Transport of Toxic Metals by Molecular Mimicry

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Intracellular concentrations of essential metals are normally maintained within a narrow range, whereas the nonessential metals generally lack homeostatic controls. Some of the factors that contribute to metal homeostasis have recently been identified at the molecular level and include proteins that mediate import of essential metals from the extracellular environment, those that regulate delivery to specific intracellular proteins or compartments, and those that mediate metal export from the cell. Some of these proteins appear highly selective for a given essential metal; however, others are less specific and interact with multiple metals, including toxic metals. For example, DCT1 (divalent cation transporter-1; also known as NRAMP2 or DMT1) is considered to be a major cellular uptake mechanism for Fe^{2+} and other essential divalent metals, but this protein also mediates uptake of Cd^{2+}, Pb^{2+}, and possibly of other toxic divalent metals. The ability of nonessential metals to interact with binding sites for essential metals is critical for their ability to gain access to specific cellular compartments and for their ability to disrupt normal biochemical or physiological functions. Another major mechanism by which metals traverse cell membranes and produce cell injury is by forming complexes whose overall structures mimic those of endogenous molecules. For example, it has long been known that arsenate and vanadate can compete with phosphate for transport and metabolism, thereby disrupting normal cellular functions. Similarly, cromate and molybdate can mimic sulfate in biological systems. Studies in our laboratory have focused on the transport and toxicity of methylmercury (MeHg) and inorganic mercury. Mercury has a high affinity for reduced sulfhydryl groups, including those of cysteine and glutathione (GSH). MeHg–L-cysteine is structurally similar to the amino acid methionine, and this complex is a substrate for transport systems that carry methionine across cell membranes. Once MeHg has entered the cell, some of it binds to GSH, and the resulting MeHg–glutathione complex appears to be a substrate for proteins that mediate cellular export of glutathione *S*-conjugates, including the apically located MRP2 (multidrug resistance-associated protein 2) transporter, a member of the adenosine triphosphate–binding cassette protein superfamily. Because other toxic metals also form complexes with endogenous molecules, comparable mechanisms may be involved in their membrane transport and disposition. *Key words:* divalent metal transporters, glutathione, membrane transport, metals, molecular mimicry. *Environ Health Perspect* 110(suppl 5):689–694 (2002).

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Metals comprise three-fourths of the elements in the periodic table, but only a few of the metals are essential for life. Most of the known metals are quite toxic to living organisms. Because even the essential metals can be toxic when present in excess, their cellular concentrations are tightly regulated. Recent studies have identified some of the human genes that control essential metal concentrations and have described how toxic metals interact with these gene products. The present report highlights these metal-transport and metal-regulatory pathways. Additional information on specific metals and their metabolic pathways is available in several recent reviews (*1–11*).

Metal Transport across Cell Membranes Occurs by Three General Mechanisms

Transport via membrane recycling: roles of transferrin, ceruloplasmin, and the gene products defective in Wilson disease (ATP7B) and Menkes syndrome (ATP7A). For certain essential metals such as copper and iron, receptor-mediated endocytotic and exocytotic mechanisms play a critical role in their homeostasis (*1*). Many of the individual steps in this process have now been characterized at the molecular level; however, the relative contribution of this process to the transport of other metals has not been examined in detail. Because the extent of vesicle trafficking is quite large in many cell types, this process may be important in facilitating movement of metals either that have a high affinity for plasma membrane binding sites or that are bound to ligands that are selectively cleared via membrane receptors (*12*). Vesicle insertion and retrieval occur by at least three mechanisms, receptor-mediated, fluid-phase, or adsorptive endocytosis, and each of these may mediate metal transport into and out of cells as well as transport into and out of intracellular organelles.

The predominant mechanism of iron transport from blood plasma into hepatocytes and certain other cell types is believed to be the transferrin receptors (Figure 1). Nearly all the iron in plasma (~99%) is normally associated with transferrin, a protein that binds iron in the Fe(III) oxidation state. Oxidation of Fe(II) to Fe(III) is catalyzed by the copper-containing enzyme ceruloplasmin (Figure 1).

Transferrin receptor–mediated endocytosis leads to the internalization of diferric transferrin, followed by release of iron within acidic vesicles and extrusion of iron-depleted transferrin (apotransferrin). The mechanism by which the released iron is subsequently transferred from the endosome/lysosome to the cytosol is not known, although recent studies implicate a role of DCT1 (divalent cation transporter-1; also known as NRAMP2 or DMT1) (*13*). A second and more speculative mechanism for iron uptake from blood plasma into the cell involves the possibility that iron is released at the plasma membrane without internalization of the transferrin–receptor complex (*1*). In this model transferrin-bound ferric iron is reduced to ferrous iron extracellularly, removed from the transferrin molecule, and transported into the cell, possibly by the recently identified DCT1 protein (Figure 1).

In addition to TfR1 and TfR2 (transferrin receptors 1 and 2), lactoferrin, melanotransferrin, and ferritin receptors also contribute to iron uptake (*4,14,15*). In the liver, Kupffer cells release a substantial fraction of the iron acquired by erythrophagocytosis in the form of ferritin, which is efficiently internalized by hepatocytes, via their ferritin receptors. Because these iron-binding proteins can bind other metal cations such as manganese, zinc, and vanadium, they may also contribute to their cellular transport. For example, most of the manganese in plasma is bound to transferrin, a finding that may explain the rapid hepatic clearance of manganese from plasma. Similarly, a substantial fraction of vanadium in rat plasma is associated with transferrin, and vanadium accumulates preferentially in tissues that are also abundant in iron (liver, spleen, and kidney). Metals also bind to

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albumin and other plasma proteins, and these complexes may also be transported by vesicle-mediated processes.

As indicated above, membrane vesicles are important not only for metal uptake into cells but also for export into the extracellular space. For example, the copper-transporting Wilson and Menkes disease proteins are P-type adenosine triphosphatases (ATPases) that are required for copper export from the cell, but these proteins are localized largely to intracellular vesicles, indicating that copper (and possibly other metals) is first compartmentalized within intracellular vesicles and that export is accomplished by vesicle fusion with the plasma membrane.

In hepatocytes, copper is transported into the *trans*-Golgi network using the ATP-dependent transporter ATP7B, the protein that is defective in Wilson disease (Figure 2). Within this intracellular compartment, copper is then used for the synthesis of copper-containing proteins such as ceruloplasmin, or it is stored for subsequent excretion. Some of the intravesicular copper is presumably sorted into vesicles destined for the lysosomal-biliary excretory pathway. According to this model, fusion of exocytic vesicles with the canalicular membrane delivers copper into bile (Figure 3); however, neither the site nor the mechanism by which this vesicular sorting occurs is known. It has been suggested that ATP7B-containing vesicles may actually fuse with the canalicular plasma membrane and thereby deliver the functional transport protein to the liver cell apical membrane (*16*–*18*). However, additional studies are needed to address this possibility.

In tissues other than the liver, a comparable P-type ATPase (ATP7A, or the Menkes disease gene product) pumps copper into endosomal/lysosomal compartments. The Wilson and Menkes diseases genes have 56% overall identity. The Menkes gene is ubiquitously expressed in adult tissues, with little or no expression in the liver (*19*), whereas the Wilson disease gene is expressed in only a few cell types, notably liver and certain neuronal cells. Mutations in *ATP7A* lead to significant copper accumulation in intestinal mucosa, kidney, and selected other tissues. This inability to deliver copper from sites of its absorption and storage results in a systemic copper insufficiency in Menkes patients. In contrast with the copper insufficiency of Menkes patients, Wilson disease patients accumulate excess copper in many tissues (e.g., liver, brain, kidney, cornea) due to the inability to excrete copper into bile, the main route of its elimination. At the cellular level, copper accumulates in the liver cell cytosol, and the cell eventually succumbs to copper toxicity.

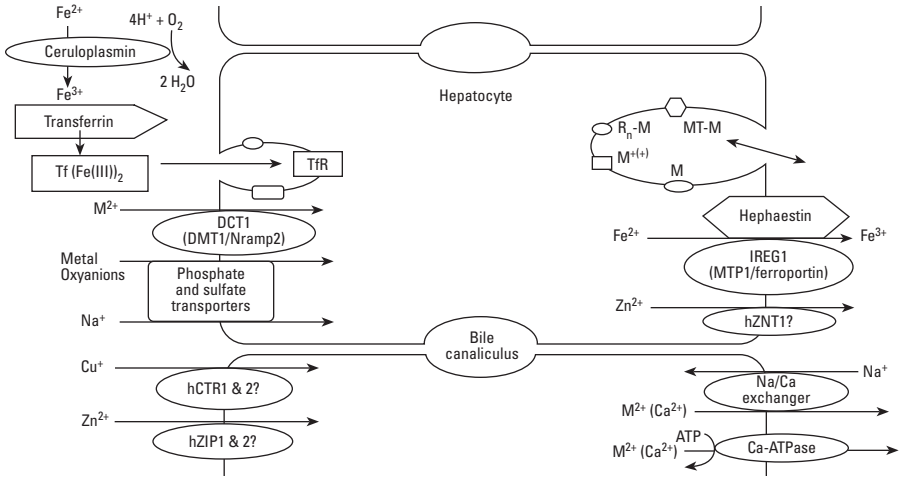

Figure 1. Plasma membrane transport mechanisms that mediate uptake of metals from blood plasma into hepatocytes and those that mediate efflux from the cell back into the bloodstream. Adapted from Ballatori (*2*).

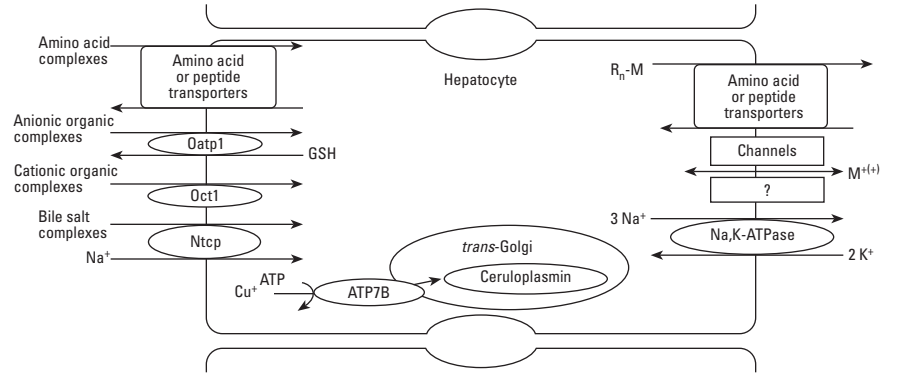

Figure 2. Additional plasma membrane transport systems that may contribute to metal homeostasis in the liver. Adapted from Ballatori (*2*).

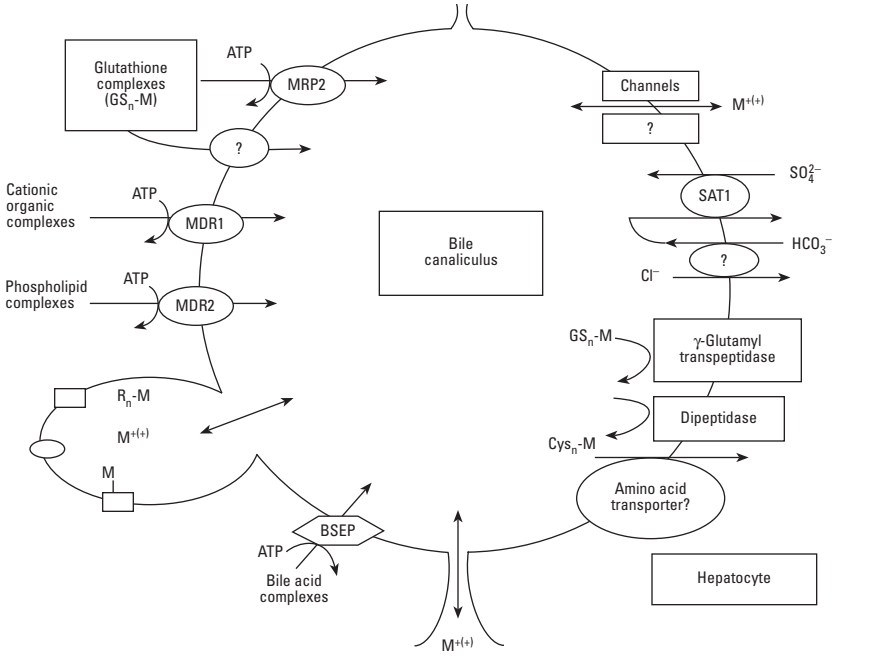

Figure 3. Mechanisms for metal export into the canalicular (apical) space of the liver. Adapted from Ballatori (*2*). BSEP, bile salt export pump.

Vesicular exocytosis may also be involved in cellular export of iron and possibly other metals (*20,21*). Regoeczi and Chindemi (*21*) measured the translocation of different forms of transferrin from blood to bile in the rat but found that only a small fraction of the metal-containing protein is excreted in bile.

Transport of metal cations and inorganic metal complexes on metal-selective proteins. Recent studies have identified and characterized several metal-selective transporters, including hCTR1 (the human equivalent of the yeast high-affinity copper transporter), members of the NRAMP (natural resistance–associated macrophage protein) family of membrane proteins (e.g., the divalent cation transporter DCT1), several putative zinc transporters (ZIP and ZNT families), and the recently described IREG1 (iron-regulatory protein-1; also called MTP1 or ferroportin) and hephaestin iron export complex, as described in more detail below. The identification of these metal transporters was made possible by the application of powerful molecular biology approaches, including complementation strategies in bacteria and yeast and the use of the *Xenopus laevis* oocyte expression system.

Uptake on phosphate or sulfate transporters. It has long been known that metal oxyanions are excellent substrates for phosphate and sulfate carriers (*22–24*). For example, vanadate and arsenate are structurally similar to phosphate and can compete with phosphate for transport as well as for intracellular binding sites. Similarly, chromate, selenate, and molybdate are structurally similar to sulfate and are substrates for sulfate transporters.

Some of the phosphate and sulfate transporters have now been identified at the molecular level, including NaP(i)1, NPT1, PiT1, PiT2, NaSi-1, and Sat-1 (*25–31*). NaSi-1 belongs to an Na^+-coupled transporter family comprising the Na^+-dicarboxylate transporters and is localized to the brush border membrane of renal proximal tubular and ileal cells. Sat-1 is an Na^+-independent transporter that mediates sulfate/bicarbonate–oxalate exchange. It is located at the basolateral membrane of proximal tubular epithelial cells and canalicular surface of hepatocytes.

hCTR1 and hCTR2, putative copper uptake transporters. Important insight into the molecular basis for copper uptake from blood plasma was provided by recent studies that took advantage of the remarkable similarities between yeast and mammalian cells in terms of their copper and iron metabolism (*32–36*). The similarities between yeast and mammalian copper and iron homeostasis are numerous and extend to the membrane transporters, the cytoplasmic chaperones, and the terminal copper acceptors (*2,37,38*).

Zhou and Gitschier (*39*) isolated a human gene involved in copper uptake by complementation of the yeast high-affinity copper uptake mutant *ctr1*. The human gene product (hCTR1; Figure 1) exhibits 29% amino acid identity with the yeast copper transporter CTR1. A database search by Zhou and Gitschier (*39*) revealed an additional human gene that was named *hCTR2*. By Northern blot analysis, *hCTR1* and *hCTR2* were expressed in all tissues examined, but the liver exhibited the highest level of expression.

Insight into the cellular localization of CTR1, its function, and its mechanism of action has been provided by studies in cells transfected with this gene (*40*) and in *Ctr1*(–/–) mice (*41,42*). Moller and co-workers (*40*) found that fibroblasts transfected with hCTR1 cDNA had dramatically increased capacity for ^{64}Cu uptake, indicating that the hCTR1 protein is functional in copper uptake in human cells, whereas hCTR2-transfected cells did not display enhanced copper uptake. *Ctr1*(–/–) mice demonstrated early embryonic lethality, indicating that this gene is required for normal development and survival (*41,42*). Heterozygous mice survived and appeared phenotypically normal, but brain copper levels were only 50% of those of control littermates, indicating that Ctr1 is particularly critical for copper entry into brain.

DCT1, a multispecific metal transporter of the NRAMP family. A major mechanism for cellular uptake of Fe^{2+} and other divalent metals was recently described by Gunshin et al. (*43*). These investigators cloned and characterized a mammalian iron and divalent cation transporter (DCT1; also known as NRAMP2 or DMT1) that is expressed in a number of tissues, including kidney and liver. DCT1 is able to transport a variety of divalent metal cations (Fe, Zn, Mn, Co, Cd, Cu, Ni, and Pb) by a proton-coupled and membrane potential–dependent mechanism. Note that this list of possible substrates includes both essential metals and toxic metals. Direct evidence for a role of DCT1 in cadmium uptake by Madin-Derby canine kidney (MDCK) cells (*44*) and human absorptive enterocytes (*45*) has now been provided. In the intestine this protein probably mediates metal uptake at the lumenal surface by co-transport with protons (with stoichiometry of $1H^+$:$1M^{2+}$). This gene is defective in mice with microcytic hypochromic anemia, a disease associated with defects in intestinal iron absorption and erythroid iron utilization (*46*). Although Figure 1 shows DCT1 mediating metal uptake into liver from sinusoidal blood, this is only speculative because the localization, orientation, and energy coupling of DCT1 in liver cell membranes have not yet been established. In the kidney, DCT1 is localized to principal and intercalated cells of the collecting ducts, the ascending limbs of Henle's loop, and distal convoluted tubules (*47*). DCT1 appears to be localized to the apical surface at these sites, suggesting that it mediates reabsorption of divalent metal ion in the distal nephron but not in the proximal tubule (*47*).

DCT1 is a member of the NRAMP family of membrane-associated proteins (*43,46,48–50*). The NRAMP family displays a high sequence conservation from yeast to humans, with many species expressing at least two discrete gene copies [e.g., rodent *Nramp1* and *Nramp2* (*48*)]. Iron uptake in *Xenopus* oocytes expressing mouse Nramp1 is also stimulated relative to control oocytes (*43*), indicating that this protein is also a metal transporter. However, its cellular localization and mechanism of action are not defined. Atkinson and co-workers (*48*) reported that murine macrophage Nramp1 is localized to a subcellular organelle that displays the structural and functional characteristics of a phagolysosome. Nramp1 is expressed only in reticuloendothelial cells, whereas Nramp2 (Dct1) is expressed in most tissues.

ZNT and ZIP, putative zinc efflux and uptake transporters, respectively. Palmiter and Findley (*51*) isolated a cDNA strand encoding a zinc transporter (ZNT1) from a rat kidney expression library by complementation of a mutated, zinc-sensitive BHK (baby hamster kidney) cell line. The transporter was localized to the plasma membrane of these cells and was found to mediate both uptake and efflux of zinc, although efflux was considered the physiological direction of transport. The energetics of transport were not identified: zinc transport was unaffected by metabolic poisons or by changes in the ionic composition of the culture medium (*51*). The metal ion specificity of ZNT1 is also not clear. Palmiter and co-workers (*52,53*) subsequently identified two other members of this family, ZNT2 and ZNT3. ZNT2 is localized to intracellular endosomal/lysosomal vesicles and appears to be relatively selective for zinc (*52*), whereas ZNT3 is expressed predominantly in brain and testes (*53*). Immunohistochemical analysis of murine brain suggests localization of ZNT3 to synaptic vesicles (*53*). Huang and Gitschier (*54*) identified the fourth member of this family of transporters, ZNT4, as the protein that is defective in the inherited zinc deficiency in the lethal milk mouse. ZNT4 was shown to confer zinc resistance to a zinc-sensitive yeast strain and was abundantly expressed in mammary epithelia and brain (*54*). The regulation of *ZNT* genes is now being examined (*55*).

Recent studies by Gaither and Eide (*56,57*) have described another family of putative zinc transporters, the ZIP family, that may function for uptake of zinc and possibly other divalent metals from the extracellular medium into the cytoplasm. There are at least 12 ZIP-encoding genes in humans, and three of them (*ZIP1*, *ZIP2*, and *ZIP3*) are closely related to fungal and plant proteins that are known to be zinc uptake transporters (*6,57*). Human ZIP1 and ZIP2 mediate a time-, temperature-, and concentration-dependent zinc uptake. Transport is saturable, with an apparent K_m of 3 μM. Human ZIP2 uptake activity is inhibited by several other transition metals, suggesting that this protein may transport other metals. Transport activity is independent of ATP content and of Na^+ or K^+ gradients but is stimulated by HCO_3^- treatment, suggesting a zinc–bicarbonate co-transport mechanism.

Export of metals mediated by the IREG1 and hephaestin proteins. Release of iron and possibly of other metals from cells into blood plasma is mediated by two proteins, IREG1 and hephaestin, that apparently work together to facilitate iron export across the basolateral membrane of cells (*35,58–61*). IREG1 is also called MTP1 or ferroportin (Figure 1). This transmembrane protein is expressed in tissues involved in body iron homeostasis, including the developing and mature reticuloendothelial system, the duodenum, and the pregnant uterus (*58*). It is localized to the basolateral membrane of the duodenal epithelial cells, and its overexpression in tissue culture cells results in intracellular iron depletion. In the adult mouse, IREG1 expression in the liver and duodenum is reciprocally regulated (*58*). The complementary protein hephaestin is a transmembrane-bound ceruloplasmin homologue that functions as a multicopper ferroxidase. This protein is mutated in the sex-linked anemic (sla) mouse, is highly expressed in intestine, and is necessary for iron egress from intestinal enterocytes into the circulation (*35*).

Uptake and efflux on membrane channels and pumps. The role of ion channels and primary active pumps in facilitating transport of heavy metals appears to be relatively minor overall, although there may be some exceptions. For example, in excitable tissues Cd^{2+} and Pb^{2+} may enter cells via voltage-sensitive channels (*62,63*). Studies by Hinkle and coworkers (*62*) in a pituitary cell line demonstrate that one route of cadmium uptake in these cells is via voltage-gated dihydropyridine-sensitive calcium channels. The voltage-gated calcium channels also admit Ba^{2+} and Sr^{2+} and are inhibited by a number of divalent metal cations. Receptor-activated calcium channels may also allow other divalent cations to enter the cell, as suggested by the observation that receptor-activated calcium channels are inhibited by Zn, Cd, Ni, Co, and Mn (*64*); however, the nature of the inhibition by the metals is unknown. Crofts and Barritt (*65*) indicate that Mn^{2+} can move into hepatocytes through the receptor-activated Ca^{2+} inflow system, identifying a potential regulated mechanism for hepatic manganese uptake. Metals also interact with plasma membrane Ca^{2+}-ATPases, the predominant proteins for Ca^{2+} efflux, raising the possibility that other metals may use these ATPases to exit cells and enter blood plasma (Figure 1).

Transport of metal complexes on organic solute transporters. In biological fluids and tissues, most metals are present largely as complexes with amino acids, peptides, proteins, phospholipids, and other tissue constituents rather than as the free metal cations. Binding of reactive heavy metals to metallothioneins, ferritin, transferrin, lactoferrin, melanotransferrin, hemosiderin, ceruloplasmin, citrate, ascorbate, glutathione (GSH), cysteine, or other amino acids is a major protective mechanism. Likewise, the biological reactivity of essential metals is regulated by interaction with specific ligands, and in particular with prosthetic groups on proteins. Thus, the disposition of both essential and toxic metals is regulated to a large extent by the availability and relative concentrations of the biological ligands as well as by the ability of the resulting metal complexes to serve as substrates for the various organic solute transporters (*2,12,66*). Because most organic solute carriers have a broad substrate specificity, it is likely that they would readily accept substrates whose only modification is the presence of a metal ion.

Several families of multispecific organic solute transporters have now been identified, and the possible contribution of these proteins to metal homeostasis is being examined (*2*). Transporters that facilitate uptake from blood plasma into the cell include OATPs (organic anion-transporting polypeptides), OATs (organic anion transporters), OCTs (organic cation transporters), and NTCP (Na^+-taurocholate [bile acid]–cotransporting polypeptide) (Figure 2). Amino acid transporters and peptide transporters have also been identified that mediate cellular uptake and export. Theoretically, these plasma membrane carriers may facilitate cellular uptake of metals that are bound to their endogenous substrates (Figure 2). Indeed, several groups have provided evidence for cellular copper or zinc uptake as histidine complexes on amino acid carriers (*67,68*). In erythrocytes, zinc may also be taken us as an anionic complex ($[Zn(HCO_3)_2\ Cl]^-$) through the anion exchanger (*69*). Studies in our laboratory and in other laboratories have demonstrated transport of methylmercury as a cysteine complex on the L-type neutral amino acid carriers (*2,12,70–72*).

Although considerable information has recently been obtained on proteins that mediate organic solute uptake into cells, less is known about organic solute export. One major class of proteins that may contribute to cellular export of metal complexes is the ATP-binding cassette (ABC) superfamily of proteins and, in particular, the MDR (multidrug resistance) and MRP (multidrug resistance protein) families within the ABC superfamily (*2*) (Figure 3). MDR and MRP transport proteins are generally multispecific, transporting a variety of structurally unrelated molecules, including organometallic complexes. Evidence has been presented that MRP2 mediates biliary transport of metals complexed with GSH, including arsenic and cisplatin, and possibly copper, cadmium, and mercury, whereas cells overexpressing MDR1 are more resistant to cationic lipophilic metal complexes, indicating transport of the metal complexes on MDR1 (*2,73–84*). Whether other multispecific transporters also mediate transport of organometallic complexes has not yet been examined.

Summary

Several metal transport mechanisms have recently been identified at the molecular level, and investigators are beginning to examine their structure, function, and regulation. Many of these transporters have thus far been identified only at the cDNA level, and there is comparatively little information on their cellular and subcellular localization, their functional orientation in the membrane (uptake or efflux), driving force, substrate selectivity, or regulation under physiological and pathophysiological conditions. It is likely that additional families of metal-specific transporters will be identified in the near future and that many new members of existing families will be described.

References and Notes

1. Aisen P, Enns C, Wessling-Resnick M. Chemistry and biology of eukaryotic iron metabolism. Int J Biochem Cell Biol 33:940–959 (2001).
2. Ballatori N. Molecular mechanisms of hepatic metal transport. In: Molecular Biology and Toxicology of Metals (Zalups RK, Koropatnick DJ, eds). London:Taylor & Francis, 2000;346–381.
3. Cousins RJ, McMahon RJ. Integrative aspects of zinc transporters. J Nutr 130(suppl 5S):1384S–1387S (2000).
4. Roy CN, Andrews NC. Recent advances in disorders of iron metabolism: mutations, mechanisms and modifiers. Hum Mol Genet 10:2181–2186 (2001).
5. Eide DJ. Metal ion transport in eukaryotic microorganisms: insights from *Saccharomyces cerevisiae*. Adv Microb Physiol 43:1–38 (2000).
6. Guerinot ML. The ZIP family of metal transporters. Biochim Biophys Acta 1465:190–198 (2000).
7. Meneton P, Oh YS, Warnock DG. Genetic renal tubular disorders of renal ion channels and transporters. Semin Nephrol 21:81–93 (2001).
8. Paulsen IT, Saier MH Jr. A novel family of ubiquitous

heavy metal ion transport proteins. J Membr Biol 156:99–103 (1997).
9. Rolfs A, Hediger MA. Intestinal metal ion absorption: an update. Curr Opin Gastroenterol 17:177–183 (2001).
10. Solioz M, Vulpe C. CPx-type ATPases: a class of P-type ATPases that pump heavy metals. Trends Biochem Sci 21:237–241 (1996).
11. Tsai K-J, Hsu C-M, Rosen BP. Efflux mechanisms of resistance to cadmium, arsenic and antimony in prokaryotes and eukaryotes. Zool Stud 36:1–16 (1997).
12. Ballatori N. Mechanisms of metal transport across liver cell plasma membranes. Drug Metab Rev 23:83–132 (1991).
13. Tabuchi M, Yoshimori T, Yamaguchi K, Yoshida T, Kishi F. Human NRAMP2/DMT1, which mediates iron transport across endosomal membranes, is localized to late endosomes and lysosomes in Hep-2 cells. J Biol Chem 275:22220–22228 (2000).
14. Joshi JG, Sczekan SR, Fleming JT. Ferritin—a general metal detoxicant. Biol Trace Elem Res 21:105–110 (1989).
15. Osterloh K, Aisen P. Pathways in the binding and uptake of ferritin by hepatocytes. Biochim Biophys Acta 1011:40–45 (1989).
16. Dierick HA, Adam AN, Escara-Wilke JF, Glover TW. Immunocytochemical localization of the Menkes copper transport protein (ATP7A) to the trans-Golgi network. Human Mol Genet 6:409–416 (1997).
17. Nagano K, Nakamura K, Urakami K-I, Umeyama K, Uchiyama H, Koiwai K, Hattori S, Yamamoto T, Matsuda I, Endo F. Intracellular distribution of the Wilson's disease gene product (ATPase7B) after *in vitro* and *in vivo* exogenous expression in hepatocytes from the LEC rat, an animal model of Wilson's disease. Hepatology 27:799–807 (1998).
18. Roelofsen H, Wolters H, Van Luyn MJ, Miura N, Kuipers F, Vonk RJ. Copper-induced apical trafficking of ATP7B in polarized hepatoma cells provides a mechanism for biliary copper excretion. Gastroenterology 119:782–793 (2000).
19. Kuo YM, Gitschier J, Packman S. Developmental expression of the mouse mottled and toxic milk genes suggests distinct functions for the Menkes and Wilson disease copper transporters. Human Mol Genetics 6:1043–1049 (1997).
20. LeSage GD, Kost LJ, Barham SS, LaRusso NF. Biliary excretion of iron from hepatocyte lysosomes in the rat. A major excretory pathway in experimental iron overload. J Clin Invest 77:90–97 (1986).
21. Regoeczi E, Chindemi PA. Translocation of different forms of transferrin from blood to bile in the rat. Hepatology 21:1063–1069 (1995).
22. Wetterhahn-Jennette K. The role of metals in carcinogenesis: biochemistry and metabolism. Environ Health Perspect 40:233–252 (1981).
23. Clarkson TW. Molecular and ionic mimicry of toxic metals. Annu Rev Pharmacol Toxicol 33:545–571 (1993).
24. van Veen HW, Abee T, Kortstee GJ, Konings WN, Zehnder AJ. Translocation of metal phosphate via the phosphate inorganic transport system of *Escherichia coli*. Biochemistry 33:1766–1770 (1994).
25. Beck L, Silve C. Molecular aspects of renal tubular handling and regulation of inorganic sulfate. Kidney Int 59:835–845 (2001).
26. Chong SS, Kristjansson K, Zoghbi HY, Hughes MR. Molecular cloning of the cDNA encoding a human renal sodium phosphate transport protein and its assignment to chromosome 6p21.3-p23. Genomics 18:355–359 (1993).
27. Hernando N, Forster IC, Biber J, Murer H. Molecular characteristics of phosphate transporters and their regulation. Exp Nephrol 8:366–375 (2000).
28. Kavanaugh MP, Kabat D. Identification and characterization of a widely expressed phosphate transporter/retrovirus receptor family. Kidney Int 49:959–963 (1996).
29. Markovich D, Forgo J, Stange G, Biber J, Murer H. Expression cloning of rat renal Na^+/SO_4^{2-} cotransport. Proc Natl Acad Sci USA 90:8073–8077 (1993).
30. Markovich D. Molecular regulation and membrane trafficking of mammalian renal phosphate and sulphate transporters. Eur J Cell Biol 79:531–538 (2000).
31. Miyamoto K, Tatsumi S, Sonoda T, Yamamoto H, Minami H, Taketani Y, Takeda E. Cloning and functional expression of a Na^+-dependent phosphate co-transporter from human kidney: cDNA cloning and functional expression. Biochem J 305:81–85 (1995).
32. Bull PC, Cox DW. Wilson disease and Menkes disease: new handles on heavy-metal transport. Trends Genet 10:246–252 (1994).
33. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat Genet 3:7–13 (1993).
34. Vulpe CD, Packman S. Cellular copper transport. Annu Rev Nutr 15:293–322 (1995).
35. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, Gitschier J, Anderson GJ. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat Genet 21:195–199 (1999).
36. Yamaguchi Y, Heiny ME, Suzuki M, Gitlin JD. Biochemical characterization and intracellular localization of the Menkes disease protein. Proc Natl Acad Sci USA 93:14030–14035 (1996).
37. Kaplan J, O'Halloran T. Iron metabolism in eukaryotes: Mars and Venus at it again. Science 271:1510–1512 (1996).
38. Valentine JS, Gralla EB. Delivering copper inside yeast and human cells. Science 278:817–818 (1997).
39. Zhou B, Gitschier J. hCTR1: a human gene for copper uptake identified by complementation in yeast. Proc Natl Acad Sci USA 94:7481–7486 (1997).
40. Moller LB, Petersen C, Lund C, Horn N. Characterization of the *hCTR1* gene: genomic organization, functional expression, and identification of a highly homologous processed gene. Gene 257:13–22 (2000).
41. Kuo YM, Zhou B, Cosco D, Gitschier J. The copper transporter CTR1 provides an essential function in mammalian embryonic development. Proc Natl Acad Sci USA 98:6836–6841 (2001).
42. Lee J, Prohaska JR, Thiele DJ. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proc Natl Acad Sci USA 98:6842–6847 (2001).
43. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482–488 (1997).
44. Olivi L, Sisk J, Bressler J. Involvement of DMT1 in uptake of Cd in MDCK cells: role of protein kinase C. Am J Physiol 281:C793–C800 (2001).
45. Tallkvist J, Bowlus CL, Lonnerdal B. DMT1 gene expression and cadmium absorption in human absorptive enterocytes. Toxicol Lett 122:171–177 (2001).
46. Fleming MD, Trenor CC III, Su MA, Foernzler D, Beier DR, Dietrich WF, Andrews NC. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet 16:383–386 (1997).
47. Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D. Cellular localization of divalent metal transporter DMT-1 in rat kidney. Am J Physiol 280:F803–F814 (2001).
48. Atkinson PG, Blackwell JM, Barton CH. Nramp1 locus encodes a 65 kDa interferon-gamma-inducible protein in murine macrophages. Biochem J 325:779–786 (1997).
49. Cellier M, Prive G, Belouchi A, Kwan T, Rodrigues V, Chia W, Gros P. Nramp defines a family of membrane proteins. Proc Natl Acad Sci USA 92:10089–10093 (1995).
50. Supek F, Supekova L, Nelson H, Nelson N. Function of metal-ion homeostasis in the cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain function. J Exp Biol 200:321–330 (1997).
51. Palmiter RD, Findley SD. Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. EMBO J 14:639–649 (1995).
52. Palmiter RD, Cole TB, Findley SD. ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. EMBO J 15:1784–1791 (1996).
53. Palmiter RD, Cole TB, Quaife CJ, Findley SD. ZnT-3, a putative transporter of zinc into synaptic vesicles. Proc Natl Acad Sci USA 93:14934–14939 (1996).
54. Huang LP, Gitschier J. A novel gene involved in zinc transport is deficient in the lethal milk mouse. Nature Genetics 17:292–297 (1997).
55. Liuzzi JP, Blanchard RK, Cousins RJ. Differential regulation of zinc transporter 1, 2, and 4 mRNA expression by dietary zinc in rats. J Nutr 131:46–52 (2001).
56. Gaither LA, Eide DJ. Functional expression of the human hZIP2 zinc transporter. J Biol Chem 275:5560–5564 (2000).
57. Gaither LA, Eide DJ. The human ZIP1 transporter mediates zinc uptake in human K562 erythroleukemia cells. J Biol Chem 276:22258–22264 (2001).
58. Abboud S, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 275:19906–19912 (2000).
59. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, et al . Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 403:776–781 (2000).
60. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, Miret S, Bomford A, Peters TJ, Farzaneh F, 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).
61. McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, Mudaly M, Richardson C, Barlow D, Bomford A, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 291:1755–1759 (2001).
62. Hinkle PM, Kinsella PA, Osterhoudt KC. Cadmium uptake and toxicity via voltage-sensitive calcium channels. J Biol Chem 262:16333–16337, (1987).
63. Simons TJ, Pocock G. Lead enters bovine adrenal medullary cells through calcium channels. J Neurochem 48:383–389 (1987).
64. Hughes BP, Barritt GJ. Inhibition of the liver cell receptor-activated Ca^{2+} inflow system by metal ion inhibitors of voltage-operated Ca^{2+} channels but not by other inhibitors of Ca^{2+} inflow. Biochim Biophys Acta 1013:197–205 (1989).
65. Crofts JN, Barritt GJ. The liver cell plasma membrane Ca^{++} inflow systems exhibit a broad specificity for divalent metal ions. Biochem J 269:579–587 (1990).
66. Ballatori N. Glutathione mercaptides as transport forms of metals. Adv Pharmacol 27:271–298 (1994).
67. Aiken SP, Horn NM, Saunders NR. Effects of amino acids on zinc transport in rat erythrocytes. J Physiol 445:69–80 (1992).
68. Horn NM, Thomas AL, Tompkins JD. The effect of histidine and cysteine on zinc influx into rat and human erythrocytes. J Physiol 489:73–80 (1995).
69. Torrubia JOA, Garay R. Evidence for a major route for zinc uptake in human red blood cells: $[Zn(HCO_3)_2\ Cl]$--- influx through the $[Cl---/HCO_3---]$ anion exchanger. J Cell Physiol 138:316–322 (1989).
70. Kerper LE, Ballatori N, Clarkson TW. Methylmercury transport across the blood-brain barrier by an amino acid carrier. Am J Physiol 262:R761–R765 (1992).
71. Mokrzan EM, Kerper LE, Ballatori N, Clarkson TW. Methylmercury-thiol uptake into cultured brain capillary endothelial cells on amino acid system L. J Pharmacol Exp Ther 272:1277–1284 (1995).
72. Kajiwara Y, Yasutake A, Adachi T, Hirayama K. Methylmercury transport across the placenta via neutral amino acid carrier. Arch Toxicol 70:310–314 (1996).
73. Ballatori N, Clarkson TW. Developmental changes in the biliary excretion of methylmercury and glutathione. Science 216:61–63 (1982).
74. Ballatori N, Gatmaitan Z, Truong AT. Impaired biliary excretion and whole body elimination of methylmercury in rats with congenital defect in biliary glutathione excretion. Hepatology 22:1469–1473 (1995).
75. Dutczak WJ, Ballatori N. Transport of the glutathione-methylmercury complex across liver canalicular membranes on reduced glutathione carriers. J Biol Chem 269:9746–9751 (1994).
76. Broeks A, Gerrard B, Allikmets R, Dean M, Plasterk RH. Homologues of the human multidrug resistance genes MRP and MDR contribute to heavy metal resistance in the soil nematode Caenorhabditis elegans. EMBO J 15:6132–6143 (1996).
77. Callahan HL, Beverley SM. Heavy metal resistance: a new role for P-glycoprotein in Leishmania. J Biol Chem 266:18427–18430 (1991).
78. Chen Z-S, Mutoh M, Sumizawa T, Furukawa T, Haraguchi M, Tani A, Akiyama S. Reversal of heavy metal resistance in multidrug-resistant human KB carcinoma cells. Biochem Biophys Res Commun 236:586–590 (1997).
79. Deeley RG, Cole SPC. Function, evolution and structure of multidrug resistance protein (MRP). Semin Cancer Biol 8:193–204 (1997).
80. Ishikawa T, Bao JJ, Yamane Y, Akimaru K, Frindrich K, Wright CD, Kuo MT. Coordinated induction of MRP/GS-X pump and γ-glutamylcysteine synthetase by heavy metals

in human leukemia cells. J Biol Chem 271:14981–14988 (1996).
81. Sharma V, Crankshaw CL, Piwnica-Worms D. Effects of multidrug resistance (MDR1)P-glycoprotein expression levels and coordination metal on the cytotoxic potency of multidentate (N_4O_2) (ethylenediamine)bis[propyl(R-benzylimino)]metal(III) cations. J Med Chem 39:3483–3490 (1996).
82. Sugawara N, Lai YR, Arizono K, Kitajima T, Inoue H. Lack of biliary excretion of Cd linked to an inherent defect of the canalicular isoform of multidrug resistance protein (cMrp) does not abnormally stimulate accumulation of Cd in the Eisai hyperbilirubinemic (EHB) rat liver. Arch Toxicol 71:336–339 (1997).
83. Sugawara N, Lai YR, Sugaware C, Arizono K. Decreased hepatobiliary secretion of inorganic mercury, its deposition and toxicity in the Eisai hyperbilirubinemic rat with no hepatic canalicular organic anion transporter. Toxicology 126:23–31 (1998).
84. Vernhet L, Allain N, Payen L, Anger JP, Guillouzo A, Fardel O. Resistance of human multidrug resistance-associated protein 1-overexpressing lung tumor cells to the anticancer drug arsenic trioxide. Biochem Pharmacol 61:1387–1391 (2001).