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Manganese Homeostasis in the Nervous System

Pan Chen[1], **Sudipta Chakraborty**[2], **Somshuvra Mukhopadhyay**[3], **Eunsook Lee**[4], **Monica MB Paoliello**[5], **Aaron B Bowman**[6], and **Michael Aschner**[2]

[1]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY, USA

[2]Neuroscience Graduate Program, Vanderbilt University Medical Center, Nashville, TN, USA

[3]Division of Pharmacology & Toxicology, College of Pharmacy; Institute for Cellular & Molecular Biology; and Institute for Neuroscience, The University of Texas at Austin Austin, TX, USA

[4]Department of Physiology, Meharry Medical College, Nashville, TN, USA

[5]Graduate Program in Public Health, Department of Pathology, Clinical and Toxicological Analysis, Center of Health Science, State University of Londrina, Parana, Brazil

[6]Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, USA

Abstract

Manganese (Mn) is an essential heavy metal that is naturally found in the environment. Daily intake through dietary sources provides the necessary amount required for several key physiological processes, including antioxidant defense, energy metabolism, immune function and others. However, overexposure from environmental sources can result in a condition known as manganism that features symptomatology similar to Parkinson's disease (PD). This disorder presents with debilitating motor and cognitive deficits that arise from a neurodegenerative process. In order to maintain a balance between its essentiality and neurotoxicity, several mechanisms exist to properly buffer cellular Mn levels. These include transporters involved in Mn uptake, and newly discovered Mn efflux mechanisms. This review will focus on current studies related to mechanisms underlying Mn import and export, primarily the Mn transporters, and their function and roles in Mn-induced neurotoxicity.

Introduction

Manganese (Mn) is a heavy metal found naturally in the earth's crust. This essential metal is the 12th most abundant element and typically exists as oxides, carbonates and silicates. Earth erosion results in the pervasive presence of Mn in air, soil and waterways. Moreover, the natural properties of Mn have resulted in its extensive use in several industrial settings. Mn is used in the manufacturing of batteries, ceramics, steel, cosmetics, leather, fireworks, glass and other textiles. Mn is also a component of an antiknock gasoline additive, known as methylcyclopentadienyl Mn tricarbonyl (MMT), and combustion results in release of Mn phosphates into the ambient air. Additionally, Mn can be found in pesticides and fungicides, smoke inhibitors, and as a contrast reagent for medical magnetic resonance imaging (MRI)

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purposes (ATSDR 2008). Furthermore, in neonates receiving total parenteral nutrition, the addition of a Mn-containing trace element solution causes a 100-fold increase in the Mn burden compared to those human milk (Aschner & Aschner 2005). Excess Mn exposure is also a concern in drug addicts who illicitly abuse methcathinone, a substance produced from the oxidation of ephedrine and pseudoephedrine via potassium permanganate. Intravenous usage of methcathinone can expose individuals to high levels of Mn derived from the potassium permanganate, which is used as an oxidant in the synthesis of methcathonine (Sikk *et al.* 2013).

Despite the abundance of Mn in the environment, the primary route of typical human Mn intake is through dietary sources. Mn is found in several foods that compose daily human diets. Legumes, nuts, rice and whole grains contain the highest levels of Mn, while leafy green vegetables, tea, chocolate and some fruits contain moderate levels. Mn is found as a component of some daily multivitamins. The plentiful dietary sources of Mn help ensure adequate levels are reached in humans, with 2.3 mg/day required for men and 1.8 mg/day for women (Aschner & Aschner 2005). The requirement of daily Mn uptake is reflected in its role as a necessary cofactor for several important enzymes, including glutamine synthetase, arginase, pyruvate carboxylase and Mn superoxide dismutase (MnSOD). These metalloproteins are crucial for several enzymatic processes that help regulate development, energy metabolism, digestion, immune function, reproduction and antioxidant defenses (Kanyo *et al.* 1996, Jitrapakdee *et al.* 2008, Reddi *et al.* 2009, Wedler *et al.* 1982).

The long list of Mn-containing foods, as well as its presence in multivitamins, makes Mn deficiency a rare problem. Moreover, only 3-5% of ingested Mn is absorbed through the gastrointestinal tract (Finley *et al.* 1994, Davis *et al.* 1993). However, certain groups can be susceptible to excess Mn from nutritional sources. These include unhealthy neonates receiving total parenteral nutrition (TPN), which is typically supplemented with a trace element solution containing Mn. Importantly, intravenous TPN administration bypasses the gastrointestinal control of Mn absorption, resulting in 100% Mn retention (Aschner & Aschner 2005). Another population at risk of nutritional exposure to excess Mn includes patients suffering from hepatic encephalopathy and/or liver failure, as Mn is excreted from the body predominantly through the biliary system (Zeron *et al.* 2011, Klos *et al.* 2005). Finally, individuals with iron (Fe) deficiency (e.g. iron deficiency anemia), a highly prevalent nutritional condition, are at risk for increased Mn body burden, because Mn and Fe use common transporters for uptake, and Fe deficiency increases the expression of these transport systems (Smith *et al.* 2013).

In addition to nutritional toxicity, excess Mn exposure occurs in occupational settings. Mn-containing fumes, especially in poorly ventilated spaces, can directly affect welders, smelters and other industrial workers (Park 2013). Studies have also found cognitive deficits in populations living close to factories and refineries with high Mn levels (Guarneros *et al.* 2013, Lucchini *et al.* 2012). Regardless of the source, excess Mn exposure can lead to a state of Mn poisoning known as “manganism,” an irreversible, progressive condition that resembles Parkinson's disease (PD). PD is a neurodegenerative, motor disorder that results from selective loss of the dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNpc) (Lees *et al.* 2009). In contrast, manganism initially targets a different brain

region, as Mn shows preferential accumulation in the globus pallidus, but also affects the SNpc to a lesser extent. Early stages of both conditions are characterized by cognitive and emotional problems, including intellectual deficits, mood changes, irritability, restlessness and sleep disturbances (Guilarte 2010). However, likely due to the difference in primary target sites, the motor symptoms are distinctive between the two conditions. The hallmark symptoms of PD include bradykinesia, tremor, rigidity and postural instability (Lees et al. 2009). On the other hand, while still showing signs of rigidity, Mn toxicity typically presents with dystonia, a more upright stance, milder tremors at rest and a signature “cock-like” walk (Guilarte 2010). Another distinguishing feature is the lack of the efficacy that levodopa has in treating patients suffering from manganism (Koller *et al.* 2004). Current treatment options utilize a combination of levodopa and chelation therapy with either edetate calcium disodium (EDTA) (Herrero Hernandez *et al.* 2006) or the tuberculosis antibiotic para-aminosalicylic acid (PAS) (Jiang *et al.* 2006). However, beneficial effects are transient in nature and thereby require prolonged use and high dosage if the source of exposure is not removed.

In order to maintain balance between the essentiality of Mn and its neurotoxicity, several transport mechanisms exist to buffer Mn levels for proper homeostasis. In this review, we will highlight the major uptake mechanisms followed by the more recently discovered efflux mechanisms.

Manganese Import

Mn enters cells through multiple transporters, including the divalent metal transporter 1 (DMT1), the zinc transporters ZIP8 and ZIP14, the citrate transporter, the choline transporter, the dopamine transporter (DAT), the transferrin receptor (TfR) and calcium (Ca) channels (Chen *et al.* 2014). Among these importers, DMT1 is the primary transporter for divalent Mn and TfR is the primary transporter for trivalent Mn. Notably, in addition to Mn, these transporters also transport other metals, such as Fe, copper (Cu), zinc (Zn), and calcium (Ca), to name a few. Given that excessive cytosolic Mn is toxic to cells, mechanisms regulating the activity and expression of these transporters are noteworthy to investigate within the context of Mn-induced cytotoxicity.

DMT1

DMT1 is also known as natural resistance-associated macrophage protein 2 (NRAMP 2) or divalent cation transporter 1 (DCT1), and is encoded by the *SLC11A2* (solute carrier family 11, member 2) gene in humans (Vidal *et al.* 1995). DMT1 was first identified and characterized in rats and shown to mediate transport of a wide range of substrates, including divalent Fe, Zn, Mn, cobalt (Co), cadmium (Cd), Cu, nickel (Ni) and lead (Pb) (Salazar *et al.* 2008, Garrick *et al.* 2006). DMT1 is highly expressed in the basal ganglia of the brain, including SN, GP, hypothalamic nucleus and striatum (Huang *et al.* 2004, Williams *et al.* 2000, Burdo *et al.* 2001), where high levels of Mn accumulation are noted. In addition, its expression level may increase with age (Ke *et al.* 2005), which may explain the adult-onset (∼ 46 years) of occupational manganism in welders (Racette *et al.* 2001). DMT1 has a higher transport affinity for Mn than Fe. Indeed, its transport affinities for metals are as follows: Mn>Cd>Fe>Pb∼Co∼Ni>Zn (Garrick et al. 2006). DMT1 preferentially transports

Mn into the brain through the blood-brain barrier (BBB), especially under low Fe conditions [due to iron-responsive element (IRE)-mediated regulation], which increases DMT1 expression (Erikson *et al.* 2005b, Crossgrove & Yokel 2004). In addition, DMT1 also transports Mn from endosomes into cytosol in a TfR dependent manner, which is discussed below.

Changes in DMT1 are associated with several diseases. In the Han Chinese population, the CC haplotype in the DMT1 gene is associated with increased susceptibility for PD (He *et al.* 2011b). In PD patients, increased expression of DMT1 has been found in the SNpc of brains (Kong *et al.* 2014, Tsunemi & Krainc 2014, Peng *et al.* 2009, Peng *et al.* 2007). In the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of PD, it is reported that DMT1 expression is upregulated, associated with Fe accumulation, as well as increased oxidative stress and DAergic cell death (Salazar et al. 2008). In addition to PD, alterations in DMT1 are associated with spinal onset amyotrophic lateral sclerosis (ALS) (Blasco *et al.* 2011), Alzheimer's disease (AD) onset in males (Jamieson *et al.* 2005), iron anemia and restless legs syndrome (RLS) (Xiong *et al.* 2007). Although Fe has been implicated to play an important role in these diseases, data on Fe accumulation in the brain in these diseases is lacking. Whether DMT1-induced changes in Mn contribute to the onset of any of these diseases remains to be investigated.

Transferrin/Trasnferrin Receptor

While DMT1 transports primarily divalent Mn, the transferrin (Tf)/transferrin receptor (TfR) system is responsible for transporting trivalent Mn, which consists of about 20% of total blood Mn. Tf is synthesized in the liver and secreted into the blood plasma (Aisen *et al.* 1978), where it binds Mn^{3+}. The TfR is expressed in most cells, including neurons, microglia, astrocytes and the endothelial cells of the BBB (Moos & Morgan 2000). Moreover, it has five IREs that sense cytosolic Fe/Mn levels and accordingly regulate the expression of TfR on the plasma membrane (Subramaniam *et al.* 2002). Tf-bound Mn in the blood is transported by TfR into cells through the ligand-receptor endocytosis mechanism. Using a fluorescent label bound to the Mn^{3+}Tf complex, Gunter and colleagues observed the endocytic transport of Mn^{3+}/Tf through TfR into mouse hippocampal and striatal neuronal cells and into a region adjacent to the mitochondrial network, presumably endosomes (Gunter *et al.* 2013). Further, available results suggest that, after endocytosis, as endosomes undergo acidification, Mn^{3+} is released from the Tf/TfR complex (Roth *et al.* 2002, Tuschl *et al.* 2013, Gunter et al. 2013). Endosomal Mn^{3+} is then reduced to Mn^{2+} by ferrireductase (probably to avoid Mn^{3+} transport to the cytosol, which can cause oxidative stress), and the Mn^{2+} is transported to the cytosol by endosomal DMT1 (Tuschl et al. 2013, Au *et al.* 2008).

Other Manganese Importers

Zinc transporters ZIP8 and ZIP14

ZIP8 (SLC39A8) and ZIP14 (SLC39A14) were first identified as Zn transporters, as cells transfected with these two genes accumulated more Zn than controls (Begum *et al.* 2002, Liuzzi *et al.* 2006). ZIP8 is expressed most abundantly in lung, testis and kidney, while ZIP14 expression is highest in the liver, but is also detected in the pancreas and heart; the

expression level of both proteins in the brain is relatively low (Jenkitkasemwong *et al.* 2012). ZIP8 was later found to import Cd and Mn as well, as ZIP8 overexpression stimulated intracellular accumulation of Cd and Mn (He *et al.* 2006, Dalton *et al.* 2005). Subsequent studies showed that ZIP14 was able to transport Fe, Mn and Cd as well (Girijashanker *et al.* 2008, Liuzzi et al. 2006). Both ZIP8 and ZIP14 have high affinity for Mn (He et al. 2006, Girijashanker et al. 2008), e.g., a metal cation competition assay for ZIP8 revealed the following order of affinity: $Mn^{2+} > Hg^{2+} \gg Pb^{2+} = Cu^{2+} = Zn^{2+} = Cs^{2+}$ (He et al. 2006). Though these transporters may not regulate Mn transport in the brain to a great degree, they may regulate Mn absorption through the liver and lung to help regulate body Mn levels. Notably, ZIP8 and ZIP14 are expressed in the nasal respiratory epithelium and olfactory receptor neuron dendrites, where Mn from inhaled dust can be directly absorbed into the blood or into the brain (Genter *et al.* 2009). This may facilitate Mn toxicity in industrial workers suffering from manganism who have inhaled fumes containing high levels of Mn.

Dopamine transporter (DAT)

DAT is a membrane-spanning protein that acts to induce reuptake of dopamine into presynaptic vesicles. In rat brain, this protein is highly expressed in the axons, dendrites and cell bodies of neurons in the substantia nigra pars compacta, globus pallidus and striatum (Ciliax *et al.* 1995). In 1999, Ingersoll and colleagues found that the DAT inhibitor cocaine induced a 10-fold inhibition of Mn uptake in rat brain (particularly in the ventral mesencephalon) (Ingersoll *et al.* 1999). Subsequently, a study found decreased DAT density and activity in patients chronically exposed to Mn (Kim *et al.* 2002, Huang *et al.* 2003). Direct evidence of DAT as a Mn transporter comes from studies using DAT knockout mice, which accumulate significantly less Mn in the striatum compared to wildtype (WT) mice after Mn exposure. Interestingly, the absence of DAT did not affect Mn accumulation in other brain regions not expressing DAT (Erikson *et al.* 2005a). Indeed, Mn accumulation was selectively decreased by DAT inhibition, but not by inhibition of the serotonin transporter (SERT) or norepinephrine transporter (NET); this decrease was only seen in the globus pallidus of rats upon chronic Mn exposure (Anderson *et al.* 2007). Surprisingly, DAT levels were significantly increased in the striatum of baboon and rats after acute Mn administration (Chen *et al.* 2006).

Ca channels

The role of Ca in Mn homeostasis was first reported by Mason et al., noting that Mn uptake was decreased by Ca inhibitors in thymic lymphocytes (Mason *et al.* 1993). In a human hepato-carcinoma cell line, Mn uptake was shown to be dependent upon Ca concentrations (Finley 1998). Moreover, in human erythrocytes, Mn uptake was shown to be inhibited by nifedipine, a Ca channel blocker (Lucaciu *et al.* 1997). Later, Mn was found to enter cell membranes through store-operated Ca channels (SOCCs) in rat mast cells, osteoblast-like cells, human platelets and cultured bovine brain microvascular endothelial cells (Baldi *et al.* 2002, Dobrydneva & Blackmore 2001, Fasolato *et al.* 1993, Crossgrove & Yokel 2005). SOCCs are expressed in brain endothelial cells (Kim *et al.* 2004), and therefore, may play a role in Mn accumulation in the brain.

Choline transporters

There are a few different types of choline transporters. In adult rat tissues, a specific choline transporter is expressed in the nervous system, whereas three other forms are primarily distributed in peripheral tissues (Traiffort *et al.* 2005). Using an *in situ* brain perfusion technique in rats choline uptake was found to be significantly inhibited in the presence of Cd and Mn, but not Cu or aluminum (Al) (Lockman *et al.* 2001). Further studies are needed to determine if choline transporters play a direct role in Mn uptake.

Citrate transporters

Citrate transporters have been found in rat liver, testis and brain (Inoue *et al.* 2002). Similar to the discovery of the choline transporters in regulating Mn uptake using *in situ* brain perfusion technique, Crogressgrove et al. found that Mn citrate was able to cross the BBB and entered the brain (Crossgrove *et al.* 2003), indicating the citrate transporters as another putative Mn transporter.

Manganese Export

Efflux is a fundamental process that plays a crucial role in regulating cellular levels of essential metals such as Cu, Zn and Fe, and genetic defects in efflux transporters cause hereditary disorders of metal metabolism (e.g. Wilson's disease and Menke's disease due to mutations in the Cu-transporting pumps, ATP7B and ATP7A, respectively) (Brissot *et al.* 2011, Donovan *et al.* 2000, Kitzberger *et al.* 2005, La Fontaine & Mercer 2007, Liuzzi & Cousins 2004, McKie *et al.* 2000, Nemeth *et al.* 2004, Troadec *et al.* 2010). However, it is only over the last few years that the role of efflux in regulating Mn homeostasis in mammalian systems has begun to be appreciated. Thus far, four transporters have demonstrated Mn efflux activity in various experimental systems: ATPase 13A2 (ATP13A2), SLC30A10, ferroportin and secretory pathway Ca^{2+}-ATPase 1 (SPCA1) (Leyva-Illades *et al.* 2014, Madejczyk & Ballatori 2012a, Mukhopadhyay *et al.* 2010, Mukhopadhyay & Linstedt 2011, Tan *et al.* 2011, Yin *et al.* 2010). Importantly, the first hereditary or familial form of Mn-induced parkinsonism was recently discovered and genetic studies revealed that there was an association between homozygous loss-of-function mutations in *SLC30A10* and the development of this disease (Quadri *et al.* 2012, Tuschl *et al.* 2012). The fact that loss-of-function of an efflux transporter is the only genetic factor associated with hereditary Mn-induced parkinsonism underlines the importance of efflux in the regulation of Mn homeostasis and detoxification. The role of ferroportin and SPCA1 in mediating Mn efflux in neuronal systems and in the pathobiology of Mn-induced neurotoxicity are unclear; therefore, these transporters will not be discussed further in this review.

ATPase 13A2 (ATP13A2 or PARK9)

ATP13A2 is a vacuolar/lysosomal transmembrane cation transporting ATPase, and mutated ATP13A2 has been associated with early-onset parkinsonism and Kufor-Rakeb syndrome (Gitler *et al.* 2009, Behrens *et al.* 2010, Di Fonzo *et al.* 2007). Expression of ATP13A2 protected yeast and mammalian cells, and primary rat neurons against Mn-induced lethality (Tan et al. 2011, Gitler et al. 2009). In addition, expression of ATP13A2 reduced

intracellular Mn levels in cells (Tan et al. 2011). These results raise the possibility that ATP13A2 may transport Mn from the cytosol to the lumen of lysosomes, and thus, function as a Mn efflux transporter. However, direct Mn efflux activity for ATP13A2 has not yet been demonstrated. Further, it is not known whether patients who harbor mutations in this gene also exhibit Mn deposition the brain. Therefore, further studies are needed to determine whether ATP13A2 is a *bona fide* Mn efflux transport.

SLC30A10

To date, loss of function mutations in *SLC30A10* are the only known cause of a hereditary or familial Mn-induced parkinsonian syndrome. Genetic studies in human patients who developed familial Mn-induced parkinsonism led to the eventual identification of SLC30A10 as a Mn efflux transporter. The first detailed case report of this form of familial parkinsonism was published by Tuschl and colleagues in 2008 (Tuschl *et al.* 2008). In this study, the authors reported findings from a 12 y old female patient who presented with parkinsonian symptoms (Tuschl et al. 2008). Clinical analyses revealed that the patient had ∼10-fold increase in blood Mn levels. Plasma copper and zinc levels were within the normal range. Magnetic resonance imaging studies provided evidence for Mn deposition in the basal ganglia (Tuschl et al. 2008). Importantly, the individual had no history of exposure to elevated Mn (Tuschl et al. 2008). These findings suggested that the patient suffered from parkinsonism as a consequence of Mn accumulation, and that the increased retention of Mn occurred due to a defect in Mn metabolism.

A few years later, in 2012, two separate studies, one by Tuschl and colleagues and the other by the group of Vincenzo Bonifati, reported findings from an additional set of patients (Quadri et al. 2012, Tuschl et al. 2012). All patients had 10-20 fold increases in blood Mn with no history of exposure to elevated Mn from environmental or occupational sources (Quadri et al. 2012, Tuschl et al. 2012). Whole genome homozygosity mapping revealed that affected patients had one region of homozygosity that mapped to the region coding for the *SLC30A10* gene on chromosome 1. Sanger sequencing then determined that patients carried homozygous mutations in the *SLC30A10* gene (Quadri et al. 2012, Tuschl et al. 2012). The disease exhibited autosomal recessive inheritance (Tuschl et al. 2012, Quadri et al. 2012). Affected patients were born to consanguineous parents and unaffected siblings and parents of patients, when studied, were found to be heterozygous for *SLC30A10* mutations (Tuschl et al. 2012). These studies suggested that mutations in SLC30A10 affected Mn metabolism in a manner that caused increased Mn retention in the body.

The above findings are similar to a prior case report published by Gospe et al in 2000 (Gospe *et al.* 2000). These authors followed the patient described in their report from age 14 y till death at age 38 y, and then described autopsy findings in a second manuscript (Lechpammer *et al.* 2014). The patient had homozygous mutations in *SLC30A10* (Lechpammer et al. 2014, Tuschl et al. 2012). Important features observed during autopsy included severe neuronal loss in the basal ganglia, particularly in the globus pallidus, along with a 16-fold increase in basal ganglia and a 9-fold increase in liver Mn levels (Lechpammer et al. 2014).

SLC30 proteins belong to the cation diffusion facilitator superfamily of ion transporters (Huang & Tepaamorndech 2013). There are 10 members in the SLC30 family, SLC30A1-A10 (Huang & Tepaamorndech 2013). SLC30A1-A8 transport Zn from the cytosol to the cell exterior or the lumen of various organelles and the function of SLC30A9 is unclear (Huang & Tepaamorndech 2013). The fact that patients with SLC30A10 mutations exhibit increased Mn retention raised the possibility that SLC30A10-WT mediated efflux of Mn, instead of Zn, while disease-causing SLC30A10 mutants lacked this Mn efflux activity. This idea was supported by a heterologous expression experiment performed by Tuschl et al, in which expression of human SLC30A10-WT, but not two separate disease-causing mutants, rescued the enhanced sensitivity to Mn-induced cell death seen in yeast strains that lack *pmr1* (Tuschl et al. 2012).

Mechanistic insights into the cellular function of SLC30A10 and the reasons why mutations in this gene affect Mn metabolism were obtained by a recent collaborative study performed by the groups of Michael Aschner and Somshuvra Mukhopadhyay (Leyva-Illades et al. 2014). Localization studies in cell culture, including in GABAergic AF5 cells and primary midbrain neurons, and in *C. elegans* revealed that SLC30A10-WT trafficked to the cell surface. In contrast, disease-causing mutants tested (L89P, Δ98-134, Δ105-107, T196P and Q308Stop) exhibited a trafficking defect and were trapped in the endoplasmic reticulum (Leyva-Illades et al. 2014). The mutant proteins also underwent increased proteasomal turn-over. Importantly, Mn measurement assays in cell culture revealed that expression of SLC30A10-WT, but not the disease-causing mutant Δ105-107, reduced intracellular Mn levels, and a pulse-chase assay confirmed that this reduction in intracellular Mn was due to an increase in Mn efflux and not a block in Mn influx (Leyva-Illades et al. 2014). Subsequent studies revealed that expression of SLC30A10-WT, but not Δ105-107, protected GABAergic AF5 cells and primary midbrain neurons against Mn toxicity. In contrast, knockdown of SLC30A10 in GABAergic AF5 cells caused Mn accumulation and increased the sensitivity of cells to Mn-induced death (Leyva-Illades et al. 2014). At the organism level, in *C. elegans*, expression of SLC30A10-WT, but not L89P (another disease-causing mutant), protected dopaminergic neurons against Mn-induced neurodegeneration, rescued a Mn-induced functional defect in locomotion and enhanced the viability of worms exposed to elevated Mn (Leyva-Illades et al. 2014). Put together, the above data strongly suggest that SLC30A10-WT is a cell surface localized Mn efflux transporter and that mutations in the protein that cause familial parkinsonism block the efflux activity of the transporter.

SLC30A10 was initially thought to be a Zn efflux transporter (Bosomworth *et al.* 2012). A 3-fold increase in liver Zn was reported in one patient of familial Mn-induced parkinsonism (Lechpammer et al. 2014). However, in this patient, there was no change in basal ganglia Zn levels, and, in the liver, there was a 2-fold increase in Cu as well (Lechpammer et al. 2014). A mild increase in liver Cu was also reported in another patient, but the plasma Cu levels in this patient were normal (Tuschl et al. 2008). The above clinical findings suggested that the observed increase in hepatic Zn or Cu levels in a few patients with SLC30A10 mutations was due to compromised liver function, secondary to Mn deposition, and not a primary effect of SLC30A10 on Zn or Cu metabolism. Consistent with this idea, in cell culture

assays, expression of SLC30A10-WT did not affect intracellular levels of Zn or Cu or alter viability of cells exposed to elevated Zn or Cu (Leyva-Illades et al. 2014).

How can mutations in SLC30A10 contribute to the development of a familial parkinsonian syndrome? SLC30A10 is expressed in the basal ganglia and the liver (Quadri et al. 2012). Mn is primarily excreted by the liver via transport into bile (Butterworth 2013). Loss-of-function mutations in SLC30A10 will be expected to block the biliary excretion of Mn, and lead to excess Mn retention in the body. The retained Mn will then be expected to cross the blood brain barrier, and eventually accumulate in the basal ganglia. Indeed, increased Mn levels in blood and basal ganglia are seen in patients with SLC30A10 mutations (Quadri et al. 2012, Tuschl et al. 2012). The reasons why basal ganglia neurons, particularly those in the globus pallidus, selectively accumulate Mn and are more sensitive to Mn-induced damage is as yet unclear. However, the fact that SLC30A10 is expressed in basal ganglia neurons, including in the globus pallidus (Quadri et al. 2012), suggests that loss-of-function of SLC30A10 will likely increase the sensitivity of these neurons to Mn accumulation and subsequent damage. This Mn-induced damage likely culminates in the development of parkinsonism.

In sum, among the known Mn efflux transports, SLC30A10 appears to have the most physiological relevance because mutations in this protein cause a hereditary parkinsonian syndrome. The relative contribution of SPCA1 and ferroportin in mediating Mn detoxification at the whole animal level is not yet clear and must be elucidated. Moreover, while mutations in ATP13A2 cause parkinsonism, defects in Mn metabolism are unlikely to be the sole cause of motor defects produced from mutations in this gene. The biology of SLC30A10 has only now begun to be investigated. Fundamental questions, such as what is the mechanism that confers selective Mn transport capability to SLC30A10 and how does Mn control SLC30A10 activity, are as yet unknown. Addressing these issues is an essential step in understanding how SLC30A10 regulates Mn homeostasis and detoxification. It will also be important to determine whether polymorphisisms in SLC30A10 exist in the population and whether these polymorphisms alter the risk of developing Mn-induced parkinsonism. Search for SLC30A10 polymorphisms is supported by the observation that polymorphisms in DMT1 increase the risk of developing Parkinson's disease (He *et al.* 2011a). Finally, the finding that over-expression of SLC30A10 enhanced Mn efflux and protected against Mn-induced toxicity, (Leyva-Illades et al. 2014), is important from a clinical perspective. Currently, there is no definitive treatment for Mn-induced parkinsonism. Indeed, the only treatment available for patients with SLC30A10 mutations is chelation therapy, which provided partial symptomatic relief (Quadri et al. 2012, Tuschl et al. 2012). The protective effect of enhanced efflux on Mn toxicity raises the possibility that increasing Mn efflux may be a useful strategy for the management of familial and non-familial forms of Mn-induced parkinsonism. It will be important to determine whether increasing Mn efflux can protect against Mn toxicity in a vertebrate animal model without causing side-effects, such as Mn deficiency. Demonstration of a protective effect will justify screening for or generating efflux enhancing small molecules or drugs. Such efflux enhancing drugs, as and when identified, may work by increasing SLC30A10 activity or levels, or by an independent mechanism (e.g. by increasing the efflux activity of SPCA1 or

ferroportin). Drugs that require SLC30A10 for activity will likely not be beneficial for patients of familial Mn-induced parkinsonism, where SLC30A10 is mutated. However, such drugs may prove to be useful for non-familial forms of Mn-induced parkinsonism, including forms of the disease that occur due to occupational or environmental over-exposure to Mn. Drugs that work in a SLC30A10-independent manner may help patients with familial and non-familial forms of Mn-induced parkinsonism. Discovery of a viable means to increase Mn efflux combined with advances being made in the identification of non-invasive biomarkers of Mn toxicity [such as hair Mn levels (Eastman *et al.* 2013)] may eventually come together to create a feasible therapeutic strategy for managing Mn-induced parkinsonism.

Other Manganese Exporters

Ferroportin and SPCA1

Ferroportin is a transmembrane protein expressed in the duodenum, liver, spleen and intestine (Donovan et al. 2005), as well as in the endothelial cells of the BBB, neurons, oligodendrocytes, astrocytes, the choroid plexus and ependymal cells (Wu *et al.* 2004). SPCA1 is widely expressed in human tissues, with the highest expression in keratinocytes, liver and brain (Hu *et al.* 2000). It is primarily localized to the Golgi apparatus (Ton *et al.* 2002), although an additional endosomal/vesicular pool was observed (Leitch *et al.* 2011). Both ferroportin and SPCA1 are able to mediate Mn efflux (Madejczyk & Ballatori 2012b, Ton et al. 2002). As the role of ferroportin and SPCA1 in the nervous system has yet to be established, it will not be further discussed herein.

Conclusions

The maintenance of Mn homeostasis involves a complex network of proteins that mediate Mn import or export. The major uptake mechanisms identified include DMT1 and Tf-TfR-mediated endocytosis. Other proteins that may play a role in uptake are Ca^{2+} channels; choline transporters; citrate transporters; and the ZIP family of Zn transporters. Notably, none of these transporters is specific for Mn as they transport other metals/substrates as well. Further work is needed to determine whether changes in cellular Mn controls expression and activity of these transporters in a manner that impacts Mn homeostasis. Compared to the influx transporters, discovery of Mn efflux transporters is relatively recent. SLC30A10 appears to be the most relevant Mn efflux transporter. The roles of ATP13A2, SPCA1 and ferroportin in mediating Mn efflux in neuronal systems and in protecting against Mn-induced neurotoxicity need additional work. Mechanistic insights of Mn transport mechanisms in relevant neurons will likely provide a better understanding of the biology of Mn-induced parkinsonism and contribute to the development of new therapeutic strategies for this devastating disease.

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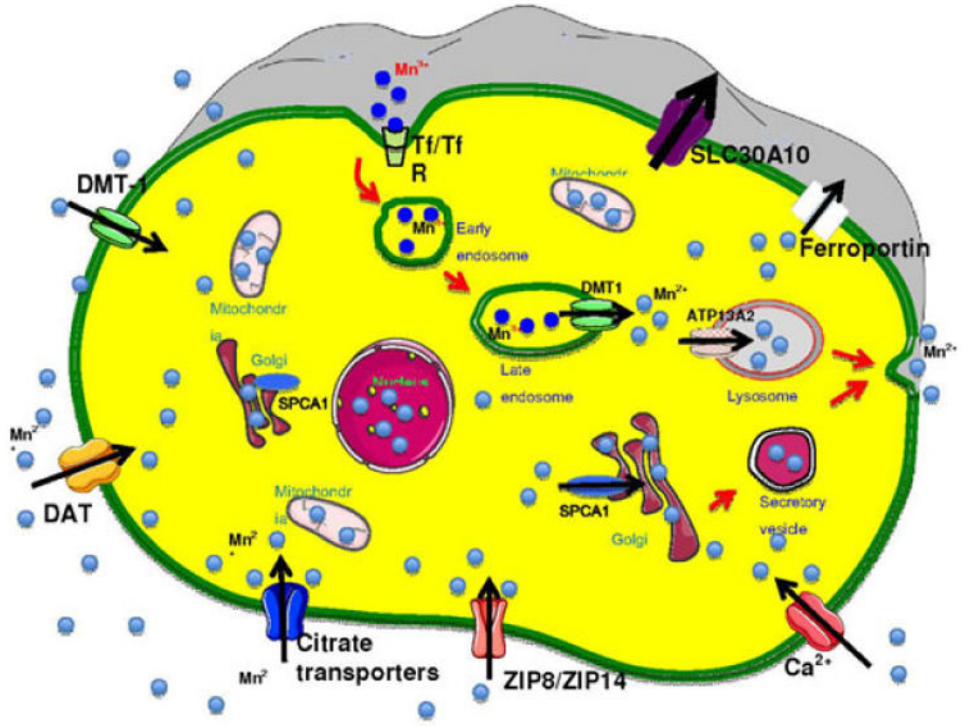

Figure.

In cells, the intracellular Mn level is tightly regulated by transporters on the cell surface and the intracellular transporters localized on the membrane of internal organelles. On the cell surface, DMT-1, DAT, ZIP8/ZIP14, citrate transporters and Ca channels facilitate Mn^{2+} influx in to the cytosol, while SLC30A10 and ferroportin mediate efflux of Mn^{2+}. Mn^{3+} can be directly transported in cells through Tf/TfR. In addition, Mn^{3+} also enters cells through Tf/TfR mediated endocytosis and finally released into cytoplasm as Mn^{2+} by DMT1. When the cytosolic Mn reaches a threshold, SPCA1 on the Golgi membrane and ATP13A2 on the lysosome membrane will transport Mn into the Golgi and lysosomes, respectively, which facilitate Mn^{2+} efflux into the extracellular matrix.