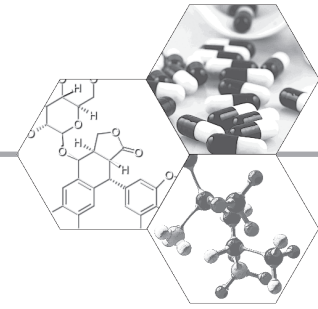

Mechanisms of brain iron transport: insight into neurodegeneration and CNS disorders

Trace metals, such as iron, copper, zinc, manganese and cobalt, are essential cofactors for many cellular enzymes. Extensive research on iron, the most abundant transition metal in biology, has contributed to an increased understanding of the molecular machinery involved in maintaining its homeostasis in mammalian peripheral tissues. However, the cellular and intercellular iron-transport mechanisms in the CNS are still poorly understood. Accumulating evidence suggests that impaired iron metabolism is an initial cause of neurodegeneration and several common genetic and sporadic neurodegenerative disorders have been proposed as being associated with dysregulated CNS iron homeostasis. This review aims to provide a summary of the molecular mechanisms of brain iron transport. Our discussion is focused on iron transport across endothelial cells of the blood–brain barrier and within the neuro- and glial-vascular units of the brain, with the aim of revealing novel therapeutic targets for neurodegenerative and CNS disorders.

The brain iron axis, oxidative stress & neurodegeneration

Iron is likely an integral part of metabolism because it can gain (ferric to ferrous, or Fe^{3+} to Fe^{2+}) or lose (Fe^{2+} to Fe^{3+}) electrons relatively easily. Interestingly, iron has a functional split personality in the nervous system, where it is essential for life but toxic if levels are perturbed. At the cellular level, iron is required for cell growth, however excessive iron (iron overload) causes oxidative stress and cell death. Perhaps not surprisingly, iron levels are tightly regulated in a process referred to as iron homeostasis. The principal protective strategy to avoid iron overload in the brain is the blood–brain barrier (BBB), which limits iron entry to the brain from the blood via highly regulated, selective transport systems [1–3]. Within the brain, multiple feedback loops form an elaborate control system for cellular iron levels, ensuring that a precisely balanced iron level exists for normal function of the nervous system [4,5].

Iron is critical for a host of basic cellular processes, such as mitochondrial ATP generation and DNA replication [6]. Iron deficiency in the brain likely affects the normal division of brain cells, including neuronal precursor cells, astrocytes and oligodendrocytes [5,7]. In addition, iron is required for several neuronal specific functions, such as dopaminergic neurotransmitter synthesis [5,8] and myelination of axons [6]. Not surprisingly, iron deficiency in early development leads to neurological abnormalities [9], contributing to the so-called 'early anemia, later mental retardation' effect [10].

Iron accumulation in the adult brain is known to cause neurodegeneration [5,7,8]. Owing to iron's ability to donate electrons to oxygen, increased iron levels can lead to the formation of hydroxyl radicals and hydroxyl anions via the Fenton reaction **(Equation 1 & Figure 1)**:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

Equation 1

Increased iron levels can also generate peroxyl/alkoxyl radicals due to Fe^{2+}-dependent lipid peroxidation [11]. These reactive oxygen species (ROS) can damage cellular macromolecules, including proteins, lipids and DNA. Under normal conditions, several detoxification systems and antioxidant defense mechanisms exist to prevent this damage; for example, catalase and glutathione peroxidase quickly convert H_2O_2 to water, helping to ensure that ROS-induced damage is minimal in the brain [12]. However, when the formation of ROS exceeds cells' detoxification/antioxidant systems, cells can experience oxidative stress. Iron-induced oxidative stress is particularly dangerous because it can cause further iron release from iron-containing proteins, such as ferritin (Ft), heme proteins and iron–sulfur (Fe–S) clusters, forming a destructive intracellular positive-feedback loop that exacerbates the toxic effects of brain iron overload [13].

Eric Mills[1], Xian-ping Dong[1], Fudi Wang[2] & Haoxing Xu[1†]
[†]Author for correspondence
[1]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, 3089 Natural Science Building (Kraus), 830 North University, Ann Arbor, MI 48109, USA
E-mail: haoxingx@umich.edu
[2]Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, PR China

Blood–brain barrier
Vascular barrier that prevents dissolved solutes in the blood from freely entering the CNS. It is formed by tight junctions between adjacent brain vascular endothelial cells lining CNS vasculature

FUTURE SCIENCE part of fsg

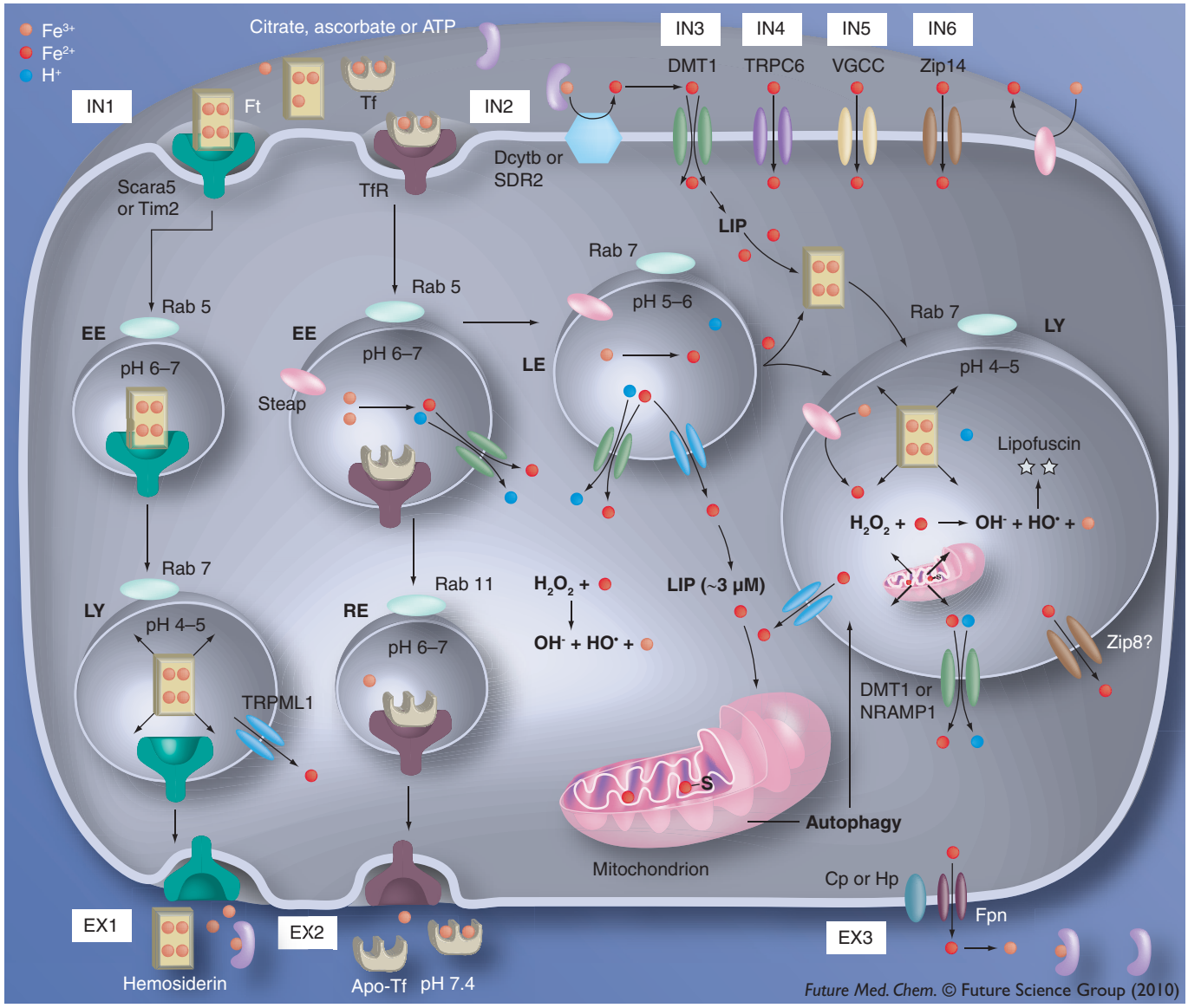

Figure 1. Molecular mechanisms of intracellular iron transport. Most cellular uptake of ferric iron (Fe^{3+}) occurs via receptor-mediated endocytosis of Tf (vesicular import pathway IN2). Fe^{3+} ions form complexes with the high affinity iron binding protein, Tf, which then binds to specific membrane-bound TfR. After endocytosis, the acidic environment of the early endosomes (compartment specificity defined by a small GTPase Rab5) triggers the release of Fe^{3+} from the Tf–TfR complex, which is recycled to the plasma membrane via Rab11-positive recycling endosomes (vesicular export pathway EX2). Members of the Steap family of ferric reductases localize to the endosome and reduce Fe^{3+} (ferric) to its Fe^{2+} (ferrous) form before Fe^{2+} is released into the cytosol by the DMT1 in an H^+-dependent manner. Fe^{3+} may also be sorted into Rab7-positive LE and LY, where it is reduced into Fe^{2+} by Steap proteins in LE or LY. In LE and LY, Fe^{2+} can also be released by other endolysosomal iron-release channels/transporters such as TRPML1, Nramp1 (in macrophages), or Zip8. Other sources of lysosomal Fe^{3+} include Fe^{3+}-laden Ft complexes and autophagic ingestion of damaged organelles such as mitochondria. Other mechanisms for receptor-mediated iron uptake exist in oligodendrocytes and other cells, where Fe^{3+} ions bind the Ft receptors (Scara5 or Tim-2) and undergo receptor-mediated endocytosis (vesicular iron import pathway IN1). This pathway depends on lysosomal degradation of Ft–Fe complexes before iron is released into the cytosol. Lysosomal Fe^{3+} or Ft–Fe complexes may also be exported out of the cells via lysosomal exocytosis (vesicular export pathway EX1). In some cell types, uptake of non-Tf bound iron (NTBI) in its Fe^{2+} form can be mediated by plasma membrane-localized iron importers such as DMT1 (nonvesicular import pathway IN3), TRPC6 (IN4), voltage-gated Ca^{2+} channels (VGCC; IN5), and Zip14 (Slc39a14; IN6). Free Fe^{2+} in the cytosol constitutes a 'labile iron' pool (LIP; ~3 µM) for cellular utilization. If not immediately used, it can also be rapidly sequestered by cytosolic Ft into a nonreactive state. Finally, iron can be released from cells by the iron exporter Fpn. Cellular export of Fe^{2+} usually is coupled to a membrane bound or cytosolic ferroxidase, such as Hp or Cp, which oxidizes reactive Fe^{2+} to its less reactive Fe^{3+} form before diffusing throughout the extracellular space. Fe^{3+} can be bound in the extracellular space by Tf, citrate, ascorbate or ATP. Cytosolic or intralysosomal iron overload may catalyze the production of free radical oxides via the Fenton reaction. Radical oxides may cause cellular damage by oxidizing macromoleucles such as lipids, DNA, and proteins. Cp: Ceruloplasmin; Dcytb: Duodenal cytochrome b561; DMT1: Divalent metal transporter-1; EE: Early endosome; Fpn: Ferroportin; Ft: Ferritin; Hp: Hephaestin; LE: Late endosome; LIP: Labile iron pool; LY: Lysosome; SDR2: Stromal cell-derived receptor-2; Tf: Transferrin; TfR: Transferrin receptor; VGCC: Voltage-gated Ca^{2+} channels.

A cardinal pathology associated with several common neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease, is iron accumulation in the brain, coincident with sites of extensive neuronal cell death [5,7,8,14–17]. Normally, iron-rich regions in the brain, such as the globus pallidus, red nucleus, substantia nigra (SN), putamen and caudate nucleus, seem to be most susceptible to neurodegenerative processes [14]. During the typical aging process, iron accumulation also occurs in multiple regions of the brain known to be more vulnerable to age-dependent neurodegeneration [8,9]. For example, iron invasion with age predominates in motor areas of the brain, such as the basal ganglia, which may explain the motor deterioration commonly exhibited in many neurodegenerative diseases.

The question remains whether iron invasion and subsequent radical formation is a primary or secondary event in neurodegenerative diseases. Recent evidence suggests that iron invasion is an initial cause of neuronal cell death and axonal degeneration [5,8,18]. Several inherited disorders of iron metabolism in humans are known to display neurodegeneration with brain-iron accumulation (NBIA) [14]. This group of genetically heterogeneous progressive disorders includes NBIA type I (formerly Hallervorden–Spatz syndrome), neuroferritinopathy, infantile neuroaxonal dystrophy-1 (INAD1), and aceruloplasminemia **(Table 1)**. Detailed studies of these disorders suggest that an iron gradient formed in brain tissues is causal for triggering neuronal toxicity [19–22]. Other CNS disorders that have been proposed to have an iron-dependent component include Friederich's ataxia [17], restless leg syndrome [23], ischemic/hemorrhagic stroke [24] and multiple sclerosis [16]. Dong *et al.* recently demonstrated that transient receptor potential mucolipin 1 (TRPML1), the gene *MCOLN1* that is mutated in mucolipidosis type IV (ML4)

Reactive oxygen species

Highly reactive products of oxygen metabolism that form in high amounts when cells are under stress conditions. Reactive oxygen species includes oxygen ions, free radicals and peroxides. Accumulation can lead to damage of several cellular structures including DNA, proteins and lipids

Ferritin

Globular intracellular iron storage protein consisting of 24 protein subunits. Ferritin maintains the solubility of iron by storing it in its ferric (Fe^{3+}) form

Table 1. Iron-related genes involved in neurodegeneration.

Gene	Functions	Involvement in neurodegeneration	Human disorders	Ref.
PANK2; Pantothenate kinase-2	Mitochondrial protein essential for coenzyme A synthesis and fatty acid metabolism	*Pank2*$^{-/-}$ mice show retinal degeneration. Human patients show NBIA in the basal ganglia.	NBIA type I; PKAN, Hallervorden–Spatz Syndrome; MIM #243200	[19,96]
FLT1; L-Ferritin (ferritin light chain)	Iron storage; lacks ferroxidase activity of H-ferritin	Patients show low serum ferritin levels but normal iron, hemoglobin and transferrin levels. Dense ferritin–Fe spheroid inclusions throughout forebrain and cerebellum; neurons, oligodendrocytes and microglia.	Neuroferritinopathy; hereditary ferritinopathy; MIM 606159	[13,22]
PLA2G6; *iPLA*$_2$; Ca^{2+}-independent group VI phospholipase A_2	Arachidonic acid release	Phospolipase A2 is important for myelin breakdown and phagocytosis during axonal degeneration.	NBIA type II; MIM #610217 Infantile neuroaxonal dystrophy-1; MIM #256600 Karak syndrome; MIM 608395	[20,97,98]
Cp; Ceruloplasmin	Cu-dependent ferroxidase converting Fe^{2+} into Fe^{3+}	*Cp*$^{-/-}$ mice exhibit brain iron accumulation in astrocytes.	Aceruloplasminemia; MIM #604290	[21]
Slc11a2; *DMT1*	H^+-dependent Fe^{2+} transporter	Upregulated in models of Parkinson's	None described	
MCOLN1; mucolipin-1; TRPML1	Ca^{2+}/Fe^{2+} release channel in late endosome and lysosome	Loss-of-function mutations cause decreased cytosolic iron levels and intralysosomal iron accumulation; iron deficiency anemia; psychomotor retardation	Mucolipidosis type-IV; MIM #252650	
IRP2; Iron-regulatory protein-2; IRP2	Cytosolic iron sensor; Post-transcriptional regulator of iron-management protein expression	*IRP2*$^{-/-}$ mice exhibit ataxia, bradykinesia and tremor; brain iron accumulation precedes neurodegeneration	None described	[28]

DMT: Divalent metal transporter; MIM: Mendelian inheritance in man; NBIA: Neurodegeneration with brain-iron accumulation; PKAN: Pantothenate kinase-associated neurodegeneration; TRPML: Transient receptor potential mucolipin.

neurodegenerative disease [25], encodes an iron-release channel found in the late endosome and lysosomes (LELs) [26]. Furthermore, several studies of iron regulatory proteins using transgenic rodents suggest that iron may be a pathogenic factor for neurodegeneration [27,28]. Finally, iron chelators [8,29,30] and genetic manipulation resulting in low iron levels [29,31,32] are found to be neuroprotective.

Although we focus on iron toxicity in this review, it is worth mentioning that other transition metals have also been implicated in neurodegenerative disorders [33]. For example, copper accumulation has also been implicated in AD [33,34]. As Cu^{+} is also able to catalyze the Fenton reaction, copper accumulation may also facilitate radical formation during oxidative stress [33]. Under different pathological conditions, iron and other transition metals accumulate in different brain regions at different levels [33,34]. Therefore, it is difficult to isolate iron-specific toxicity. Furthermore, there is cross-talk between iron and copper toxicity, as ceruloplasmin (Cp), the major ferroxidase, functions in a Cu-dependent manner [35]. Nevertheless, the abundance of iron suggests a major role of iron toxicity in neurodegeneration.

Cellular iron transport in the peripheral tissues

In normal human plasma, serum iron (~20 μM) exists primarily in the Fe^{3+} form and is complexed with the high-affinity iron-binding protein transferrin (Tf; ~40 μM) in a 2:1 ratio ($Tf–Fe_2$; **Figure 1**). Hence, Tf is only partially (~25%) saturated. Under iron overload conditions, however, Tf is saturated and nontransferrin-bound iron (NTBI) can reach 1–20 μM [36]. In the cerebrospinal fluid (CSF), in which the Tf concentration is low (< 0.5 μM), NTBI can reach a sub-micromolar concentration (< 1 μM) [37,38]. Depending on the microenvironment and availability of ferric reductases, NTBI can exist in both Fe^{3+} and Fe^{2+} forms. Inside the cell, free iron in its reduced form (Fe^{2+}) constitutes the 'labile iron' pool (~2–3 μM) [39], which supplies Fe^{2+} molecules as co-factors for many Fe^{2+}-dependent enzymes in the cytosol, mitochondria and nucleus. If cytosolic iron is not needed for immediate use, it is usually stored in Fe^{3+}- Ft complexes [4].

Nonheme iron can enter mammalian cells via two distinct mechanisms: vesicular and nonvesicular iron import. Fe^{3+} forms a complex with Fe^{3+}-binding proteins, such as Tf, to enter the cell through the receptor-mediated endocytic pathway (vesicular iron import pathways IN1 and IN2; **Figure 1**). Fe^{2+}, on the other hand, may influx into the cell through transmembrane channels or secondary transporters (nonvesicular iron import pathways IN3, IN4, IN5 and IN6; **Figure 1**). Similarly, iron can leave the cell through mechanisms of vesicular and nonvesicular transport. Both Fe^{3+} and Fe^{2+} inside intracellular vesicles can exit the cell by exocytosis of recycling endosomes (RE) or lysosomes (vesicular iron export pathway EX2 and EX1; **Figure 1**). Fe^{2+} may also efflux from the cell via transmembrane channels or transporters (nonvesicular iron export pathway EX3; **Figure 1**). Most mammalian cells transport iron using a combination of these iron import and export pathways.

■ Iron transport in enterocytes

Systemic iron levels are principally regulated at the level of absorption by the small intestine [40,41]. Intestinal iron absorption involves transcellular transport mediated by enterocytes (polarized epithelial cells that line the lumen of the intestines). Iron crosses the apical brush border membrane, traverses the cell, and then exits across the basolateral membrane to enter the blood plasma [4,42,43]. Dietary iron, existing primarily in the insoluble Fe^{3+} form, is first reduced to Fe^{2+} by duodenal cytochrome B (DcytB, also called CYBRD1), a ferrireductase present on the apical membranes of enterocytes [44]. Fe^{2+} then enters the enterocytes by divalent metal transporter-1 (DMT1, Slc11a2), an H^{+}-dependent Fe^{2+} transporter (nonvesicular iron import pathway IN3; **Figure 1**) expressed on the apical membrane [45]. Once inside, Fe^{2+} can leave the enterocyte through ferroportin (Fpn), an Fe^{2+} channel/transporter (nonvesicular iron export pathway EX3; **Figure 1**) expressed on the basolateral membrane [46]. Released Fe^{2+} is quickly oxidized into the bio-unavailable Fe^{3+} form via a membrane bound or cytosolic ferroxidase, such as hephaestin (Hp) [47], or Cp [21]. In order to be available for use, Fe^{3+} enters the plasma by forming complexes with high-affinity iron-binding proteins, such as Tf, or with low-affinity iron-binding molecules, such as citrate, ascorbate or ATP **(Figure 1)**. The inability to maintain sufficient iron levels in the plasma is defined as iron deficiency, often associated with anemia, while excessive iron absorption is termed iron overload.

Transferrin

Blood plasma glycoprotein that transports Fe^{3+} through the body and delivers iron to tissues that express transferrin receptors, initiating the transferrin cycle of delivery

Nontransferrin-bound iron

Iron in the blood plasma or cerebrospinal fluid that is free or bound by molecules other than transferrin

■ Iron transport in erythroid precursor cells

In most mammalian cells, especially erythroid precursor cells, iron is sequestered in a nonreactive (Fe^{3+}) state by Tf in the blood plasma, forming a Tf–Fe_2 complex **(FIGURE 1)**. The Fe^{3+}-binding affinity for Tf is extremely high (K_d ~10^{-23} M). Since Tf is abundant (~40 µM) and only partially saturated in the plasma (~25%), Tf–Fe_2 is the predominant form of iron present in the extracellular space surrounding erythroid precursor cells. In this classical iron uptake mechanism **(FIGURE 1)**, the Tf–Fe_2 complex is taken up by transferrin receptor (TfR) 1 on erythroid precursor cells via receptor-mediated endocytosis into clathrin-coated vesicles (vesicular iron import pathway IN2; **FIGURE 1**) [40,41]. The acidic environment of Rab5-positive early endosomes triggers the release of Fe^{3+} from the Tf–TfR complex, which is sorted into Rab11-positive RE before trafficking to the cell membrane and subsequent exocytosis. In erythroid precursor cells, specifically, the endosomal ferric reductase six-transmembrane epithelial antigen of the prostate 3 (Steap3) reduces Fe^{3+} to Fe^{2+} [48], which is then released into the cytosol by DMT1 in a H^+-dependent manner [45]. Ferrous iron in the cytosol comprises the 'labile iron' pool and represents iron that is directly available for cellular utilization. The majority of cytosolic Fe^{2+} however, is transported into mitochondria by the iron importer Mfrn for the synthesis of iron–sulfur (Fe–S) clusters and biogenesis of heme [49]. If not used immediately, cytosolic Fe^{2+} can also be stored in ferritin–Fe^{3+} complexes, cytoplasmic iron storage proteins [13]. These complexes are eventually degraded in the lysosome, releasing reactive Fe^{2+} [50] via DMT1. In addition to utilization and storage, iron can also be released from the cell by the iron exporter Fpn (nonvesicular iron export pathway EX3; **FIGURE 1**) [46].

■ Iron transport in most mammalian cells

Most other mammalian cell types recruit the cellular iron-transport machinery that is used by enterocytes and erythroid precursor cells. This includes using the Tf–TfR system for iron import and Fpn for iron export. Intracellular iron-transport machinery, however, may differ significantly. As DMT1 is specifically expressed on the endosomes of erythroid precursor cells, but not all cells [45], genetic evidence suggests that a DMT1-independent iron release mechanism must exist in the endolysosomes of most cells [51]. Dong *et al.* recently characterized a

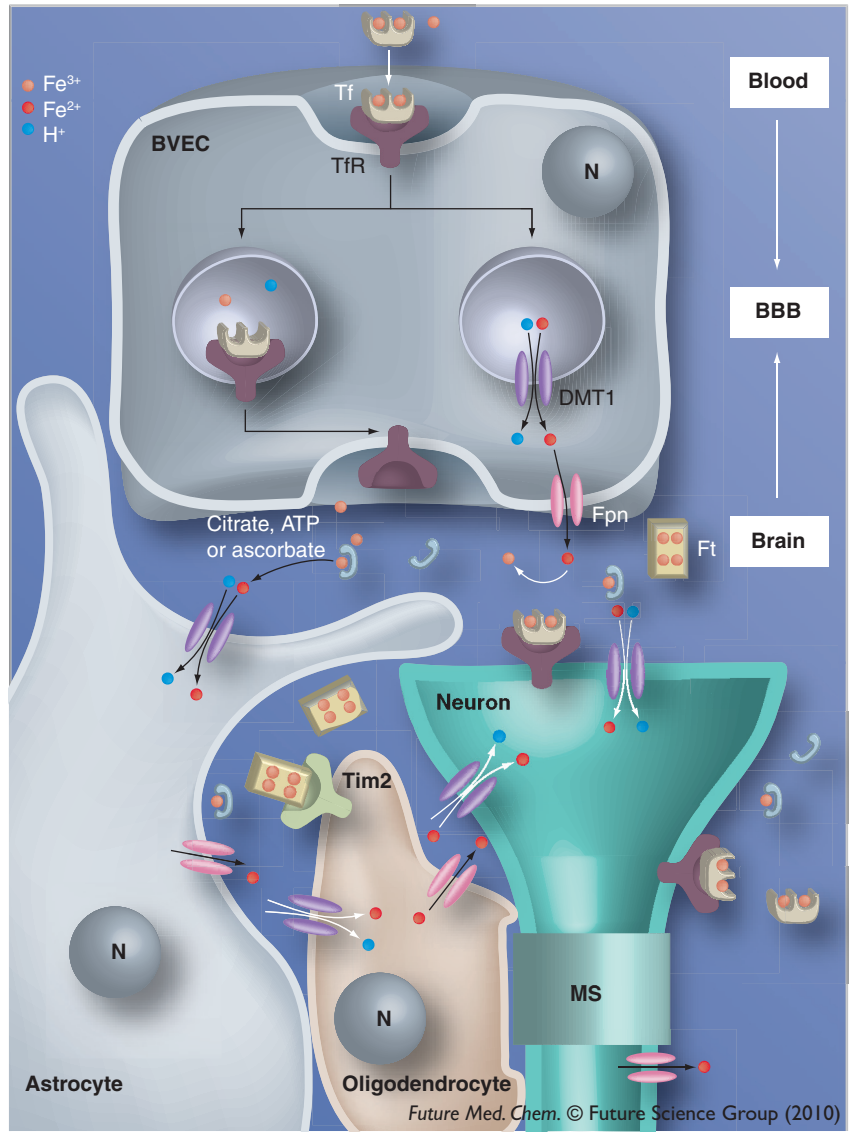

Figure 2. Iron transport across the BBB. Iron is sequestered in a nonreactive state in the blood plasma by Tf (~40 µM). Material transport from the blood plasma into the brain is limited by the BBB, which is formed by tight junctions between the BVECs. TfRs line the lumen of the brain microvasculature and bind circulating Tf–Fe_2 (~10 µM) and facilitate iron uptake into BVECs via receptor-mediated endocytosis. Two models for BVEC iron export are shown. The DMT1/Fpn-independent pathway releases Fe^{3+} or Tf–Fe_2 through the exocytosis of recycling endosomes (vesicular export pathway). Tf (mainly secreted from oligodendrocytes) concentration (<0.5 µM) is low in the cerebrospinal fluid. Thus, the majority of Fe^{3+} forms complexes with ascorbate, citrate, and ATP as NTBI (~1 µM). Citrate and ATP are released from astrocytes. DMT1/Fpn-dependent (nonvesicular export) pathway releases Fe^{2+}, which is rapidly converted into Fe^{3+} via Cp in the abluminal membranes. Alternatively, astrocytic foot processes form close associations with BVECs, and polarized DMT1 expression in these astrocytic end-feet may facilitate rapid Fe^{2+} uptake after release (from BVECs) into the perivascular space of the brain. Neither astrocytes nor oligodendrocytes express detectable levels of TfR. Oligodendrocytes can take up iron via the Ft receptor Tim-2. In addition, oligodendrocytes may also uptake NTBI via DMT1 or other nonvesicular iron import mechanisms. The axons of neurons are wrapped with the myelin sheath, which is made in oligodendrocytes in an iron-dependent manner. BBB: Blood–brain barrier; BVEC: Brain vascular endothelial cell; Cp: Ceruloplasmin; Fpn: Ferroportin; Ft: Ferritin; MS: Myelin sheath; N: Nucleus; Tf: Transferrin; TfR: Transferrin receptor.

novel pathway for iron release from the LEL, mediated by TRPML1 [26]. Mutations in TRPML1 cause the neurodegenerative disease ML4, which in some cases presents as iron-deficiency anemia **(TABLE 1)**. TRPML1 is ubiquitously expressed and functions as a conduit for endolysosomal iron release [26]. The electrophysiological properties of TRPML1 are somewhat similar to those of DMT1. Both iron-carrying currents are inwardly rectifying (cations flowing out of the endolysosome), which is consistent with a role in iron release from the vesicular lumen. Like DMT1, TRPML1 conducts Fe^{2+} but not Fe^{3+}. Hence, a putative ferric reductase, presumably one of the Steap proteins [48,52], is required for TRPML1-mediated Fe^{2+} release from LEL. Like DMT1, low pH (the environment of endolysosomes) potentiates Fe^{2+} permeation of TRPML1. Although the function of TRPML1 is similar to DMT1, its localization in specific endocytic compartments is distinct from that of DMT1. While DMT1 can be detected in lysosomes, the quasi-steady-state location for DMT1 is early or late endosomes [4,53]. On the other hand, TRPML1 is primarily localized in LELs. Therefore, TRPML1 is localized in overlapping but later endocytic compartments compared with DMT1. Consistent with a role for TRPML1 in endolysosomal iron release, TRPML1-deficient fibroblast cells exhibit lysosomal iron overload, as well as cytosolic iron deficiency [26], suggesting defective iron transport across LEL membranes **(FIGURE 1)**. However, as TRPML1 is also permeable to Ca^{2+}, it is unlikely that TRPML1 is fully dedicated to intracellular iron metabolism. Other cell-type-specific endolysosomal iron-release proteins may exist. For example, Nramp1 (Scl11a1), a DMT1-related proton-dependent iron transporter, is expressed in the lysosomes of macrophages [54]. Similarly, TRPML2, a homologue of TRPML1 and also a potential iron-release channel in LEL [26], is expressed in macrophages and B cells [55]. Additionally, ZIP8 (Slc39a8), previously known as a zinc transporter, has also recently been implicated in endolysosomal iron release [56].

The biologic reason for the existence of multiple iron release mechanisms remains unclear. For simplicity, we refer to DMT1 as an endosomal (early, recycling and late) Fe^{2+} release pathway, and TRPML1/2 and Nramp1 as LEL iron-release pathways [54]. Importantly, the source of iron may determine the primary release mechanism. For example, iron acquired through the Tf–TfR cycle is likely to be released via DMT1, which is localized in the early endocytic compartments. Thus, endosomal iron release is mainly due to DMT1. Consistent with this notion, DMT1 is crucial for iron uptake by erythroid precursor cells [51], in which the dominant uptake pathway is Tf-dependent. On the other hand, TRPML1/2 and Nramp1 pathways may be used for iron release following lysosomal degradation of Ft–Fe complexes and autophagic/phagosomal ingestion of iron-containing macromolecules [4]. In a scenario where macrophages recycle senescent red blood cells, Nramp1 may serve as the main release channel/transporter in the lysosome [4,54]. Following lysosomal degradation of Ft–Fe complexes and iron-containing macromolecules, TRPML1/2 may be the primary release channel in LEL. Additionally, both DMT1- and TRPML1-dependent mechanisms may co-exist in some cell types, such as neurons [26,45]. As endosomes undergo rapid Rab-dependent sorting, a certain amount of iron from the Tf cycle may not be released into the cytosol before being sorted into LELs. Thus, DMT1 and TRPML1 may mediate iron release in a sequential manner. In cells with dual expression of DMT1 and TRPML1, impairment in DMT1-mediated endosomal iron release may be tolerated because iron entering the late endocytic pathways can still be released via TRPML1.

The regulation of iron release pathways is poorly understood. Regulators of intracellular traffic may play a role in iron metabolism by affecting both vesicular and nonvesicular iron transport pathways. For example, it was demonstrated that Mon1a, a protein involved in multiple steps of intracellular trafficking, affects cellular iron metabolism in an Fpn-dependent manner [57]. Rab proteins are a family of small GTPases that are known to control organelle identity and the specificity of intracellular membrane traffic [58]. For example, Rab5, Rab11 and Rab7 are primarily localized in the early endosome, RE and LEL, respectively **(FIGURE 1).** Impaired Rab5 function in zebrafish reportedly causes iron-deficiency anemia [59,60]. Future research may reveal the role of vesicular trafficking in regulation of cellular iron transport.

Brain iron uptake across the BBB

The brain, unlike most other organs in the body, rests behind a vascular barrier. Unlike peripheral endothelia, endothelial cells within the brain vasculature are bound together by tight junctions, resulting in a physical barrier to

material exchange between the tissues and fluids of blood and brain. Therefore, the brain cannot take up iron directly from the circulatory system. The choroid plexus epithelium, comprised of ependymal cells, separates the blood from the ventricular CSF. Although ependymal cells can mediate iron absorption into the brain from the blood, their contribution is thought to be rather small [61]. The major uptake pathway is mediated by the BBB, which is formed by cerebrovascular endothelial cells (or brain vascular endothelial cells [BVECs]) **(Figure 2)**. Unlike small gas molecules, such as O_2 and CO_2, which can diffuse freely across lipid membranes into the brain, iron crosses BVECs in a multistep transcellular process similar to that of enterocytes in the duodenum.

■ Transferrin-dependent and -independent iron import into BVECs

The initial observation that TfR is expressed on the luminal side of brain capillaries suggested a mechanism for iron transport across the BBB through TfR-mediated endocytosis into BVECs [62]. Now, accumulated evidence from functional and genetic studies reveals that, indeed, Tf–TfR does play a primary role in iron uptake across the BBB [5,7, 37,63,64] **(Figure 2)**. The Tf–TfR-dependent iron uptake system in BVECs is similar to that used in most cell types **(Figure 1)**.

Transferrin-independent mechanisms may also contribute to iron transport across the BBB [1–3,61,64–66]. As hypotransferrinemic mice (<1% of normal circulating Tf) have reportedly normal amounts of iron in the brain, significant contributions from Tf-independent mechanism(s) of brain iron uptake must exist [67]. While iron may also be taken up to BVECs by Tf homologues, such as lactoferrin or melanotransferrin [1], the extent of this activity is still unclear [2].

Nontransferrin-bound iron can enter BVECs through both vesicular and nonvesicular import mechanisms **(Figure 1)**. Fe^{3+} may form complexes with non-Tf iron-binding proteins, such as secreted Ft in the serum [68]. Scara5 [69] and Tim-2 [70], have both been recently demonstrated to function as Ft receptors **(Figure 1)**. Because a molecule of Ft can bind as many as 4500 iron atoms, these newly identified receptors may play an important role in providing iron for highly iron-dependent processes, such as organogenesis [69] and myelination [6], during development. The existence and relative contribution of Ft receptor-dependent iron uptake in BVECs is still unclear. Nonvesicular import pathways may provide a direct mechanism to cross the BBB. Since the brains of DMT1-compromised rats containing the Belgrade hypomorphic mutation (G185R) show decreased iron levels in the brain, it was thought to play a direct role in brain iron uptake [61]. However, it is not clear whether DMT1 is actually expressed in BVECs [61,71]. Thus, it is necessary to investigate whether other nonvesicular iron importers (IN4, IN5 and IN6; **Figure 1**) are expressed on the luminal membranes of BVECs. Although DMT1 transports iron at the acidic pH of the endosome, it may also be able to function as an Fe^{2+} importer at the neutral pH of the lumen [72].

Brain vascular endothelial cells

Specialized endothelial cells that form the lining of the brain's circulatory system. Tight junctions between adjacent endothelial cells form the BBB and restrict the passage of solutes from the blood into the brain parenchyma

■ Intracellular iron transport in BVECs

While it is well established that iron enters brain endothelial cells through Tf-dependent receptor-mediated endocytosis, there is little agreement on the mechanisms of intracellular transport that ensure iron eventually traverses the abluminal membrane and enters the brain. The controversy of DMT1 expression in BVECs [61,71] may determine how iron is transported intracellularly. Given its ubiquitous expression pattern, TRPML1 is likely to play an important role in endolysosomal iron release in BVECs [26]. Whether other iron release proteins, such as ZIP8 and TRPML2, are expressed in BVEC cells is not yet known. Additionally, it is not known which ferric reductase(s) are expressed in the endosomes and lysosomes of BVECs, though they likely express one or more of the Steap proteins [48,52].

■ Iron export across the abluminal membrane

Ferroportin is currently the only known iron exporter [46], making it a natural candidate to export iron across abluminal membranes. However, it is unclear whether it is expressed [61,73,74] in BVECs. It is also possible that the expression level of Fpn is low in these cells. Since knock-out of Fpn globally in mice results in embryonic lethality [75], an endothelium- or BVEC-specific inactivation of Fpn in mice may be necessary to provide a definite answer on whether Fpn plays a role in iron transport across the BBB.

DMT1/Fpn-independent export mechanisms may also account for iron transport across the abluminal membranes of BVECs [74]. Moos *et al.* propose that exocytosis of the RE containing

$Tf–Fe_2$ complexes to the abluminal surface of the BVEC, leading to the detachment of iron from Tf in the slightly lower pH of brain interstitial fluid (ISF) compared with blood plasma (vesicular iron export pathway EX2; **Figure 1**). Compared with plasma, CSF/ISF solutions contain much lower concentrations of Tf (<0.5 μM), but several-fold higher concentrations of citrate and ascorbate [37]. Thus, citrate and ascorbate in the ISF may serve as possible coordination partners for released Fe^{3+} **(Figure 1)**. Additionally, astrocytes form close associations with the microvasculature of the brain and are known to release ATP and other nucleotides that may play a role in this exocytosis-dependent abluminal membrane iron transport [76]. Indeed, significant sources of NTBI exist in the brain bound to citrate, ascorbate and ATP [5]. Nevertheless, this model is lacking explanation of how abluminally exposed apo-Tf laden TfRs are recycled or degraded, since very little serum Tf crosses into the BBB. Another potential iron export mechanism in BVECs is lysosomal exocytosis (vesicular iron export pathway EX1; **Figure 1**). Lysosomal exocytosis has recently been demonstrated to occur in all mammalian cell types studied thus far [77]. As Ft-receptor- (Scara5 or Tim-2) mediated endocytic iron uptake and reutilization of Ft-bound storage iron require lysosomes [50], this pathway may not only export iron but also provide secreted Ft or its degraded product hemosiderin to the CSF/ISF. Direct evidence for this lysosome-mediated iron export is currently still lacking. Nevertheless, if both exocytotic types of iron-export mechanisms coexist in BVECs, manipulations of compartment-specific Rab proteins may help determine the extent to which iron absorption by BVECs relies on the Rab7-dependent lysosomal pathway versus the Rab11-dependent endosomal pathway.

Iron transport in neurons & glia

■ Iron transport in astrocytes

Astrocytic perivascular foot processes surround the vascular barrier formed by BVECs just outside the basal lamina, ensheathing the abluminal membranes of BVECs **(Figure 2)** [63]. These foot processes can also form direct connections to neurons. As such, astrocytes likely play a critical role in regulating brain iron absorption and metabolism at the junction of the BBB [78]. This unique arrangement allows astrocytes to act as gatekeepers in regulating the iron transport properties of the BBB. Astrocytes do not express TfR [78], although this is somewhat controversial [79]. They may, however, take up significant amounts of NTBI as a primary iron-uptake pathway. Interestingly, DMT1 has been shown to have polarized expression in astrocytes, being only expressed in the endfoot processes that associate with the BBB [80]. Therefore, iron released by BVECs may quickly be taken up by nearby astrocytes through DMT1 (nonvesicular iron import pathway IN3; **Figure 1** and **Figure 2**) and redistributed to the extracellular space in the brain parenchyma. Astrocytes have also been implicated in metabolizing heme, releasing iron degraded by heme oxygenase-1 [81].

Ferroportin (nonvesicular iron export pathway EX3; **Figure 1**) forms the major iron efflux pathway in astrocytes [73]. Fpn is coupled to a GPI-anchored form of Cp, the Cu-dependent ferroxidase that can rapidly oxidize released Fe^{2+} to its less reactive Fe^{3+} form [35]. Astrocytes cultured from Cp-deficient mice are unable to export iron, even though they have normal iron uptake [82]. Yet normal iron export is restored by simply supplying soluble Cp to the cell culture medium. Because astrocytes are closely associated to the brain's vasculature and have a high capacity for iron accumulation, yet store little iron, they may be involved in brain iron distribution after uptake. Consistent with this notion, patients with aceruloplasminemia **(Table 1)** are observed to have primary iron accumulation in the astrocytic foot processes along the BBB, forming inclusions known as 'grumose foamy spheroid bodies' [83].

■ Iron transport in neurons

Iron is required for many neuronal functions and is a cofactor required for the synthesis of dopaminergic neurotransmitters [5,8]. A substantial portion of oxygen consumption in the body (~20%) relies on mitochondrial respiration in the brain, of which iron is an essential trophic factor, required for oxygen consumption and ATP generation [6]. As TfR is expressed in central neurons, the Tf–TfR system may be an important pathway for neurons to acquire iron. Furthermore, both DMT1 and TRPML1 are highly expressed in neurons [37,55]. Since both proteins transport $Fe^{2+,}$ but not Fe^{3+}, a ferric reductase must exist in the endosomes and lysosomes of neurons. While Steap3 is shown to function in the endosomes of erythroid cells [48], both Steap1 and -2 are expressed in the brain [52]. In addition, the stromal cell-derived receptor-2 [84], a homologue of the duodenal ferrireductase DcytB [44], is expressed in the brain. It is

unclear whether stromal cell-derived receptor-2 is localized to the endolysosomal membranes or the plasma membrane.

Neurons are also able to utilize NTBI as an iron source, where it forms significant stores of brain iron [64]. Although citrate and ascorbate bind Fe^{3+} with affinities (K_d ~10^{-11} M) more than 10^{10}-times lower than Tf (K_d ~10^{-23} M), they are much more abundant in the CSF/ISF than the plasma [37]. Hence citrate/ascorbate-bound NTBI can reach sub-micromolar concentrations (<1 μM) in the CSF [37,38]. It has been demonstrated that NTBI may enter the neurons through several nonvesicular import mechanisms (IN3, IN4, IN5 and IN6; **FIGURE 1**). DMT1, Zip14 (Slc39a14) [85], voltage-gated Ca^{2+} channels [86] and TRPC6 [87] are all selective for Fe^{2+} over Fe^{3+}. It remains an open question how Fe^{3+} can be dissociated from ascorbate/citrate and converted into Fe^{2+} by a membrane-bound ferric reductase. Little iron is actually stored in neurons since Ft detection is relatively weak [5,7,70], suggesting that iron is taken up either for rapid utilization or secreted by the robustly detected iron exporter Fpn. Increased neuronal Ft expression, as seen in *Irp2*$^{-/-}$ mice, may be toxic and has been reportedly associated with extrapyramidal neurodegeneration [27].

Abnormal intracellular iron transport in neurons may cause neurodegeneration. Cytosolic iron overload causes oxidative stress by increasing ROS levels via the Fenton reaction. Since neuronal iron is transported along axons and dendrites, iron overload in neurons may cause both neuronal cell death and axonal degeneration. Impaired endolysosomal iron release may result in intralysosomal iron overload, which in turn increases free radical oxides and facilitates the formation of 'lipofuscin' (or 'aging pigment') [26,88]. Lipofuscin accumulation may affect the normal function of lysosomes, such as autophagy, resulting in neuronal cell death and axonal degeneration. Impaired intracellular iron transport may also cause other neuronal dysfunctions. For example, hippocampus-specific inactivation of DMT1 in mice results in iron deficiency in the hippocampal neurons and impaired learning and memory [89].

■ Iron transport of oligodendrocytes

Most of the histochemically detectable iron in the brain is found within oligodendrocytes, likely reflecting the high demand of iron required for myelination [6]. These cells are also the predominant producers of Tf in the brain [66], suggesting an indirect role for oligodendrocytes in neuronal iron transport. Ferritin, traditionally considered to be an intracellular iron storage protein, is also present in the serum [13] and is known to bind the extracellular face of oligodendrocytes *in vivo* [90]. The recent identification of Ft receptors suggests that oligodendrocytes primarily take up NTBI iron from the ISF by a Ft-dependent mechanism (vesicular import pathway IN1; **FIGURE 1**) [69,90]. Consistent with the stringent iron requirements for axon myelination **(FIGURE 2)**, the Ft receptor Tim-2 is highly expressed in oligodendrocytes, but not astrocytes [90].

CNS iron homeostasis, dyshomeostasis & novel therapies

■ Regulation of brain iron transport

Iron uptake across the BBB is dependent on the brain's iron status. During development or inadequate iron supply conditions, the transport rate of iron into the brain is significantly increased [91]. However, this is not associated with increased TfR expression by BVECs, rather TfR expression is upregulated in neurons during periods of inadequate circulating iron [64]. Therefore, multiple regulatory mechanisms may exist to control iron levels in the brain, independent of peripheral iron status. Consistent with this notion, during peripheral iron overload, such as is seen in patients with hemochromatosis, brain iron levels are not elevated or disrupted [66].

Key proteins involved in iron metabolism are regulated post-transcriptionally by the iron responsive element–iron regulatory proteins (IRE–IRP) system in mammalian cells [4,28]. IRPs are cytosolic iron-sensing proteins that, when cells are iron-depleted, interact with RNA transcripts containing a stem–loop structure known as an IRE [4]. IREs are present in many key proteins involved in iron acquisition, transport and storage. Low cytosolic iron levels cause two homologous IRPs (IRP1 and IRP2) to inhibit Ft and Fpn translation and to stabilize mRNA transcripts of TfR1 and DMT1 [13]. In iron-replete cells, neither IRP1 nor -2 binds IREs and Ft/Fpn expression increases while TfR/DMT1 expression decreases. The role of the IRE–IRP network in regulating systemic iron homeostasis has been well reviewed elsewhere [28].

Iron transport in the brain is also regulated by the IRE–IRP system. In brain tissues from *Irp2*$^{-/-}$ mice, Ft/Fpn expression is significantly increased.

Conversely, TfR levels were found to be decreased, consistent with the notion that IRP2 ablation may constitute an inability to stabilize TfR transcripts [4,28]. Although IRP double knockout mice (*Irp1*$^{-/-}$/*Irp2*$^{-/-}$) do not survive, *Irp2*$^{-/-}$ mice exhibit microcytic anemia and progressive neurodegeneration [27]. Interestingly, brain iron accumulation precedes degeneration by several months in these mice [27]. Moreover, regions of brain iron accumulation in young mice accurately predict sites of CNS neurodegeneration. This work represents another example of support for the idea that defects in iron metabolism can act in a causal manner in CNS disorders.

■ Iron dyshomeostasis in animal models of CNS disorders

In *Irp2*$^{-/-}$ mice, immunolabeling of Ft revealed subunits along the length of axons, suggesting that Ft is transported from the site of synthesis (soma) to the axons, prior to axonal degeneration. Lysosomal turnover of Ft–Fe complexes at the synaptic terminal may lead to increased reactive Fe^{2+} levels and oxidative stress [84]. Consistent with this notion, axonal iron staining of *Irp2*$^{-/-}$ mice is significantly greater than the wild-type control [27].

Increased DMT1 expression has also been shown to be associated with neurodegeneration. Salazar *et al.* report that the transporter contributes to neurodegeneration in animal models of PD [32]. Using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse intoxication model of PD, the authors demonstrate that DMT1 expression increases in the ventral mesencephalon, leading to iron accumulation, increased oxidative stress and dopaminergic cell death. A hypomorphic mutation that impairs iron transport (G185R) in DMT1 was sufficient to protect rodents against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induction of neurodegeneration, suggesting a critical role for DMT1-mediated neuronal iron uptake into regions of the brain commonly affected in animal models of PD. Consistent with a role for iron-mediated toxicity in the animal models of PD, Kaur *et al.* used transgenic expression of Ft (as a genetic iron chelator) to demonstrate its protective effect on dopaminergic neurons in the SN after injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [31]. Similar results were obtained using oral administration of the iron chelator Clioquinol [31].

Mutations in TRPML1 cause the neurodegenerative disorder ML4. Dong *et al.* propose that the excessive accumulation of intralysosomal iron leads to the formation of iron-catalyzed ROS and, eventually, to formation and accumulation of lipofuscin in post-mitotic cells, such as neurons [26]. Progressive damage to cellular macromolecules eventually leads to psychomotor retardation and retinal degeneration. It is noteworthy that ML4 cells exhibit intralysosomal iron overload and concurrent cellular iron deficiency. Cellular iron deficiency observed in ML4 cells may lead to dysregulation of the IRE–IRP regulatory system, further enhancing iron mislocalization and neurodegeneration. Thus, it is the cellular iron mislocalization, but not the overall cellular iron content, that causes neuronal cell death and degeneration.

■ Therapeutic targets

Two principal iron-related therapies for neurodegenerative disorders are anti-oxidant or chelation therapies. While anti-oxidant therapy aims to attenuate oxidative stress by decreasing the amount of ROS, chelation therapy is geared toward suppressing radical formation by decreasing the level of the catalyst, (i.e., iron). Desferrioxamine (DFO), a well-known iron chelator, has been shown to slow cognitive decline in Alzheimer's patients [29] and Clioquinol has demonstrated neuroprotective success in animal models of PD [31]. Most therapeutic agents, however, are hampered by pitfalls. For example, Clioquinol has notably neurotoxic side effects [30], and DFO crosses the BBB poorly [29].

Several new technologies may provide novel targeting methods for iron chelators. For example, DFO can be incorporated into drug-delivery systems with nanoparticles [92]. Iron chelator–nanoparticle systems allow therapeutic agents to cross the BBB by using low-density lipoprotein transport machinery for uptake [93]. This approach minimizes toxicity by allowing the chelator–nanoparticle complex to be transported, together with iron, out of the brain. These carrier systems may be able to efficiently deliver any iron-related therapeutic compound or transgenic fusion protein to the CNS, equivalent to a 'genetic iron chelator'. As iron accumulation in neurodegenerative diseases is often 'regional', research may eventually lead to targeting strategies specifically to regions of interest in the brain that are particularly vulnerable to iron accumulation and iron-mediated toxicity, such as the SN. Iron overload can also be a 'subcellular' problem, as seen in the ML4 cells, which exhibit cytosolic iron deficiency and

concurrent intralysosomal iron overload [26]. Therefore, iron chelators that target the lysosome, such as α-lipoic acid plus (LAP) may specifically reduce intralysosomal iron overload and subsequent lipofuscin formation [94]. Although LAP has the potential to cross the BBB, its conjugation with nanoparticles may greatly facilitate transport into and out of the brain. The specificity and efficacy of iron chelators can be tested in the aforementioned animal models.

Finally, gene therapy may provide an alternative neuroprotection strategy. Using genetic engineering approaches to express chimeric TfR fused to a peptidomimetic monoclonal antibody, Boado *et al.* demonstrate that TfR can serve as a viable drug-delivery mechanism to cross the BBB for therapeutic fusion proteins in mice [95]. Furthermore, Chang *et al.* have shown that Tf-bound nanoparticles interact with cells in a specific manner and enter cells via the caveolae pathway [92]. As the mechanisms of brain iron transport begin to emerge, new pharmacological targets and drug-carrier systems are likely to develop.

Future perspective

Several genetic disorders of iron metabolism clearly cause degenerative symptoms. These include inherited mutations in pantothenate kinase-2 leading to NBIA type I, mutations in *FLT1* (encoding L-Ft) leading to neuroferritinopathy, mutations in *PLA2G6* leading to INAD1, and mutations in *Cp* leading to aceruloplasminemia. Numerous neurodegenerative diseases have also been proposed as being related to changes in iron homeostasis, including PD, AD, Huntington's, Friedreich's ataxia, restless leg syndrome, ischemic/hemorrhagic stroke and multiple sclerosis. As a clearer picture of brain iron metabolism begins to come into focus, future research will be able to help develop iron-centric therapies for these CNS disorders. Continuing efforts should be made to gain a mechanistic understanding of iron transport across the abluminal membrane of BVECs, the roles of glia cells in regulating brain iron uptake and, finally, how neuronal iron accumulation may lead to neurodegeneration and CNS disease progression. Brain iron accumulation

Executive summary

- Excessive iron levels lead to oxidative stress and, in the brain, cause neurodegeneration.
- Nonheme iron can enter and exit mammalian cells by two distinct mechanisms: vesicular and nonvesicular.
- Dietary ferric iron is transported across intestinal enterocytes in a coordinated effort between the apical ferrireductase DcytB and the iron importer DMT1, followed by its export via ferroportin (Fpn) and a basolateral ferrioxidase, binding to transferrin (Tf) and entry into plasma.
- Tf-bound iron is taken into erythroid precursor cells by the membrane-bound transferrin receptor (TfR) via endocytosis, released in the early endosome, reduced by Steap3, and transported into the cytosol by DMT1, where it contributes to the labile iron pool, is transported to mitochondria, stored in ferritin or transported out of the cell via Fpn.
- Mutations in TRPML1, a protein involved in iron release from the late endosome and lysosomes, cause the neurodegenerative disease mucolipidosis type IV, which causes some patients to exhibit iron-deficiency anemia.
- The brain cannot directly take up iron from the circulatory system; rather, iron crosses brain vascular endothelia cells (BVECs) in a multistep transcellular process similar to that of enterocytes in the duodenum.
- The Tf–TfR system plays a primary role in iron uptake across the blood–brain barrier (BBB), while the extent to which iron is taken up by Tf homologues, such as lactoferrin or melanotransferrin, is still unclear.
- While there is controversy as to whether DMT1 is expressed in BVECs, TRPML1 has a ubiquitous expression pattern and is likely to play an important role in endolysosomal iron release. The role of other iron release proteins, such as ZIP8 and TRPML2, is not yet known.
- The iron-export mechanism across the abluminal membrane is undefined; however, both vesicular and nonvesicular exocytotic mechanisms may coexist in BVECs.
- As astrocytes are closely associated to the brain's vasculature and have a high capacity for iron accumulation, yet store little iron, they may be involved in brain iron distribution after uptake, acting as gatekeepers in regulating the iron transport properties of the BBB.
- Little iron is typically stored in neurons and, since neuronal iron is transported along axons and dendrites, iron overload may cause both neuronal cell death and axonal degeneration.
- Most detectable iron in the brain is found within oligodendrocytes, as is the high expression of the recently identified ferritin receptor (Tim-2), likely reflecting the high demand of iron required for axon myelination.
- Iron transport in the brain is governed by key proteins regulated post-transcriptionally by the iron-responsive element–iron regulatory proteins system.
- Cellular iron mislocalization, but not the overall cellular iron content, causes neuronal cell death and degeneration.
- New technologies for neuroprotection may provide novel targeting methods, including nanoparticle drug-delivery systems in conjunction with iron chelators as well as gene therapy strategies.

manifests at regional, cellular and subcellular levels. Understanding the cellular and intercellular transport mechanisms may not only reveal the pathogenic cause of neurodegenerative diseases, will also be necessary for designing effective therapeutic approaches to take advantage of newly developed drug-targeting methods.

Financial & competing interests disclosure

The work in the Xu laboratory is supported by startup funds to H Xu from the Department of MCDB and Biological Science Scholar Program, the University of Michigan, USA, an NIH RO1 grant (NS062792 to H Xu), pilot grants (to H Xu) from the UM Initiative on Rare Disease Research, Michigan Alzheimer's Disease Research Center (NIH grant P50-AG08671 to Gilman), and National Multiple Sclerosis Society. F Wang is supported by Chinese Academy of Sciences One Hundred Talents Program (KSCX2-YW-R-151) and National Basic Research Program of China (973 Program; 2009CB941400).

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript. We appreciate the encouragement and helpful comments from other members of the Xu laboratory.

Bibliography

1 Fillebeen C, Descamps L, Dehouck MP *et al.* Receptor-mediated transcytosis of lactoferrin through the blood–brain barrier. *J. Biol. Chem.* 274(11), 7011–7017 (1999).

2 Richardson DR, Ponka P. The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. *Biochim. Biophys. Acta.* 1331(1), 1–40 (1997).

3 Rothenberger S, Food MR, Gabathuler R *et al.* Coincident expression and distribution of melanotransferrin and transferrin receptor in human brain capillary endothelium. *Brain Res.* 712(1), 117–121 (1996).

4 Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. *Cell* 117(3), 285–297 (2004).

5 Moos T, Rosengren NT, Skjørringe T, Morgan EH. Iron trafficking inside the brain. *J. Neurochem.* 103(5), 1730–1740 (2007).

6 Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR. Oligodendrocytes and myelination: the role of iron. *Glia* 57(5), 467–478 (2009).

7 Ke Y, Qian ZM. Brain iron metabolism: neurobiology and neurochemistry. *Prog. Neurobiol.* 83(3), 149–173 (2007).

8 Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR. Iron, brain ageing and neurodegenerative disorders. *Nat. Rev. Neurosci.* 5(11), 863–873 (2004).

9 Lozoff B, Georgieff MK. Iron deficiency and brain development. *Semin. Pediatr. Neurol.* 13(3), 158–165 (2006).

10 Pollitt E. Early iron deficiency anemia and later mental retardation. *Am. J. Clin. Nutr.* 69(1), 4–5 (1999).

11 Schipper HM. Brain iron deposition and the free radical–mitochondrial theory of ageing. *Ageing Res. Rev.* 3(3), 265–301 (2004).

12 Lillig CH, Berndt C, Holmgren A. Glutaredoxin systems. *Biochim. Biophys. Acta.* 1780(11), 1304–1317 (2008).

13 MacKenzie EL, Iwasaki K, Tsuji Y. Intracellular iron transport and storage: from molecular mechanisms to health implications. *Antioxid. Redox Signal* 10(6), 997–1030 (2008).

14 Berg D, Hochstrasser H. Iron metabolism in Parkinsonian syndromes. *Mov. Disord.* 21(9), 1299–1310 (2006).

15 Lee DW, Andersen JK, Kaur D. Iron dysregulation and neurodegeneration: the molecular connection. *Mol. Interv.* 6(2), 89–97 (2006).

16 Abo-Krysha N, Rashed L. The role of iron dysregulation in the pathogenesis of multiple sclerosis: an Egyptian study. *Mult. Scler.* 14(5), 602–608 (2008).

17 Barnham KJ, Bush AI. Metals in Alzheimer's and Parkinson's diseases. *Curr. Opin. Chem. Biol.* 12(2), 222–228 (2008).

18 Moos T, Morgan EH. The metabolism of neuronal iron and its pathogenic role in neurological disease: review. *Ann. NY Acad. Sci.* 1012, 14–26 (2004).

19 Zhou B, Westaway SK, Levinson B, Johnson MA, Gitschier J, Hayflick SJ. A novel pantothenate kinase gene (PANK2) is defective in Hallervorden–Spatz syndrome. *Nat. Genet.* 28(4), 345–349 (2001).

20 Morgan NV, Westaway SK, Morton JE *et al.* PLA2G6, encoding a phospholipase A2, is mutated in neurodegenerative disorders with high brain iron. *Nat. Genet.* 38(7), 752–754 (2006).

21 Harris ZL, Durley AP, Man TK, Gitlin JD. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. *Proc. Natl Acad. Sci. USA* 96(19), 10812–10817 (1999).

22 Curtis AR, Fey C, Morris CM *et al.* Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. *Nat. Genet.* 28(4), 350–354 (2001).

23 Clardy SL, Wang X, Boyer PJ, Earley CJ, Allen RP, Connor JR. Is ferroportin–hepcidin signaling altered in restless legs syndrome? *J. Neurol. Sci.* 247(2), 173–179 (2006).

24 Hua Y, Keep RF, Hoff JT, Xi G. Brain injury after intracerebral hemorrhage: the role of thrombin and iron. *Stroke* 38(Suppl. 2), 759–762 (2007).

25 Sun M, Goldin E, Stahl S *et al.* Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. *Hum. Mol. Genet.* 9(17), 2471–2478 (2000).

26 Dong XP, Cheng X, Mills E *et al.* The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. *Nature* 455(7215), 992–996 (2008).

27 LaVaute T, Smith S, Cooperman S *et al.* Targeted deletion of the gene encoding iron regulatory protein-2 causes misregulation of iron metabolism and neurodegenerative disease in mice. *Nat. Genet.* 27(2), 209–214 (2001).

28 Rouault TA. The role of iron regulatory proteins in mammalian iron homeostasis and disease. *Nat. Chem. Biol.* 2(8), 406–414 (2006).

29 Crapper McLachlan DR, Dalton AJ, Kruck TP *et al.* Intramuscular desferrioxamine in patients with Alzheimer's disease. *Lancet* 337(8753), 1304–1308 (1991).

30 Benvenisti-Zarom L, Chen J, Regan RF. The oxidative neurotoxicity of clioquinol. *Neuropharmacology* 49(5), 687–694 (2005).

31 Kaur D, Yantiri F, Rajagopalan S *et al*. Genetic or pharmacological iron chelation prevents MPTP-induced neurotoxicity *in vivo*: a novel therapy for Parkinson's disease. *Neuron* 37(6), 899–909 (2003).

32 Salazar J, Mena N, Hunot S *et al*. Divalent metal transporter 1 (DMT1) contributes to neurodegeneration in animal models of Parkinson's disease. *Proc. Natl Acad. Sci. USA* 105(47), 18578–18583 (2008).

33 Sayre LM, Perry G, Smith MA. Redox metals and neurodegenerative disease. *Curr. Opin. Chem. Biol.* 3(2), 220–225 (1999).

34 Smith DG, Cappai R, Barnham KJ. The redox chemistry of the Alzheimer's disease amyloid β peptide. *Biochim. Biophys. Acta.* 1768(8), 1976–1990 (2007).

35 Patel BN, David S. A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. *J. Biol. Chem.* 272(32), 20185–20190 (1997).

36 Tsushima RG, Wickenden AD, Bouchard RA *et al*. Modulation of iron uptake in heart by L-type Ca^{2+} channel modifiers: possible implications in iron overload. *Circ. Res.* 84(11), 1302–1309 (1999).

37 Bradbury MW. Transport of iron in the blood–brain-cerebrospinal fluid system. *J. Neurochem.* 69(2), 443–454 (1997).

38 Savman K, Nilssonb UA, Thoresenc M, Kjellmera I. Non-protein-bound iron in brain interstitium of newborn pigs after hypoxia. *Dev. Neurosci.* 27(2–4), 176–184 (2005).

39 Petrat, FH, de Groot, Rauen U. Determination of the chelatable iron pool of single intact cells by laser scanning microscopy. *Arch. Biochem. Biophys.* 376(1), 74–81 (2000).

40 Andrews NC. Forging a field: the golden age of iron biology. *Blood* 112(2), 219–230 (2008).

41 De Domenico I, McVey Ward D, Kaplan J. Regulation of iron acquisition and storage: consequences for iron-linked disorders. *Nat. Rev. Mol. Cell Biol.* 9(1), 72–81 (2008).

42 Andrews NC, Schmidt PJ. Iron homeostasis. *Annu. Rev. Physiol*. 2007. 69, 69–85.

43 Garrick MD, Garrick LM. Cellular iron transport. *Biochim. Biophys. Acta*. 1790(5), 309–325 (2009).

44 McKie AT, Barrow D, Latunde-Dada GO *et al*. An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* 291(5509), 1755–1759 (2001).

45 Gunshin H, Mackenzie B, Berger UV *et al*. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388(6641), 482–488 (1997).

46 Donovan A, Brownlie A, Zhou Y *et al*. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 403(6771), 776–781 (2000).

47 Vulpe CD, Kuo YM, Murphy TL *et al*. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the SLA mouse. *Nat. Genet.* 21(2), 195–199 (1999).

48 Ohgami RS, Campagna DR, Greer EL *et al*. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nat. Genet.* 37(11), 1264–1269 (2005).

49 Shaw GC, Cope JJ, Li L *et al*. Mitoferrin is essential for erythroid iron assimilation. *Nature* 440(7080), 96–100 (2006).

50 Kidane TZ, Sauble E, Linder MC. Release of iron from ferritin requires lysosomal activity. *Am. J. Physiol. Cell Physiol.* 291(3), C445–C455 (2006).

51 Gunshin H, Fujiwara Y, Custodio AO, Direnzo C, Robine S, Andrews NC. Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. *J. Clin. Invest*. 115(5), 1258–1266 (2005).

52 Ohgami RS, Campagna DR, McDonald A, Fleming MD. The Steap proteins are metalloreductases. *Blood* 108(4), 1388–1394 (2006).

53 Lam-Yuk-Tseung S, Gros P. Distinct targeting and recycling properties of two isoforms of the iron transporter DMT1 (NRAMP2, Slc11A2). *Biochemistry* 45(7), 2294–2301 (2006).

54 Jabado N, Jankowski A, Dougaparsad S, Picard V, Grinstein S, Gros P. Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. *J. Exp. Med.* 192(9), 1237–1248 (2000).

55 Puertollano R, Kiselyov K. TRPMLs: in sickness and in health. *Am. J. Physiol. Renal Physiol.* 296(6), F1245–F1254 (2009).

56 Nguyen A, Zhao N, Morrison C *et al*. Mechanisms of iron release from lysosomes. *FASEB.* 921, 11 (2009).

57 Wang F, Paradkar PN, Custodio AO *et al*. Genetic variation in Mon1a affects protein trafficking and modifies macrophage iron loading in mice. *Nat. Genet.* 39(8), 1025–1032 (2007).

58 Zerial M, McBride H. Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell Biol.* 2(2), 107–117 (2001).

59 Pal A, Severin F, Lommer B, Shevchenko A, Zerial M. Huntingtin–HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease. *J. Cell Biol.* 172(4), 605–618 (2006).

60 Lumsden AL, Henshall TL, Dayan S, Lardelli MT, Richards RI. Huntingtin-deficient zebrafish exhibit defects in iron utilization and development. *Hum. Mol. Genet.* 16(16), 1905–1920 (2007).

61 Burdo JR, Menzies SL, Simpson IA *et al*. Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. *J. Neurosci. Res.* 66(6), 1198–1207 (2001).

62 Jefferies WA, Brandon MR, Hunt SV, Williams AF, Gatter KC, Mason DY. Transferrin receptor on endothelium of brain capillaries. *Nature* 312(5990), 162–163 (1984).

63 Abbott NJ, Ronnback L, Hansson E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nat. Rev. Neurosci.* 7(1), 41–53 (2006).

64 Moos T, Morgan EH. Evidence for low molecular weight, non-transferrin-bound iron in rat brain and cerebrospinal fluid. *J. Neurosci. Res.* 54(4), 486–494 (1998).

65 Burdo JR, Antonetti DA, Wolpert EB, Connor JR. Mechanisms and regulation of transferrin and iron transport in a model blood–brain barrier system. *Neuroscience* 121(4), 883–890 (2003).

66 Burdo JR, Connor JR. Brain iron uptake and homeostatic mechanisms: an overview. *Biometals* 16(1), 63–75 (2003).

67 Beard JL, Wiesinger JA, Li N, Connor JR. Brain iron uptake in hypotransferrinemic mice: influence of systemic iron status. *J. Neurosci. Res.* 79(1–2), 254–261 (2005).

68 Fisher J, Devraj K, Ingram J *et al*. Ferritin: a novel mechanism for delivery of iron to the brain and other organs. *Am. J. Physiol. Cell Physiol.* 293(2), C641–C649 (2007).

69 Li JY, Paragas N, Ned RM *et al*. Scara5 is a ferritin receptor mediating non-transferrin iron delivery. *Dev. Cell* 16(1), 35–46 (2009).

70 Todorich B, Zhang X, Slagle-Webb B, Seaman WE, Connor JR. Tim-2 is the receptor for H-ferritin on oligodendrocytes. *J. Neurochem.* 107(6), 1495–1505 (2008).

71 Siddappa AJ, Rao RB, Wobken JD, Leibold EA, Connor JR, Georgieff MK. Developmental changes in the expression of

iron regulatory proteins and iron transport proteins in the perinatal rat brain. *J. Neurosci. Res.* 68(6), 761–775 (2002).

72 Mackenzie B, Ujwal ML, Chang MH, Romero MF, Hediger MA. Divalent metal-ion transporter DMT1 mediates both H^+-coupled Fe2+ transport and uncoupled fluxes. *Pflugers Arch.* 451(4), 544–558 (2006).

73 Wu LJ, Leenders AG, Cooperman S *et al.* Expression of the iron transporter ferroportin in synaptic vesicles and the blood–brain barrier. *Brain Res.* 1001(1–2), 108–117 (2004).

74 Moos T, Skjoerringe T, Gosk S, Morgan EH. Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. *J. Neurochem.* 98(6), 1946–1958 (2006).

75 Donovan A, Lima CA, Pinkus JL *et al.* The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab.* 1(3), 191–200 (2005).

76 Montana V, Malarkey EB, Verderio C, Matteoli M, Parpura V. Vesicular transmitter release from astrocytes. *Glia* 54(7), 700–715 (2006).

77 Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by Ca^{2+}-regulated exocytosis of lysosomes. *Cell* 106(2), 157–169 (2001).

78 Dringen R, Bishop GM, Koeppe M, Dang TN, Robinson SR. The pivotal role of astrocytes in the metabolism of iron in the brain. *Neurochem. Res.* 32(11), 1884–1890 (2007).

79 Qian ZM, To Y, Tang PL, Feng YM. Transferrin receptors on the plasma membrane of cultured rat astrocytes. *Exp. Brain Res.* 129(3), 473–476 (1999).

80 Xu J, Ling EA. Studies of the ultrastructure and permeability of the blood–brain barrier in the developing corpus callosum in postnatal rat brain using electron dense tracers. *J. Anat.* 184 (2), 227–237 (1994).

81 Ham D, Schipper HM. Heme oxygenase-1 induction and mitochondrial iron sequestration in astroglia exposed to amyloid peptides. *Cell Mol. Biol.* 46(3), 587–596 (2000).

82 Jeong SY, David S. Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. *J. Biol. Chem.* 278(29), 27144–27148 (2003).

83 Kaneko K, Yoshida K, Arima K *et al.* Astrocytic deformity and globular structures are characteristic of the brains of patients with aceruloplasminemia. *J. Neuropathol. Exp. Neurol.* 61(12), 1069–1077 (2002).

84 Vargas JD, Herpers B, McKie AT. Stromal cell-derived receptor 2 and cytochrome b561 are functional ferric reductases. *Biochim. Biophys. Acta.* 1651(1–2), 116–123 (2003).

85 Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. *Proc. Natl Acad. Sci. USA* 103(37), 13612–13617 (2006).

86 Oudit GY, Sun H, Trivieri MG *et al.* L-type Ca^{2+} channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. *Nat. Med.* 9(9), 1187–1194 (2003).

87 Mwanjewe J, Grover AK. Role of transient receptor potential canonical 6 (TRPC6) in non-transferrin-bound iron uptake in neuronal phenotype PC12 cells. *Biochem. J.* 378(3), 975–982 (2004).

88 Kurz T, Terman A, Gustafsson B, Brunk UT. Lysosomes in iron metabolism, ageing and apoptosis. *Histochem. Cell Biol.* 129(4), 389–406 (2008).

89 Carlson ES, Tkac I, Magid R *et al.* Iron is essential for neuron development and memory function in mouse hippocampus. *J. Nutr.* 139(4), 672–679 (2009).

90 Hulet SW, Hess EJ, Debinski W *et al.* Characterization and distribution of ferritin binding sites in the adult mouse brain. *J. Neurochem.* 72(2), 868–874 (1999).

91 Taylor EM, Crowe A, Morgan EH. Transferrin and iron uptake by the brain: effects of altered iron status. *J. Neurochem.* 57(5), 1584–1592 (1991).

92 Chang J, Jallouli Y, Kroubi M *et al.* Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood–brain barrier. *Int. J. Pharm.* 379(2), 285–292 (2009).

93 Liu G, Men P, Kudo W, Perry G, Smith MA. Nanoparticle-chelator conjugates as inhibitors of amyloid-β aggregation and neurotoxicity: a novel therapeutic approach for Alzheimer disease. *Neurosci. Lett.* 455(3), 187–190 (2009).

94 Persson HL, Yu Z, Tirosh O, Eaton JW, Brunk UT. Prevention of oxidant-induced cell death by lysosomotropic iron chelators. *Free Radic. Biol. Med.* 34(10), 1295–1305 (2003).

95 Boado RJ, Zhang Y, Wang Y, Pardridge WM. Engineering and expression of a chimeric transferrin receptor monoclonal antibody for blood–brain barrier delivery in the mouse. *Biotechnol. Bioeng.* 102(4), 1251–1258 (2009).

96 Boado RJ, Zhang Y, Wang Y, Pardridge WM. Deficiency of pantothenate kinase 2 (Pank2) in mice leads to retinal degeneration and azoospermia. *Hum. Mol. Genet.* 14(1), 49–57 (2005).

97 Balsinde J, Balboa MA. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A2 in activated cells. *Cell Signal.* 17(9), 1052–1062 (2005).

98 De S, Trigueros MA, Kalyvas A, David S. Phospholipase A2 plays an important role in myelin breakdown and phagocytosis during Wallerian degeneration. *Mol. Cell Neurosci.* 24(3), 753–765 (2003).