divalent metal ions	BHAPI is converted to HAPI after reaction with hydrogen peroxide or paraquat (228).	
SWH	Lipophilic, contains metal chelating and amyloid binding moieties	SWH is converted to copper chelator CP after enzyme modification by β-secretase (155). Inhibits Aβ aggregation (155).	
Aroylhydrazones			
PCIH	PIH analog, lipophilic, forms more stable complex with copper than iron (18, 268)	Induces iron mobilization and prevents iron uptake from Tf (268, 475), permeates mitochondrion to induce iron release (475)	
PCTH	PCIH analog, high oral bioavailability	Faster membrane penetration than DFO or PCIH (268), able to penetrate inner and outer membrane of mitochondria, and prevents hydrogen peroxide-induced cytotoxicity (268)	

6-OHDA, 6 hydroxydopamine; 8HQ, 8-hydroxyquinolone; Aβ, amyloid β; AD, Alzheimer's disease; APP, amyloid precursor protein; BBB, blood brain barrier; CSF, cerebrospinal fluid; CQ, clioquinol; DFO, desferrioxamine; DFP, deferiprone; DFX, deferasirox; FA, Friedreich's ataxia; Fe^{3+}, ferric iron; HIF-1α, hypoxia-inducible factor-1α; PCTH, 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone; PCIH, 2-pyridylcarboxaldehyde isonicotinoyl hydrazine; PD, Parkinson's disease; PHD, prolyl-4-hydroxylases; ROS, reactive oxygen species; SIH, salicylaldehyde isonicotynoyl hydrazine.

neuroprotective, and has shown beneficial effects in animal models of PD (242).

The development of therapeutic options for the treatment of CJD and other prion disorders has been limited by the lack of knowledge concerning PrP^C and its role in metal homeostasis, and the contribution of PrP^{Sc} in disturbing brain iron metabolism. In the absence of a clear understanding of the mechanism of neurotoxicity by PrP^{Sc}, the current target of therapy involves blocking the conversion of PrP^C to PrP^{Sc}. Toward this end, PrP autoantibodies have been shown to block PrP^{Sc} formation, possibly by downregulating PrP expression (456). It is also suggested that manganese plays a role in disease pathogenesis by facilitating the refolding of normal PrP^C to PrP^{Sc} and promoting subsequent aggregation; this is supported by higher levels of manganese in prion-infected brains as well as evidence of PrP^{Sc} reduction in mice treated with the manganese chelator $Na_2CaCDTA$ (55). Likewise, a modest delay in disease onset has been observed in scrapie-inoculated mice treated with the copper chelator D-penicillamine (404). Although metal imbalance appears to trigger the conversion of normal prion protein to its scrapie isoform, it is unclear whether chelation can modify its metal ion occupancy and revert PrP^{Sc} to its α-helical rich form.

Lipid peroxidation and decreased levels of SOD in prion-infected animals and cell cultures implicates oxidative stress in disease pathogenesis (167). Contributing factors are increased concentration of total iron in the brains of scrapie-infected mice, and the ability of an internal fragment of PrP ($PrP^{106-126}$) to promote hydroxyl-radical formation through Fenton chemistry in the presence of copper (162). Loss of the antioxidant SOD-like activity of PrP^C due to aggregation to the PrP^{Sc} form may also contribute to the overall state of oxidative stress in diseased brains. This is likely to be compounded by reduced iron export due to reduction of microtubule affinity regulating kinase-4 that decreases the phosphorylation of tau (170), thereby destabilizing tubulin and impairing the transport of APP to the plasma membrane (259). Thus, therapies targeted at restoring brain iron homeostasis and reducing oxidative stress in prion-infected brains may prove useful.

VIII. Conclusions

Iron is a well-known initiator of toxic hydroxyl-radicals, necessitating intricate mechanisms of uptake, utilization, and storage in systemic organs and the brain. Regulation of iron homeostasis in systemic organs has become increasingly clear over the past few years, and has provided important information on the transport of iron across the BBB and regulation within the brain. Although most iron-modulating proteins are synthesized locally in the brain, their functional similarity has helped in understanding their role in the complex environment of the brain. Unlike most systemic organs, the brain is unique in its region- and cell-specific distribution of iron. Moreover, the mechanism of iron uptake, storage, utilization, and vulnerability to changes in the intra- and extracellular environment is specific to each cell type within the brain. For example, astrocytes are more resistant to toxicity by iron compared with neurons and brain vascular endothelial cells, and may even provide transient protection to the neurons. Cell-specific expression of certain iron-modulating proteins has clarified these observations and the role of different cell types in maintaining brain iron homeostasis. Absence of certain proteins, such as Cp, results in iron accumulation and associated pathology in systemic organs as well as the brain. In others, such as hereditary ferritinopathy, the disease is limited to the brain; while in still others such as hemochromatosis, iron accumulation is mainly in systemic organs. The cause of organ- and cell-specific accumulation of iron and associated pathology is clear for some of these disorders; while for others, additional information on functional interaction among different iron-modulating proteins and overall maintenance of iron homeostasis within the brain is necessary. Nevertheless, these disorders provide proof of concept that excess iron in the brain is neurotoxic, especially as unlike serum Tf that is ~30% saturated, brain Tf is ~100% saturated with iron, and has minimal buffering capacity when faced with excess iron.

For neurodegenerative conditions such as AD, PD, sCJD, and HD, the underlying cause of brain iron dyshomeostasis is not entirely clear. The debate over whether iron is the cause or consequence of disease pathogenesis, therefore, continues.
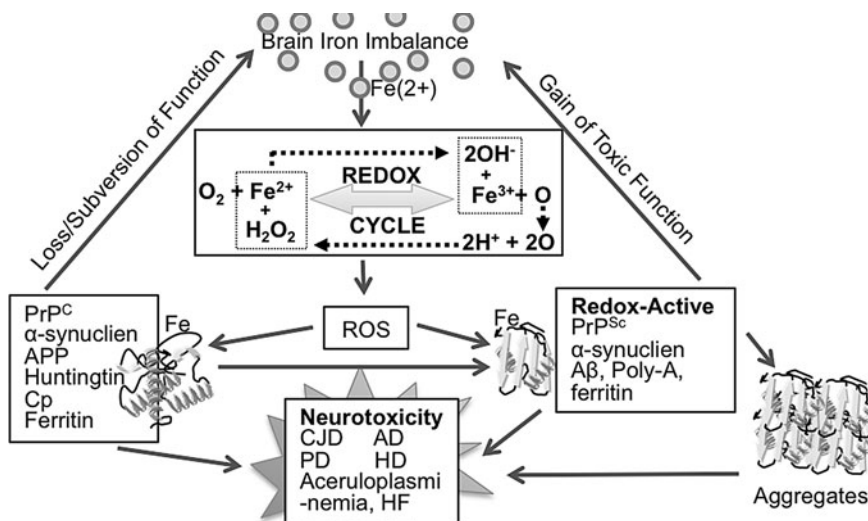
FIG. 14. **Brain iron dyshomeostasis in neurodegenerative conditions.** Excess Fe^{2+} iron in the brain leads to the generation of ROS, resulting in the aggregation of certain proteins. Some of these proteins acquire pro-oxidant characteristics. Neuronal death ensues due to the combined effect of loss of function of these proteins in iron metabolism and gain of toxic function by redox-active protein aggregates. AD, Alzheimer's disease; CJD, Creutzfeldt-Jakob disease; HD, Huntington's disease; PD, Parkinson's disease; PrP^C, prion protein; PrP^{Sc}, PrP-scrapie.

However, cumulative evidence implicating key proteins involved in the pathogenesis of these disorders in iron metabolism favors the former possibility. The presence of 5′ UTR sequences in the APP and α-synuclein transcripts, the activity of APP as a ferroxidase, α-synuclein and PrP^C as a FR, the functional role of PrP^C in iron transport, and sequestration of iron in PrP^{Sc}-protein complexes leaves little doubt that iron plays a significant role in the neurotoxicity associated with AD, PD, and sCJD. The accuracy of CSF ferroxidase activity and Tf in distinguishing sCJD from AD and other dementias suggests that the underlying cause of iron mis-metabolism is distinct in these disorders. Accumulated iron may induce direct neurotoxicity, or increase the vulnerability of affected neurons to toxic insults. The phenotype of iron deficiency in the SN of PD brains and neurons of sCJD cases despite accumulation of iron in these areas and consequent up-regulation of iron import proteins DMT1 and TfR1, respectively, suggests mis-regulation of the iron homeostatic machinery, emphasizing the underlying complexity (Fig. 14). Thus, chelation of iron alone is unlikely to ameliorate the neurotoxicity associated with these disorders. It is, therefore, imperative to understand the cause of iron mis-metabolism in these conditions, and device therapeutic strategies to restore brain iron homeostasis and halt the ongoing state of oxidative stress.

Several gaps in our understanding of brain iron metabolism have hampered progress toward this goal. For example, it is unclear how the iron transport machinery at the BBB senses brain iron levels and regulates import of iron from systemic circulation to the brain. The cross-talk between different cell types and pathways regulating brain iron levels at the inter-cellular level are still unclear. Thus, the neurotoxic signals generated by the absence of an astrocyte specific ferroxidase Cp are difficult to understand except for the general hypothesis of oxidative stress and lack of trophic support due to degenerating astrocytes. Likewise, the functional role of ferritin as an iron transport protein is unclear. Receptors for ferritin have been identified in endothelial cells lining the BBB and in oligodendrocytes, and radiolabeled ferritin is absorbed by rat intestinal segments (294, 426). However, further studies are necessary to elucidate the functional role of ferritin in brain iron regulation. Since ferritin is rich in iron, is fairly resistant to degradation by proteases, and co-aggregates with denatured proteins such as PrP^{Sc}, it is likely to influence brain iron levels significantly. The role of other iron-modulating proteins such as Fpn, Hp, and hepcidin or a hepcidin-like molecule in maintaining brain iron homeostasis is still emerging.

A complete understanding of these pathways and their disruption by pathogenic processes involved in various brain disorders is necessary to understand the cause of iron mis-metabolism in familial and sporadic neurodegenerative disorders associated with iron imbalance. The initial trigger of iron accumulation or deficiency is likely to vary with each condition. The subsequent cascade of events resulting in the generation of ROS and the ensuing self-enhancing vicious cycle is likely to be similar among the various brain disorders. Differences among the brain regions and cell types involved in each disorder probably reflect the proteins and biochemical pathways involved in a specific disorder. Thus, a common strategy of iron chelation is unlikely to succeed as a therapeutic strategy for all disorders associated with brain iron imbalance, though it may thwart the neurotoxicity caused by redox-active iron to some extent. However, iron chelators have provided neuroprotection for some brain disorders. Continued efforts at understanding the cause of iron imbalance and search for drugs that restore brain iron homeostasis are necessary to identify therapeutic drugs which can benefit the increasing population suffering from neurodegenerative and other disorders associated with iron imbalance.

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Abbreviations Used

6-OHDA	= 6 hydroxydopamine
8HQ	= 8-hydroxyquinolone
Aβ	= amyloid β
ABCB7	= ATP-binding cassette (ABC) protein
AD	= Alzheimer's disease
ALAS2	= δ-aminolevulinate synthase 2
APP	= amyloid precursor protein
ATP13A2	= ATPase
BBB	= blood brain barrier
CJD	= Creutzfeldt-Jakob disease
Cp	= ceruloplasmin
CSF	= cerebrospinal fluid
CQ	= clioquinol
DA	= dopaminergic
Dcytb	= duodenal cytochrome b
DFO	= desferrioxamine
DMT1	= divalent metal transporter 1
EGCG	= epigallocatechin gallate
FA	= Friedreich's ataxia
FAHN	= fatty acid hydroxylase-associated neurodegeneration
Fe^{2+}	= ferrous iron
Fe^{3+}	= ferric iron
FECH	= ferrochelatase
FLVCR	= feline leukemia virus subgroup C. receptor
Fpn	= ferroportin
FR	= ferrireductase
Ft-H	= ferritin H-chain
Ft-L	= ferritin light chain
Fxn	= frataxin
HD	= Huntington's disease
HFE	= hereditary hemochromatosis gene product
HIF-1α	= hypoxia-inducible factor
HO-1	= heme-oxygenase-1
Hp	= hephaestin
IREs	= iron-responsive elements
Irp1/Irp2	= iron regulatory proteins 1 and 2
ISCs	= iron-sulfur clusters
IscU	= ISC assembly protein U
LDLR	= low density lipoprotein receptor
LRRK2	= leucine rich repeat kinase-2
LTF	= lactotransferrin
MPAN	= mitochondrial membrane protein associated neurodegeneration
MPTP	= 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NBIA	= neurodegeneration with brain iron accumulation
Nfs1	= nitrogen fixation 1 homologue
NFT	= neurofibrillary tangles
NTBI	= non-transferrin bound iron
PCIH	= 2-pyridylcarboxaldehyde isonicotinoyl hydrazine
PCTH	= 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone
PD	= Parkinson's disease
PHD	= prolyl-4-hydroxylases
PKAN	= pantothenate kinase-associated neurodegeneration
PLAN	= phospholipase A2-associated neurodegeneration
PrP^C	= prion protein
PrP^{Sc}	= PrP-scrapie
PINK1	= PTEN-induced putative kinase 1
ROS	= reactive oxygen species
S. cerevisiae	= *Saccharomyces cerevisiae*
sCJD	= sporadic Creutzfeldt-Jakob disease
SENDA	= static encephalopathy of childhood with neurodegeneration in adulthood syndrome
SIH	= salicylaldehyde isonicotynoyl hydrazine
SN	= *substantia nigra pars compacta*
SOD	= superoxide dismutase
Tf	= transferrin
TfR1	= transferrin receptor 1
TfR2	= transferrin receptor 2
XLSA/A	= X-linked sideroblastic anemia with ataxia
Yfh1	= yeast frataxin homologue