

# Brain Iron Metabolism

Tracey A. Rouault, MD, and Sharon Cooperman MD, PhD

Brain iron uptake is regulated by the expression of transferrin receptor 1 in endothelial cells of the blood-brain barrier. Transferrin-bound iron in the systemic circulation is endocytosed by brain endothelial cells, and elemental iron is released to brain interstitial fluid, likely by the iron exporter, ferroportin. Transferrin synthesized by oligodendrocytes in the brain binds much of the iron that traverses the blood-brain barrier after oxidation of the iron, most likely by a glycoposphosinositide-linked ceruloplasmin found in astrocytic foot processes that ensheath the brain endothelial cells. Neurons acquire iron from diferric transferrin, but it is less clear how glial cells acquire iron. In aging mammals, iron accumulates in the basal ganglia, and iron accumulation is believed to contribute to neurodegenerative diseases, including Parkinson and Alzheimer disease. Here we consider the possibility that iron accumulations, which are often thought to facilitate free radical generation and oxidative damage, may contain insoluble iron that is unavailable for cellular use, and the pathology associated with iron accumulations may result from functional iron deficiency in some diseases.

Semin Pediatr Neurol 13:142-148 © 2006 Elsevier Inc. All rights reserved.

**KEYWORDS** blood brain barrier, transferrin receptor, ferroportin, GPI-linked ceruloplasmin, Parkinson disease

In mammals, iron is essential for the functions of many enzymes and prosthetic groups, including heme and iron-sulfur clusters. In the last decade, many of the proteins required for uptake of dietary iron from the duodenum, transport of iron in the serum, uptake of iron by individual cells, and retrieval of iron from senescent red cells have been identified. In the intestinal mucosa, a proton-coupled ferrous iron transporter, DMT1<sup>1</sup> (previously DCT1<sup>2</sup> or Nramp2<sup>3</sup>), is located on the apical membrane of the duodenal epithelium, the site of dietary iron uptake, along with a reductase known as Dcytb that is thought to reduce ferric (Fe<sup>3+</sup>) to ferrous (Fe<sup>2+</sup>) iron<sup>4</sup> for transport by DMT1. On the basolateral membrane, an iron exporter known as ferroportin<sup>5</sup> (also known as IREG1<sup>6</sup> or MTP1<sup>7</sup>) exports iron to the circulation aided by the membrane-bound ferroxidase hephaestin.<sup>8</sup> Duodenal mucosal cells also express a dedicated heme transporter, HCP1, that transports intact heme across the apical

epithelium,<sup>9</sup> and intact heme may also be transported across the basolateral membrane into the systemic circulation, although there is no direct evidence for basolateral heme transport.<sup>10,11</sup> Ferroportin, Dcytb, and HCP1 messenger RNA levels increase in iron-deficient animals, implying that these intestinal iron-uptake proteins are partially regulated at the transcriptional level.<sup>12</sup> When iron enters the mammalian circulation, it binds tightly to serum transferrin (Tf),<sup>13</sup> and cells acquire iron from serum by expressing transferrin receptor 1 (TfR1), which binds iron-loaded Tf<sup>14</sup> and internalizes the Tf-TfR complex in endosomes. Acidification of the endosome facilitates the release of ferric iron, and endosomal reductases<sup>15</sup> generate ferrous iron for transport into the cytosol by endosomal DMT1.

The cytosolic iron pool is highly regulated because it is an important source of iron for numerous cytosolic and nuclear iron proteins and is also the likely source from which mitochondria and other organelles derive iron. In developing erythroid cells, iron may bypass the cytosolic pool, moving directly from endosomes into mitochondria,<sup>16</sup> but in most cells, it appears that the iron needed by organelles is absorbed from the cytosol. A duplicate homologous pair of regulatory proteins known as iron regulatory proteins 1 and 2 (IRP1 and IRP2) sense cytosolic iron levels and regulate expression of genes that affect cytosolic iron levels, including expression of TfR, which increases iron uptake, and ferritin, which reduces

Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Supported by the intramural program of the National Institute of Child Health and Human Development.

Address reprint requests to Tracey A. Rouault, MD, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, 900 Rockville Pike, Bethesda, MD 20892. E-mail: trou@helix.nih.gov

cytosolic iron levels by sequestering iron. IRP1 registers cytosolic iron levels through an iron-sulfur “switch” mechanism, whereas the highly homologous IRP2 undergoes iron-dependent degradation in iron-replete cells. In iron-depleted cells, IRPs bind to RNA stem-loop elements found in the transcripts of ferritin, TfR1, ferroportin, mitochondrial aconitase, and erythroid ALA synthase, decreasing translation of proteins that would lower cytosolic iron levels by sequestering, using, or exporting iron, while simultaneously stabilizing the TfR transcript and thereby allowing increased TfR1 synthesis and iron uptake.<sup>17,18,19</sup>

In mammals, hepatocytes coordinately regulate absorption of dietary iron and reutilization of iron after senescent red cells are phagocytosed and digested by macrophages by secreting a regulatory peptide hormone, hepcidin. Hepatocytes gauge total-body iron stores by integrating information derived from iron uptake and signaling pathways, and they secrete hepcidin in direct proportion to hepatocytic iron stores. Macrophages regulate the return of iron to the circulation by regulating expression of ferroportin, which appears to be the sole mammalian iron exporter.<sup>20</sup> In the iron-replete liver, hepcidin transcription increases, and secreted hepcidin binds to ferroportin on the plasma membrane of duodenal epithelial cells and macrophages throughout the body, causing internalization and lysosomal degradation of ferroportin.<sup>21</sup> Decreased expression of intestinal ferroportin reduces intestinal iron absorption, and decreased expression of ferroportin in macrophages simultaneously reduces return of iron to the circulation. By decreasing iron absorption from the intestine and increasing macrophage iron sequestration, hepcidin causes a decrease in serum iron levels.<sup>22</sup>

The question of how hepatocytes regulate hepcidin transcription is a key issue in systemic iron homeostasis. To correctly assess iron levels, hepatocytes require function of several other proteins, including a second liver-specific transferrin receptor, TfR2,<sup>23</sup> the expression of which is not regulated by iron. In addition, appropriate hepatocytic hepcidin expression requires a TfR-binding protein, HFE<sup>24</sup> and hemojuvelin,<sup>25</sup> a coreceptor for bone morphogenetic protein that activates hepcidin transcription by using SMAD transcription factors.<sup>26</sup> Mutations in TfR2, HFE, and hemojuvelin (HJV) cause systemic iron overload by impairing normal hepcidin expression, and mutations in these genes cause different types of hereditary hemochromatosis.<sup>27</sup> Hepcidin deficiency and systemic iron overload also develop in mice that lack the transcriptional coactivator, SMAD 4, a member of the SMAD family of transcription factors responsible for signal transduction in the transforming growth factor (TGF)- $\beta$ -signaling pathway.<sup>28</sup>

Thus, the iron homeostasis of most mammalian tissues served by the systemic circulation, including bone marrow, kidney, muscle, and liver, is governed by a regulatory system in which the liver secretes hepcidin, which in turn coordinates iron metabolism by regulating iron absorption and reutilization in the multiple tissues served by the systemic circulation. The hepcidin-regulatory system ensures that Tf-bound iron is sufficiently abundant to meet tissue nutritional

needs, whereas individual cells appropriately regulate iron uptake mainly by altering TfR1 expression.

There are 3 sites in the mammalian body that are excluded from the liver-dependent macroregulatory axis: the central nervous system (CNS), testis, and retina. Each site is separated from the systemic circulation by a tight epithelial barrier analogous to that of the mammalian duodenum. Iron that enters any of these compartments must cross an apical and basolateral membrane before it disperses to various iron-consuming cells on the other side of the epithelium. The barrier that separates the CNS from the systemic circulation is known as the blood-brain barrier.<sup>29</sup>

## How Does Iron Cross the Blood-Brain Barrier and Move Within the CNS?

For many years, it was believed that iron entered the brain mainly during infancy before the blood-brain barrier matured. However, in the last decade, it has become apparent that brain-iron uptake is mediated by endothelial TfR expression in the blood-brain barrier of adult animals, and this TfR expression on the luminal endothelial surface is regulated by the iron status of the CNS. The blood-brain barrier is an unusual structure composed of endothelial cells, a basal lamina, pericytes, and astrocytic foot processes. In other parts of the body outside the CNS, fenestrations of the endothelial cells that line blood vessels allow serum substances to easily pass into interstitial fluids of tissues. However, in the CNS, endothelial cells are joined by tight junctions, and substances that enter the CNS must use dedicated endothelial transport systems.<sup>29</sup> An important key to blood-brain barrier formation is the interaction between astrocytes, star-shaped cells distributed throughout the CNS that extend long processes that ensheath blood vessels, and endothelial cells, which are induced to polarize by contacts with astrocytes. Unlike other blood vessel endothelia, the endothelial cells of the blood-brain barrier express receptors and proteins on the luminal endothelial membrane side of the systemic circulation that differ from those that are expressed on the abluminal membrane, which is surrounded by astrocytic foot processes, neuronal processes, and brain interstitial fluid. Pericytes found within the endothelial basement membrane near the endothelial tight junctions that seal the blood-brain barrier are crucial to barrier formation.<sup>30</sup> In animals that cannot express the platelet-derived growth factor receptor, pericytes are dysfunctional and the blood-brain barrier does not form correctly.<sup>31</sup>

The first step of iron entry into the CNS is mediated by TfR1 expressed on the luminal membrane of the endothelial cell (Figure 1).<sup>32-34</sup> Initially, it was thought that Tf that entered the cell with the aid of the TfR could be transcytosed and released from the abluminal membrane.<sup>35</sup> However, radiolabeling of Tf with <sup>60</sup>Fe iron showed that transferrin-bound iron enters the CNS, whereas systemic Tf is excluded from brain interstitial fluid.<sup>33,36</sup> The import of iron from endosomes is generally believed to depend on DMT1, but there

is some disagreement about whether DMT1 is expressed in the endothelial cells of the blood-brain barrier.<sup>37,38</sup> Once iron has been imported into the cytosol of endothelial cells, it can then be exported into the CNS by ferroportin, which has been immunohistochemically detected in the blood-brain barrier, although its exact membrane location has not been ascertained by immunoelectron microscopy.<sup>39</sup> The end-foot processes of astrocytes that contribute to the blood-brain barrier express a special form of the ferroxidase, ceruloplasmin. Through alternative splicing, astrocytes generate ceruloplasmin that attaches to the membrane via a glycosphosphoinositide (GPI) linkage.<sup>40</sup> The linkage of ceruloplasmin to membranes near its site of activity may allow the CNS to express ample ceruloplasmin without increasing the concentration of soluble proteins in brain interstitial fluid. Importantly, GPI-linked proteins can transfer to neighboring membranes, and perhaps GPI-linked ceruloplasmin facilitates ferroportin activity of endothelial cells by oxidizing newly released ferrous iron and allowing it to bind to the Tf in brain interstitial fluid.

In the CNS, Tf is synthesized and secreted mainly by oligodendrocytes, cells found throughout the CNS that elaborate the myelin sheathes around axons, and Tf transports iron throughout the CNS, and although the cells of the CNS are closely packed together, fluid moves in the interstitial areas between cells primarily by convection or bulk flow rather than simple diffusion.<sup>41</sup> Notably, in patients with aceruloplasminemia, iron accumulates in astrocytic foot processes in deposits known as "grumose foamy spheroid bodies."<sup>42</sup> Absence of ceruloplasmin or the related ferroxidase, hephaestin, at the blood-brain barrier would mean that ferrous iron would not be oxidized to ferric iron, the form that extracellular Tf binds, and the Tf secreted by oligodendrocytes could not transport iron to other sites in the brain interstitium. Glial cells such as astroglia are believed to have a metal import system that does not depend on Tf.<sup>43,44</sup> Thus, the absence of ceruloplasmin could allow excess ferrous iron to accumulate near astrocytic foot processes, and uptake of ferrous iron by nonspecific metal transport pathways in astrocytes could lead to the observed iron overload in the astrocytic foot processes of aceruloplasminemic patients.

Although blood vessels throughout brain parenchyma could theoretically permit the return of iron from the CNS interstitial fluid to the circulation, the well-established topology of TfR expression in the blood-brain barrier suggests that parenchymal blood vessels permit iron entry into the CNS, whereas iron likely exits the brain by crossing the arachnoid membrane in the arachnoid granulations and entering the venous drainage system.<sup>32</sup> The cerebrospinal fluid that fills the ventricles is elaborated by the choroids plexus, which consists of capillary tufts that protrude into ventricular spaces and are covered by a tightly connected layer of epithelial cells. Tf synthesized within the CNS does not return to the systemic circulation, most likely because egress from the CNS involves crossing the arachnoid membrane, a tight epithelial cell layer across which substances must be transported before they can reenter the systemic circulation.<sup>32,45</sup>

Diferric Tf is likely the main source of iron for neurons in

the CNS because both TfR1 and DMT1 are highly expressed in neurons.<sup>46,47</sup> Whether TfR1 is expressed in astrocytes,<sup>48</sup> oligodendrocytes,<sup>49</sup> and microglia<sup>50</sup> has been debated,<sup>46</sup> but it is important to remember that proteins expressed at low levels may be difficult to detect, and results may be difficult to interpret correctly. In addition, TfR2, a second TfR that is not regulated by iron,<sup>23</sup> is expressed in the brain according to the unigene expression profile for human TfR2, but it is not known which cells express TfR2 in the CNS. HFE, hephaestin,<sup>51</sup> and HJV<sup>52</sup> are also expressed in brain, but their expression patterns are also not yet well defined. Interestingly, HFE-/- mice have been reported to have a mild movement disorder,<sup>53</sup> and some patients with HFE hemochromatosis appear to be more likely to develop Parkinson disease.<sup>54,55</sup> Heparin is expressed in the spinal cord,<sup>56-58</sup> but the cell of origin is unknown. Interestingly, mutations in TfR2, HFE, HJV, and hepcidin are not known to cause diseases of the CNS, even though mutations in these genes cause iron overload in the periphery. The absence of apparent brain iron overload in mouse models of hemochromatosis<sup>53</sup> as well as in most humans with HFE-related hemochromatosis implies that regulation of brain iron homeostasis differs from the regulation of peripheral iron homeostasis. However, there are important similarities between peripheral and CNS regulation because TfR expression increases in brain endothelial cells of iron-deficient animals,<sup>33</sup> leading to appropriately increased iron uptake in the CNS of iron-deficient animals.

Iron that crosses the abluminal membrane of the blood-brain barrier endothelial cell binds to Tf in interstitial fluid, (Figure 1) but brain Tf concentrations are about 10% of serum Tf concentrations, and measurements of interstitial iron concentrations imply that the Tf may be highly saturated by iron; significant amounts of nontransferrin-bound iron (NTBI) may exist.<sup>59</sup> NTBI is rarely found in serum because Tf is in great excess, and NTBI can be uniquely damaging, perhaps because it can enter cells through unregulated channels. NTBI may be an important iron source for cells such as oligodendrocytes that do not express much TfR1.<sup>43</sup> It is also possible that NTBI enters astrocytes and neuronal processes and is transported across the brain by trafficking of intracellular metalloproteins. Astrocytes are connected by gap junctions, which can allow transport of ions between cells.<sup>60</sup> Manganese, which is similar to iron in many of its properties, can be directly imported by astrocytes.<sup>44</sup> Manganese transport in the olfactory system has been used to trace neuronal tracts that include multiple synapses.<sup>61,62</sup> If manganese can be transported along neuronal tracts, it is also theoretically possible that iron can be similarly transported, although experimental evidence does not support this possibility.<sup>63</sup> In addition, ferritin is present in the axons of normal neurons in the retina<sup>64,65</sup> and in the brain,<sup>66</sup> implying that there may be a trafficking mechanism that permits ferritin to carry iron from the neuronal cell body to the synapse. Ferroportin is present in synaptic vesicles, suggesting that ferrous iron may be released into synapses.<sup>39</sup> It is not clear how post-synaptic neurons or glial cells would take up elemental iron, but expression of DMT1 on the plasma membrane is a good candidate

for uptake of non-transferrin bound iron, as it can function at neutral pH<sup>67</sup> as well as in acidified endosomes.<sup>2</sup>

## Iron Redistribution With the Brain

After initial uptake in the brain, iron redistributes into various areas of the brain,<sup>68,69</sup> but the mechanisms by which iron redistributes in the brain are poorly understood. Studies in hypotransferrinemic mice suggest that redistribution of iron depends on the presence of intact Tf in brain interstitial fluid.<sup>70</sup> As previously stated, Tf synthesized by oligodendrocytes does not return to the systemic circulation,<sup>71</sup> presumably because CNS Tf cannot cross the tight epithelial cells of the arachnoid granulations in which fluid and ions exit the brain and return to the systemic circulation.<sup>29</sup>

During the aging process, iron accumulates in the substantia nigra, and iron accumulation during aging may contribute to the development of Parkinson and Alzheimer disease.<sup>72,73</sup> However, one of the most important unknowns about iron redistribution is that it is not known which cells accumulate iron within various regions of the brain. Increased iron levels have been measured repeatedly in the substantia nigra of Parkinson patients, but it is not clear whether a change in cellular composition, such as an increase in microglia or the small macrophages of the brain, accounts for the increase. In general, identifying cell types that accumulate iron in the brain is much more challenging than in other tissues because astrocytes, oligodendrocytes, and neurons have long processes, and it can be hard to discern where one cell ends and another begins.

Once acquired, it appears that the brain can conserve iron very well because brain iron contents decrease minimally in adult animals that develop severe systemic iron deficiency on a low-iron diet,<sup>74</sup> implying that mechanisms are in place to allow the brain to efficiently conserve its iron.<sup>32</sup> Although little is known about potential mechanisms for iron export from the brain, the fact that the arachnoid membrane contains tight junctions implies that egress of iron can be a regulated process, analogous to brain iron uptake. However, it is not known if the arachnoid membrane expresses TfR, DMT1, ferroportin, and other iron metabolism proteins. Immunoelectron microscopy and localization of these proteins in the arachnoid could greatly aid in formulation of hypotheses about brain iron homeostasis.

## Misregulation of Brain Iron Metabolism and Neurodegeneration

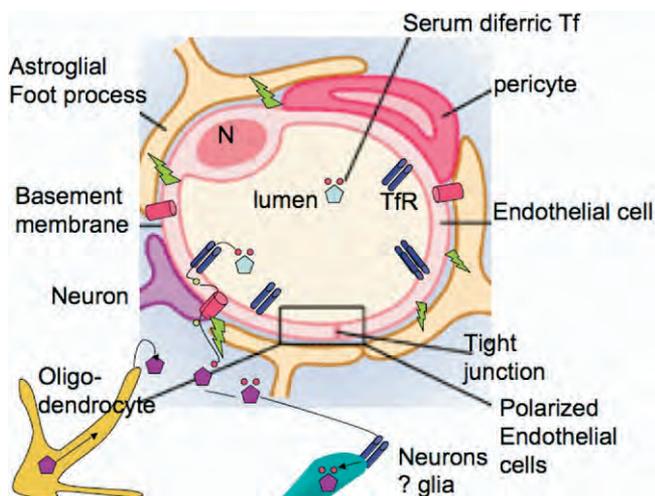
In addition to the observation that iron accumulates abnormally in the brain of patients with sporadic Parkinson and Alzheimer diseases, abnormal accumulations of iron are also found in diseases with known genetic causes, including aceruloplasminemia,<sup>75</sup> neurodegeneration with brain iron accumulation,<sup>76</sup> and neuroferritinopathy.<sup>77</sup> The usual hypothesis associated with these diseases is that abnormal ac-

cumulations of iron lead to increased formation of reactive oxygen species because ferrous iron reacts with endogenously generated hydrogen peroxide to yield damaging hydroxyl radicals.

Although it is possible that excess iron is the culprit, another intriguing possibility is that the iron observed in pathologic sections represents iron that is unavailable for use in normal cellular metabolism, and mislocalization of iron results in functional iron deficiency. An example of misregulation of iron metabolism resulting in functional iron deficiency occurs in animals with targeted deletions of IRP2.<sup>78</sup> In these animals, abnormal accumulations of ferric iron were detected in the cell bodies of oligodendrocytes and in their extensions. Axonal degeneration was present in areas in which increased ferric iron was detected by iron stains, and it initially seemed likely that the iron detected within the axonal framework, which was mainly sequestered in ferritin, could be a cause of axonal degeneration, with loss of neurofilament structures and collapse of the axon. However, on more rigorous analysis performed by using electron tomography, it turned out that the increased iron detected within degenerating axons was mainly contained in invaginations of oligodendrocytic processes into the space that should have been occupied by axonal structures.<sup>67</sup> The study confirmed that ferritin is present in normal axons, but it also revealed that increased ferritin in the axonal shaft of IRP2<sup>-/-</sup> animals was within oligodendrocyte processes. Total brain iron was not statistically increased,<sup>78</sup> and the nonheme iron content of IRP2<sup>-/-</sup> animals may be decreased (Rouault, TA, unpublished). Thus, increased ferric (3+) iron staining detected in the Prussian blue reaction may be misleading because it may represent aggregates of unavailable ferric iron in cells that lack sufficient ferrous iron. IRP2<sup>-/-</sup> animals have an iron-insufficiency anemia<sup>79,80</sup> associated with increased protoporphyrin IX levels. The iron-insufficiency anemia of IRP2<sup>-/-</sup> mice is probably caused by expression of inadequate levels of TfR on developing erythroid precursor cells to allow sufficient iron uptake for hematopoiesis. In addition, overexpression of ferritin likely results in the sequestration of iron making it unavailable for use in normal metabolism.<sup>79</sup> Thus, it is worth considering whether iron insufficiency associated with inappropriate sequestration of iron within ferritin or the ferritin breakdown product, hemosiderin, is a common problem in diseases in which “iron overload” has been reported. In human patients, measurements of cerebrospinal fluid iron and Tf saturations may be informative about brain iron status, but this procedure is very difficult to accomplish in mice because mice have so little cerebrospinal fluid and brain interstitial fluid. Although there are no reports of its detection, it would be interesting to measure hepcidin levels in the cerebrospinal fluid and brain interstitial fluid of humans and other mammals.

## Future Directions

The axis that governs brain iron homeostasis has been partially described because it seems clear that endothelial TfR determines how much iron the brain will absorb. However, it



**Figure 1** The blood-brain barrier is composed of endothelial cells joined by tight junctions surrounded by a basement membrane in which pericytes are found and which is in close apposition with astrocytic foot processes. TfR1 is expressed on the luminal membrane of endothelial cells, which have nuclei (N) and which likely express ferroportin (cylinders). Astrocytic foot processes express GPI-linked ceruloplasmin (lightening). On endocytosis of the Tf-TfR complex, ferric iron (dots) is reduced to ferrous iron (pale dot); exported to cytosol, most likely by DMT1; exported from the cell, presumably by ferroportin; and oxidized to ferric iron by GPI-linked ceruloplasmin within brain interstitium. Transferrin synthesized by oligodendrocytes in the brain binds ferric iron, and neurons and probably many other brain cells acquire iron by expressing the TfR, although TfR expression is low in nonneuronal cells. To exit the brain interstitial fluid and cerebrospinal fluid, iron must cross the arachnoid membrane (not shown), a tight epithelial layer that brain Tf does not cross. (Modified with permission from Francis K, Van Beek J, Canova C, et al: Innate immunity and brain inflammation: The key role of complement. *Expert Rev Mol Med* 2003;1-19, 2003.)<sup>86</sup> (Color version is available online.)

is unclear how the brain and spinal cord gauge iron status and communicate this information to the blood brain barrier. To identify candidates for this role, the cells that express TfR2 should be identified because cells that express TfR2 could be functionally similar to hepatocytes. In addition, identifying the site of hepcidin synthesis by *in situ* hybridization could also facilitate identification of important CNS iron-sensing cells. It is likely that the mechanisms that maintain homeostasis in the systemic circulation will be recreated with some modifications in the CNS (ie, a cell analogous to the hepatocyte will gauge iron availability and will secrete hepcidin according to iron status).

The mechanisms by which iron moves and accumulates in the brain require elucidation. Are there intracellular trafficking pathways? Why does iron accumulate in regions of the brain such as the globus pallidus and substantia nigra? Do the basal ganglia function as iron repositories in the central nervous system? The highest levels of iron in the brain are found in the globus pallidus, followed by the putamen, substantia nigra, and caudate nucleus.<sup>32</sup> Notably, iron concentrations in these brain regions are comparable to iron concentrations in

the mammalian liver, a recognized iron-storage tissue that supplies iron to tissues served by the systemic circulation. A possible reason for high iron concentrations in the substantia nigra is that tyrosine hydroxylase,<sup>81</sup> crucial in dopamine synthesis, is an iron enzyme.<sup>82</sup> The substantia nigra appears to be particularly vulnerable to iron deficiency,<sup>83</sup> which can impair dopamine production and cause motor problems in adults.<sup>84</sup>

Can iron export from the brain be accurately measured and is export regulated? Are TfR1, TfR2, hemojuvelin, and HFE expressed on the arachnoid membrane? Does NTBI in the brain have a significant function, and, if so, how does the brain protect itself from the reactivity and toxicity of unbound ferrous and ferric iron?

The fact that iron misregulation is often observed in neurodegenerative diseases underscores the need to understand basic brain iron homeostasis. Application of high-resolution imaging technologies such as electron energy-loss spectroscopy<sup>85</sup> and electron tomography<sup>67</sup> may permit identification of the cell type, intracellular location, and bioavailability of iron deposits in patients with Parkinson disease, NBIA, and aceruloplasminemia. High-resolution imaging of iron deposits in patient samples before the onset of end-stage disease could help to guide future research on iron misregulation and neurodegeneration by focusing attention on the cells and organelles that accumulate iron early in disease.

## References

- Andrews NC: Metal transporters and disease. *Curr Opin Chem Biol* 6:181-186, 2002
- Gunshin H, Mackenzie B, Berger UV, et al: Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 388:482-488, 1997
- Fleming MD, Trenor CC, Su MA, et al: Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* 16:383-386, 1997
- McKie AT, Barrow D, Latunde-Dada GO, et al: An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* 291:1755-1759, 2001
- Donovan A, Brownlie A, Zhou Y, et al: Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 403:776-781, 2000
- McKie AT, Marciani P, Rolfs A, et al: A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell* 5:299-309, 2000
- Abboud S, Haile DJ: A novel mammalian iron-regulated protein involved in intracellular iron metabolism. *J Biol Chem* 275:19906-19912, 2000
- 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* 21:195-199, 1999
- Shayeghi M, Latunde-Dada GO, Oakhill JS, et al: Identification of an intestinal heme transporter. *Cell* 122:789-801, 2005
- Quigley JG, Yang Z, Worthington MT, et al: Identification of a human heme exporter that is essential for erythropoiesis. *Cell* 118:757-766, 2004
- Latunde-Dada GO, Simpson RJ, McKie AT: Recent advances in mammalian haem transport. *Trends Biochem Sci* 31:182-188, 2006
- Rouault TA: The intestinal heme transporter revealed. *Cell* 122:649-651, 2005
- Aisen P: Transferrin, the transferrin receptor, and the uptake of iron by cells. *Met Ions Biol Syst* 35:585-631, 1998
- Aisen P: Transferrin receptor 1. *Int J Biochem Cell Biol* 36:2137-2143, 2004
- Ohgami RS, Campagna DR, Greer EL, et al: Identification of a ferric-

- ductase required for efficient transferrin-dependent iron uptake in erythroid cells. *Nat Genet* 37:1264-1269, 2005
16. Napier I, Ponka P, Richardson DR: Iron trafficking in the mitochondrion: novel pathways revealed by disease. *Blood* 105:1867-1874, 2005
  17. Pantopoulos K: Iron metabolism and the IRE/IRP regulatory system: An update. *Ann N Y Acad Sci* 1012:1-13, 2004
  18. Hentze MW, Muckenthaler MU, Andrews NC: Balancing acts: Molecular control of mammalian iron metabolism. *Cell* 117:285-297, 2004
  19. Rouault TA: The role of iron regulatory proteins in mammalian iron homeostasis and disease. *Nat Chem Biol* 2:406-414, 2006
  20. Donovan A, Lima CA, Pinkus JL, et al: The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab* 1:191-200, 2005
  21. Nemeth E, Tuttle MS, Powelson J, et al: Hfeclidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 306:2090-2093, 2004
  22. Ganz T, Nemeth E: Iron imports.IV. Hfeclidin and regulation of body iron metabolism *Am J Physiol Gastrointest Liver Physiol* 290:G199-G203, 2006
  23. Kawabata H, Yang R, Hiramata T, et al: Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family. *J Biol Chem* 274:20826-20832, 1999
  24. Fleming RE, Britton RS: Iron Imports. VI. HFE and regulation of intestinal iron absorption *Am J Physiol Gastrointest Liver Physiol* 290:G590-G594, 2006
  25. Papanikolaou G, Samuels ME, Ludwig EH, et al: Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nat Genet* 36:77-82, 2004
  26. Babbitt JL, Huang FW, Wrighting DM, et al: Bone morphogenetic protein signaling by hemojuvelin regulates hfeclidin expression. *Nat Genet* 38:531-539, 2006
  27. Beutler E: Hemochromatosis: Genetics and pathophysiology. *Annu Rev Med* 57:331-347, 2006
  28. Wang RH, Li C, Xu X, et al: A role of SMAD4 in iron metabolism through the positive regulation of hfeclidin expression. *Cell Metab* 2:399-409, 2005
  29. Abbott NJ, Ronnback L, Hansson E: Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 7:41-53, 2006
  30. Lai CH, Kuo KH: The critical component to establish in vitro BBB model: Pericyte. *Brain Res Brain Res Rev* 50:258-265, 2005
  31. Hellstrom M, Gerhardt H, Kalen M, et al: Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol* 153:543-553, 2001
  32. Bradbury MW: Transport of iron in the blood-brain-cerebrospinal fluid system. *J Neurochem* 69:443-454, 1997
  33. Moos T, Morgan EH: Transferrin and transferrin receptor function in brain barrier systems. *Cell Mol Neurobiol* 20:77-95, 2000
  34. Enerson BE, Drewes LR: The rat blood-brain barrier transcriptome. *J Cereb Blood Flow Metab* 26:959-973, 2005
  35. Burdo JR, Antonetti DA, Wolpert EB, et al: Mechanisms and regulation of transferrin and iron transport in a model blood-brain barrier system. *Neuroscience* 121:883-890, 2003
  36. Beard JL, Wiesinger JA, Li N, et al: Brain iron uptake in hypotransferrinemic mice: Influence of systemic iron status. *J Neurosci Res* 79:254-261, 2005
  37. 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* 66:1198-1207, 2001
  38. Moos T, Morgan EH: The significance of the mutated divalent metal transporter (DMT1) on iron transport into the Belgrade rat brain. *J Neurochem* 88:233-245, 2004
  39. Wu LJ, Leenders AG, Cooperman S, et al: Expression of the iron transporter ferroportin in synaptic vesicles and the blood-brain barrier. *Brain Res* 1001:108-117, 2004
  40. Jeong SY, David S: Glycosylphosphatidylinositol-anchored ceruloplasmin is required for iron efflux from cells in the central nervous system. *J Biol Chem* 278:27144-27148, 2003
  41. Oide T, Yoshida K, Kaneko K, et al: Iron overload and antioxidative role of perivascular astrocytes in aceruloplasminemia. *Neuropathol Appl Neurobiol* 32:170-176, 2006
  42. Brightman MW, Kaya M: Permeable endothelium and the interstitial space of brain. *Cell Mol Neurobiol* 20:111-130, 2000
  43. Takeda A, Devenyi A, Connor JR: Evidence for non-transferrin-mediated uptake and release of iron and manganese in glial cell cultures from hypotransferrinemic mice. *J Neurosci Res* 51:454-462, 1998
  44. Erikson KM, Aschner M: Increased manganese uptake by primary astrocyte cultures with altered iron status is mediated primarily by divalent metal transporter. *Neurotoxicology* 27:125-130, 2006
  45. Abbott NJ: Dynamics of CNS barriers: Evolution, differentiation, and modulation. *Cell Mol Neurobiol* 25:5-23, 2005
  46. Connor JR, Menzies SL: Cellular management of iron in the brain. *J Neurol Sci* 134(Suppl):33-44, 1995
  47. Moos T: Immunohistochemical localization of intraneuronal transferrin receptor immunoreactivity in the adult mouse central nervous system. *J Comp Neurol* 375:675-692, 1996
  48. Hoepken HH, Korten T, Robinson SR, et al: Iron accumulation, iron-mediated toxicity and altered levels of ferritin and transferrin receptor in cultured astrocytes during incubation with ferric ammonium citrate. *J Neurochem* 88:1194-1202, 2004
  49. Dickinson TK, Connor JR: Immunohistochemical analysis of transferrin receptor: Regional and cellular distribution in the hypotransferrinemic (hpx) mouse brain. *Brain Res* 801:171-181, 1998
  50. Kaur C, Ling EA: Increased expression of transferrin receptors and iron in amoeboid microglial cells in postnatal rats following an exposure to hypoxia. *Neurosci Lett* 262:183-186, 1999
  51. Hahn P, Qian Y, Dentchev T, et al: Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. *Proc Natl Acad Sci U S A* 101:13850-13855, 2004
  52. Rodriguez Martinez A, Niemela O, Parkkila S: Hepatic and extrahepatic expression of the new iron regulatory protein hemojuvelin. *Haematologica* 89:1441-1445, 2004
  53. Golub MS, Germann SL, Araiza RS, et al: Movement disorders in the Hfe knockout mouse. *Nutr Neurosci* 8:239-244, 2005
  54. Dekker MC, Giesbergen PC, Njajou OT, et al: Mutations in the hemochromatosis gene (HFE), Parkinson's disease and parkinsonism. *Neurosci Lett* 348:117-119, 2003
  55. Costello DJ, Walsh SL, Harrington HJ, et al: Concurrent hereditary haemochromatosis and idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 75:631-633, 2004
  56. Park CH, Valore EV, Waring AJ, et al: Hfeclidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 276:7806-7810, 2001
  57. Nicolas G, Chauvet C, Viatte L, et al: The gene encoding the iron regulatory peptide hfeclidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 110:1037-1044, 2002
  58. Cardarelli G, Anatra GM: Hfeclidin: A key peptide in iron metabolism. *Minerva Med* 94:135-139, 2003
  59. Moos T, Morgan EH: Evidence for low molecular weight, non-transferrin-bound iron in rat brain and cerebrospinal fluid. *J Neurosci Res* 54:486-494, 1998
  60. Bennett MV, Contreras JE, Bukauskas FF, et al: New roles for astrocytes: gap junction hemichannels have something to communicate. *Trends Neurosci* 26:610-617, 2003
  61. Slood WN, Gramsbergen JB: Axonal transport of manganese and its relevance to selective neurotoxicity in the rat basal ganglia. *Brain Res* 657:124-132, 1994
  62. Tjalve H, Henriksson J: Uptake of metals in the brain via olfactory pathways. *Neurotoxicology* 20:181-195, 1999
  63. Rao DB, Wong BA, McManus BE, et al: Inhaled iron, unlike manganese, is not transported to the rat brain via the olfactory pathway. *Toxicol Appl Pharmacol* 193:116-126, 2003
  64. Hahn P, Dentchev T, Qian Y, et al: Immunolocalization and regulation of iron handling proteins ferritin and ferroportin in the retina. *Mol Vis* 10:598-607, 2004
  65. Dentchev T, Hahn P, Dunaief JL: Strong labeling for iron and the iron-handling proteins ferritin and ferroportin in the photoreceptor

- layer in age-related macular degeneration. *Arch Ophthalmol* 123:1745-1746, 2005
66. Zhang P, Land W, Lee S, et al: Electron tomography of degenerating neurons in mice with abnormal regulation of iron metabolism. *J Struct Biol* 150:144-153, 2005
67. Mackenzie B, Ujwal ML, Change MH, Romero MF, Hediger MA: Divalent metal-ion transporter DMT1 mediates both H<sup>+</sup>-coupled Fe<sup>2+</sup> transport and uncoupled fluxes. *Pflugers Arch* 451:544-558, 2006
68. Dwork AJ, Lawler G, Zybert PA, et al: An autoradiographic study of the uptake and distribution of iron by the brain of the young rat. *Brain Res* 518:31-39, 1990
69. Dwork AJ: Effects of diet and development upon the uptake and distribution of cerebral iron. *J Neurol Sci* 134(Suppl):45-51, 1995
70. Malecki EA, Cook BM, Devenyi AG, et al: Transferrin is required for normal distribution of <sup>59</sup>Fe and <sup>54</sup>Mn in mouse brain. *J Neurol Sci* 170:112-118, 1999
71. de Arriba Zerpa GA, Saleh MC, Fernandez PM, et al: Alternative splicing prevents transferrin secretion during differentiation of a human oligodendrocyte cell line. *J Neurosci Res* 61:388-395, 2000
72. Bartzokis G, Tishler TA, Shin IS, et al: Brain ferritin iron as a risk factor for age at onset in neurodegenerative diseases. *Ann N Y Acad Sci* 1012:224-236, 2004
73. Zecca L, Youdim MB, Riederer P, et al: Iron, brain ageing and neurodegenerative disorders. *Nat Rev Neurosci* 5:863-873, 2004
74. Beard JL, Felt B, Schallert T, et al: Moderate iron deficiency in infancy: Biology and behavior in young rats. *Behav Brain Res* 170:224-232, 2006
75. Xu X, Pin S, Gathinji M, et al: Aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis. *Ann N Y Acad Sci* 1012:299-305, 2004
76. Gregory A, Hayflick SJ: Neurodegeneration with brain iron accumulation. *Folia Neuropathol* 43:286-296, 2005
77. 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* 28:350-354, 2001
78. LaVaute T, Smith S, Cooperman S, et al: Targeted deletion of iron regulatory protein 2 causes misregulation of iron metabolism and neurodegenerative disease in mice. *Nat Genet* 27:209-214, 2001
79. Cooperman SS, Meyron-Holtz EG, Olivier-Wilson H, et al: Microcytic anemia, erythropoietic protoporphyria, and neurodegeneration in mice with targeted deletion of iron-regulatory protein 2. *Blood* 106:1084-1091, 2005
80. Galy B, Ferring D, Minana B, et al: Altered body iron distribution and microcytosis in mice deficient in iron regulatory protein 2 (IRP2). *Blood* 106:2580-2589, 2005
81. Frantom PA, Seravalli J, Ragsdale SW, et al: Reduction and oxidation of the active site iron in tyrosine hydroxylase: Kinetics and specificity. *Biochemistry* 45:2372-2379, 2006
82. Perry TL, Norman MG, Yong VW, et al: Hallervorden-Spatz disease: Cysteine accumulation and cysteine dioxygenase deficiency in the globus pallidus. *Ann Neurol* 18:482-489, 1985
83. Beard JL, Erikson KM, Jones BC: Neurobehavioral analysis of developmental iron deficiency in rats. *Behav Brain Res* 134:517-524, 2002
84. Levenson CW, Cutler RG, Ladenheim B, et al: Role of dietary iron restriction in a mouse model of Parkinson's disease. *Exp Neurol* 190:506-514, 2004
85. Leapman RD: Detecting single atoms of calcium and iron in biological structures by electron energy-loss spectrum-imaging. *J Microsc* 210:5-15, 2003
86. Francis K, Van Beek J, Canova C, et al: Innate immunity and brain inflammation: the key role of complement. *Expert Rev Mol Med* 2003:1-19, 2003