Mammalian Zinc Transporters: Nutritional and Physiologic Regulation

Louis A. Lichten and Robert J. Cousins

Nutritional Genomics Laboratory, Food Science and Human Nutrition Department and Center for Nutritional Sciences, University of Florida, Gainesville, Florida 32611-2710; email: cousins@ufl.edu

Annu. Rev. Nutr. 2009. 29:153–76

First published online as a Review in Advance on April 28, 2009

The *Annual Review of Nutrition* is online at nutr.annualreviews.org

This article's doi: 10.1146/annurev-nutr-033009-083312

0199-9885/09/0821-0153$20.00

Key Words

zinc metabolism, inflammation, gene regulation, zinc deficiency

Abstract

Research advances defining how zinc is transported into and out of cells and organelles have increased exponentially within the past five years. Research has progressed through application of molecular techniques including genomic analysis, cell transfection, RNA interference, kinetic analysis of ion transport, and application of cell and animal models including knockout mice. The knowledge base has increased for most of 10 members of the ZnT family and 14 members of the Zrt-, Irt-like protein (ZIP) family. Relative to the handling of dietary zinc is the involvement of ZnT1, ZIP4, and ZIP5 in intestinal zinc transport, involvement of ZIP10 and ZnT1 in renal zinc reabsorption, and the roles of ZIP5, ZnT2, and ZnT1 in pancreatic release of endogenous zinc. These events are major factors in regulation of zinc homeostasis. Other salient findings are the involvement of ZnT2 in lactation, ZIP14 in the hypozincemia of inflammation, ZIP6, ZIP7, and ZIP10 in metastatic breast cancer, and ZnT8 in insulin processing and as an autoantigen in diabetes.

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INTRODUCTION

Interest and research advances in how cells and organelles transport zinc have increased exponentially since the previous review on this subject in the *Annual Review of Nutrition* (91). The research has progressed through application of unique molecular techniques: RNA interference (RNAi), cell transfection, genomic analysis, and application of cell and animal models including knockout mice. Provided in this review is an analysis of the current information directed at the biology of zinc transporters. This includes a discussion of the tissue specificity of expression for each zinc transporter gene; metals, hormones, and cytokines that influence their expression; and diseases that have been linked to their aberrant expression.

ZINC TRANSPORTERS

Two families of mammalian zinc transporters exist. The first is the ZnT family of transporters, which act to decrease intracellular zinc levels by transporting zinc from the cytoplasm to the lumen of organelles or the extracellular space. The second is the ZIP (Zrt-, Irt-like protein) family, which is named after the yeast Zrt1 protein and the *Arabidopsis* Irt1 protein. The signature of the ZIP family is that these proteins are responsible for increasing intracellular zinc levels by either transporting the metal from the extracellular space or organellar lumen into the cytoplasm (**Figure 1**).

ZnT (SLC30) FAMILY

More than 100 members of the SLC30 family are found in organisms at all phylogenetic levels. The ZnT family is divided into three subfamilies (113). Subfamily I contains mainly prokaryotic members, whereas subfamilies II and III contain eukaryotic and prokaryotic members in a similar ratio (91). Most ZnT proteins have six transmembrane domains (TMDs) and are predicted to have cytoplasmic amino and carboxy termini. In addition, a classic characteristic of ZnT proteins is the long histidine-rich loop between TMDs IV and V, (HX)n (n = 3 to 6), which could represent a metal-binding domain. Highly amphipathic TMDs I, II, and V are well conserved (92).

ZnT1 (SLC30A1)

The first mammalian zinc transporter to be discovered was *ZnT1* (*Slc30a1*), which was mapped to chromosome 1 in both humans and mice (112). ZnT1 was identified by isolation from a rat kidney cDNA expression library by complementation of a mutated, zinc-sensitive BHK cell line. ZnT1 displays a ubiquitous tissue

distribution; however, it is more highly expressed in tissues involved in zinc acquisition, recycling, or transfer, such as the small intestine, (91, 101). When neuronal cells were transfected with rZnT1 cDNA, the protein localized primarily to the plasma membrane with some punctate staining throughout the cell (76). In vivo immunolocalization studies indicate that, in growing male rats, ZnT1 is increasingly abundant along basolateral membranes of enterocytes where it may participate in zinc transfer into the circulation (101). Abundant expression of ZnT1 was also found on the basolateral surface in cells lining the thick ascending and distal convoluted tubules of the kidney (24). This localization of ZnT1 indicates that it may play a role in recovery of zinc from the glomerular filtrate. ZnT1 also localizes to the villous yolk sac membrane, suggesting that ZnT1 participates in zinc transport between maternal supplies and the fetus (24, 86, 90). Interestingly, homozygous targeted knockout of *ZnT1* leads to early embryonic lethality in the mouse, indicating that ZnT1 serves an essential function of transporting maternal zinc into the embryonic environment during the egg cylinder stage of development and further suggests that ZnT1 plays a role in zinc homeostasis in adult mice (4).

ZnT1 expression can be influenced differentially by the dietary zinc supply. Rats fed a diet deficient in zinc (<1 ppm) showed decreased ZnT1 mRNA expression, whereas rats fed a diet high in zinc (180 ppm) exhibited increased ZnT1 mRNA abundance (90). These findings indicate a metal responsive mode of regulation for the *ZnT1* gene (**Figure 2**). Indeed, in vitro DNA-binding assays demonstrated that mouse MTF-1 can bind avidly to two metal-response element sequences found in the *ZnT1* promoter. Using mouse embryo fibroblasts with homozygous deletions of the MTF-1 gene, it was shown that this transcription factor is essential for basal as well as metal-induced (i.e., zinc or cadmium) regulation of the ZnT1 gene in these cells. In vivo, ZnT1 mRNA was abundant in the midgestation visceral yolk sac and placenta. Dietary zinc deficiency during pregnancy leads to downregulation of ZnT1 levels in the visceral yolk sac but has little effect on the mRNA in the placenta. Homozygous knockout of the MTF-1 gene in mice also leads to a reduction in ZnT1 mRNA levels in the visceral yolk sac, suggesting that MTF-1 mediates the response of *ZnT1* to zinc in the visceral yolk sac (86).

There is a considerable body of evidence with isolated cells showing that zinc restriction (via chelation) or zinc supplementation usually found in culture medium influences expression and localization of zinc transporters. **Table 1** lists some of the zinc transporter genes that have been shown to be zinc regulated in an animal model through changes in dietary zinc intake. A number of zinc transporters that are localized to the plasma membrane in zinc-depleted cell cultures, specifically ZIP1, ZIP2, ZIP3, and ZIP5, become internalized by a zinc-induced endocytotic process.

ZnT2 (SLC30A2)

Similar to ZnT1, a cDNA encoding the second zinc transporter (ZnT2) was isolated from a rat kidney cDNA expression library by complementation of a zinc-sensitive BHK cell line (110). However, unlike ZnT1, which is in the plasma membrane and lowers cellular zinc by stimulating zinc efflux, ZnT2 is localized on vesicles and allows the zinc-sensitive BHK cells to accumulate zinc to levels that are much higher than nontransformed cells can tolerate. ZnT2 mRNA has been detected in specific tissues of rodents: small intestine, kidney, placenta, pancreas, testis, seminal vesicles, and the mammary gland (reviewed in 91).

ZnT2 is upregulated in the small intestine by high dietary Zn intake, during very late-stage gestation, and early lactation in maternal and fetal tissues (90). Similarly, the lateral lobes of the prostate have a very high Zn content, which correlates with high ZnT2 expression (62). Prolactin up-regulates ZnT2 expression in the prostate (22) (**Table 2**). The association of ZnT2 with high cellular Zn concentrations and its vesicular localization in the acinar cells of

Table 1 Known dietary zinc regulation of some ZnT (SLC30A) and Zip (SLC39A) zinc transporters

Zinc transporter	Dietary zinc (response)	Tissue/cell	Subcellular location
ZnT1	ZnD (–) ZnE (+)	Ubiquitous Blood cells	Plasma membrane, vesicles
ZnT2	ZnD (–) ZnE (+)	Small intestine, liver, pancreatic acinar cells, and kidney Mammary gland	Vesicles, secretory granules
ZnT4	ZnD (–)	Small intestine	TGN, vesicles, endosomes
ZnT5	ZnD (–)	Small intestine	Secretory granules
ZnT6	ZnD (–) ZnE (+)	Small intestine	TGN
Zip3	ZnE (–)	Blood cells	
Zip4	ZnD (+)	Small intestine/colon	Apical Plasma membrane
Zip10	ZnD (+)	Brain Liver Erythroid progenitor cells	Plasma membrane

Abbreviations: TGN, trans-golgi network; ZnD, dietary zinc restriction below the requirement; ZnE, dietary zinc in excess of requirement; +, up-regulation; -, down-regulation. This table does not include zinc transporters that may undergo post-translational regulation influenced by zinc.

the pancreas suggests that this transporter may function through an exocytotic pathway, before incorporation of Zn into secreted pancreatic proteins to control endogenous losses (92).

The role of ZnT2 in maintenance of zinc homeostasis is not entirely known. However, its production in the mammary gland, and concurrent decrease in abundance with milk zinc concentration, suggests a role for this transporter in mammary gland zinc metabolism. Interestingly, while the abundance of ZnT2 at the basolateral membrane appears to remain constant, the expression of ZnT2 at the apical membrane of the mammary gland decreases through lactation (75). These results are consistent with ZnT2 relocalizing to an intracellular compartment of mammary epithelial cells during exposure to physiologically high levels of zinc, to possibly sequester excess cellular zinc (75, 110). Furthermore, a study investigating transient neonatal zinc deficiency in two breast-fed infants as a consequence of reduced zinc secretion into breast milk identified a His to Arg mutation at amino acid residue 59 of ZnT2 in the mothers, linking this gene mutation to infant zinc deficiency (18).

ZnT3 (SLC30A3)

ZnT3 protein is predicted to have six transmembrane domains and shares 52% amino acid identity with ZnT2, with the homology extending throughout the two sequences. The *ZnT3* gene was identified and subsequently cloned by screening of a mouse λ library through homology with ZnT2 cDNA (111). ZnT3 mRNA is most abundant in the hippocampus and cortex of the mouse brain. The ZnT3 protein is detected immunologically in the mossy fibers, where zinc-containing vesicles are most abundant.

The mammalian brain contains an abundant amount of zinc, with 5%–15% concentrated in synaptic vesicles in a subset of glutamatergic neurons (43, 53, 115). Zinc is also particularly abundant in the hippocampus. Homozygous disruption of murine *ZnT3* (*ZnT3*$^{-/-}$) decreases the amount of detectable zinc in these regions of the brain (21). Timm stain is used for histochemical detection of zinc, and this method revealed that the reduction in zinc content corresponds exclusively to zinc packaged into synaptic vesicles (44). An intermediate level of both ZnT3 protein and histochemically

reactive zinc was found in *ZnT3* heterozygotes ($ZnT3^{+/-}$) when compared with wild-type and $ZnT3^{-/-}$ mice, demonstrating that the amount of zinc in synaptic vesicles is limited by the abundance of ZnT3. These results suggest that zinc is taken up into synaptic vesicles through ZnT3 located at the vesicle membrane.

ZnT4 (SLC30A4)

A disorder of zinc deficiency was identified in mouse pups unable to survive infancy nursing on milk of mice homozygous for the autosomal-recessive mutation, lethal milk (*lm*) (118). The major effect of the *lm* mutation is the production of Zn-deficient milk (1). The gene responsible for the lm phenotype was later identified as *ZnT4* by positional cloning (57). Confirmation of the zinc transport function of ZnT4 was achieved by complementation of the yeast *ZRC1* mutant. A single C to T point mutation at base 934 leads to a nonsense mutation and premature translation termination of ZnT4. This mutation leads to an approximately 50% reduction in milk of *lm* animals (1, 87). However, because milk from *lm* mice is not completely void of zinc, and maternal zinc supplementation is able to rescue the lethal milk phenotype, other zinc transporters may be active, such as the aforementioned ZnT2. Indeed, analysis of *hZnT4* gene expression in a mammary gland disorder leading to reduced zinc secretion into human milk indicated that ZnT4 was not responsible for postnatal zinc deficiency (102).

ZnT4 is not only found in mammary tissue, but it is also abundantly expressed in the mouse brain and intestinal epithelial cells (57, 90, 105). Furthermore, ZnT4 localizes to intracellular vesicles of various cells (57, 73, 105). The endogenous ZnT4 was detected in the Golgi apparatus as well as in the vesicular compartment of rat normal kidney (NRK) cells, whereas transfection of a Myc-tagged version of ZnT4 into Caco-2 cells revealed expression in an endosomal compartment (57, 105).

Expression of *ZnT4* appears to be independent of zinc status (90). However, an increased extracellular zinc concentration induces trafficking of ZnT4 from trans-golgi network (TGN) to the cytoplasmic vesicular compartment in the cultured NRK cells (105).

Overall, ZnT4 appears to facilitate entry of zinc into secretory vesicles of certain glands (mammary and submaxillary) and thereby allows secretion of zinc by these exocrine glands (reviewed in 113).

Table 2 Hormonal and cytokine regulation of ZnT (SLC30A) and Zip (SLC39A) transporters

Zinc transporter	Hormone/cytokine (response)	Tissue/cell
ZnT2	Glucorticoid hormone (+)	Pancreatic acinar cells
	Prolactin (+)	Prostate
ZnT4	Immune activation (+)	T cells
	Cell differentiation (+)	Intestinal epithelium
ZnT5	IL-6	Liver cells
Zip1	Cell differentiation (+)	Osteoblasts
	Prolactin (+)	Prostate cells
	Testosterone (+)	Prostate cells
	IL-6(+)	Liver cells
Zip3	Prolactin (+)	Mammary cells
Zip5	IL-6(+)	Liver cells
Zip6	IL-6/IL-1 (+)	Liver cells
	Lipopolysaccharide (+)	Dendritic cells
Zip8	Lipopolysaccharide (+)	Monocytes
	Immune activation (+)	T cells
	TNFα (+)	Lung epithelial cells
Zip10	Thyroid hormone (+)	Intestine
		Kidney cells
Zip14	IL-6/IL-1 (+)	Liver cells
	Nitric oxide (+)	Liver cells

+, up-regulation.

ZnT5 (SLC30A5)

A database search of DNA sequences homologous to yeast ZRC1 allowed identification of ZnT5. Human ZnT5 cDNA encodes a 765-amino acid protein with 15 predicted membrane-spanning domains (72). ZnT5 was ubiquitously expressed in all tested human tissues, but was most abundantly expressed in insulin-containing beta cells that contain zinc at the highest level in the body (72). Another report, published at almost the same time,

reported the cloning of a human zinc transporter expressed at the apical membrane of the Caco-2 human small intestinal cell line model designated hZTL1 (human ZnT-like transporter 1) (25). However, hZTL1 was subsequently identified as hZnT5.

An intriguing aspect of ZnT5 function is the observation that this transporter interacts with ZnT6 to form a complex that can transport zinc into the secretory pathway (39). Additionally, ZnT5 and ZnT6 are both located in the TGN of mammalian cells (58, 72) and are expressed in many of the same tissues (128). The formation of ZnT5/ZnT6 hetero-oligomeric complexes is considered to be essential for their functions, because both genes need to be expressed to activate tissue-nonspecific alkaline phosphatase (TNAP) (134).

Regulation of *ZnT5* is complex: Two major transcripts of *ZnT5* were identified by Northern blotting, and comparison of the two published sequences shows that they differ at both the 5′ and 3′ ends, with variant B being a shorter transcript (25, 72). Alignment of both sequences with the human genome reveals that they are splice variants of the *SLC30A5* gene, incorporating different exons at the 5′ and 3′ ends (65). Variant B was localized to the plasma membrane, where evidence for bidirectional function (145) indicates possible roles in both the uptake and efflux of zinc. Both increased and reduced expression of *ZnT5* has been found in response to zinc, including increased expression in Caco-2 cells exposed to 100 μM zinc (25), decreased expression in human intestinal mucosa in response to zinc supplementation (26), and reduced expression in the mouse placenta in response to both a zinc-restricted and zinc-supplemented diet (55). Therefore, two modes of regulation appear to exist for *ZnT5*: transcriptional repression and increased mRNA stability (65).

The importance of ZnT5 to zinc homeostasis is emphasized by deletion of *ZnT5* in mice, which leads to poor growth, abnormal bone development, weight loss, and male-specific cardiac arrhythmias (65).

ZnT6 (SLC30A6)

Search of the Expressed Sequence Tag (EST) databases with the amino acid sequence of mouse ZnT4 revealed the zinc transporter homolog ZnT6 (58). Overexpression of ZnT6 in both wild-type yeast and mutants that are deficient in cytoplasmic zinc causes growth inhibition, but this inhibition is abolished in mutant cells with high cytoplasmic zinc. ZnT6 may function in transporting cytoplasmic zinc into the Golgi apparatus as well as the vesicular compartment. ZnT5 and ZnT6 are both localized to the TGN (58, 72) and functionally interact to activate TNAP (39, 134).

ZnT6 mRNA was found in the liver, brain, kidney, and small intestine. Intriguingly, the protein was only detected in the brain and lung, suggesting that a post-transcriptional mechanism may play a role in tissue-specific expression of the ZnT6 protein (58).

ZnT7 (SLC30A7)

ZnT7 was identified by homology to the amino acid sequence of ZnT1 in the EST databases (80). The Znt7 gene is expressed in many mouse tissues including liver, kidney, spleen, heart, brain, small intestine, and lung, along with abundant expression in small intestine and liver, and less expression in the heart. However, expression of ZnT7 protein is limited to the tissues of lung and small intestine with abundant expression in the proximal segment (duodenum and part of jejunum) of the small intestine (80). When overexpressed in Chinese hamster ovary cells (CHO), ZnT7 leads to zinc accumulation in the Golgi apparatus. ZnT7 localizes to a vesicular compartment seemingly different from that of ZnT2, ZnT3, ZnT4, ZnT5, or ZnT6 in the hBRIE 380, WI-38, and transiently transfected NRK cells suggesting that ZnT7 may also be involved in transporting zinc into a unique vesicular compartment. Disruption of *ZnT7* in DT40 cells results in a 20% decrease in TNAP activity (134). Therefore, there seems to be at least partial dependence of TNAP activity on ZnT7.

Znt7 knockout mice display a zinc-deficient phenotype that is irresponsive to dietary zinc supplementation (61). Furthermore, these mice demonstrate poor growth and have decreased body-fat composition, which suggests that ZnT7 plays a critical role in maintaining cellular zinc homeostasis and may be involved in the regulation of body composition.

ZnT8 (SLC30A8)

Insulin-secreting ß-cells located in the islets of Langerhans of the pancreas accumulate very high amounts of zinc (160). Insulin is thought to be stored inside secretory vesicles as a solid hexamer bound with two Zn^{2+} ions per hexamer (40). Insulin is then released by exocytosis in response to external stimuli, such as elevated glucose concentrations. When exocytosis of insulin occurs, insulin granules fuse with the ß-cell plasma membrane, releasing insulin as well as zinc into the circulation (119). Interestingly, a complex relationship between zinc and both type 1 and type 2 diabetes arises because several complications of diabetes may be mediated through oxidative stress, which is amplified in part by zinc deficiency (reviewed in 15).

In 2004, the islets of Langerhans-specific zinc transporter, ZnT8, was identified in ß-cells and shown to facilitate the accumulation of zinc from the cytoplasm into intracellular vesicles (17). Moreover, a ZnT8-EGFP fusion protein was colocalized with insulin secretory granules in the rat insulin-secreting INS-1 cell line, suggesting that ZnT8 may be involved in providing zinc for insulin maturation and/or storage processes in insulin-secreting pancreatic ß-cells. Three years later, the importance of *ZnT8* in the etiology of diabetes became clear when the *ZnT8* gene was first associated with a novel risk locus for type II diabetes (30, 126, 131, 161). By genotyping 921 metabolically characterized German subjects for candidate single-nucleotide polymorphisms (SNPs) of *SLC30A8*, the SNP rs13266634 was associated with reduced insulin secretion stimulated by orally or intravenously administered glucose, but not with insulin resistance (132). Furthermore, the major nonsynonymous SNP at Arg325-encoding C-allele confers a minor risk (odds ratio 1.07–1.18) of disease. In nondiabetic subjects with a family history of type 2 diabetes, the C-allele was associated with increased circulating proinsulin-to-insulin ratio (79) and decreased insulin responses in intravenous glucose tolerance tests (10), indicating a dominant effect on insulin secretion, β-cell mass, or both.

Then, by utilizing microarray expression profiling of human and rodent pancreas and islet cells and screening with radioimmunoprecipitation assays using new-onset type 1 diabetes and prediabetic sera, ZnT8 was identified as a major autoantigen in human type 1 diabetes (155). However, autoantibodies to ZnT8 in human type 1 diabetic patients show little cross-reactivity to other human Zn transporters or even to mouse ZnT8, which is 82% identical in sequence. Interestingly, the amino acid encoded by a common polymorphism in human ZnT8 at aa325 (either Arg or Trp) is a key determinant of two of the three major conformational epitopes in the protein (154). The autoantibody responses to the ZnT8 Arg- and Trp-restricted isoepitopes segregated with the alleles encoding the respective variant amino acids, which indicates that humoral type 1 diabetes autoimmunity to ZnT8 is directed against self, and not nonself, epitope determinants (155). Recently, mutagenesis of mZnT8 Q324 to arginine (equivalent to R325 in the human protein) allowed for reactivity with human autoimmune sera, which further indicates that the hZnT8 epitope is critically dependent upon the arginine residue at position 325 (154).

Finally, the implications of hZnT8 identification as an autoantigen for type I diabetes are far reaching and show that hZnT8 autoantibodies can be used as an additional and independent predictive marker for type I diabetes (155).

ZnT9 (SLC30A9)

The *ZnT9* gene was originally isolated from human embryonic lung cells (130). The 569 aa protein has a putative cation efflux motif,

a DNA excision repair motif, and a nuclear receptor interaction sequence. Although the protein is predicted to have six transmembrane domains, it associates with cytosol and nuclear fractions, not membranes (130). To date, no studies have assessed the function of this protein.

ZnT10 (SLC30A10)

Investigators utilizing DNA sequence homology with *ZnT1* found *ZnT10* (128). From EST analysis results, ZnT10 was discovered to have a restricted expression profile to the fetal liver and fetal brain. This is the first ZnT predicted to have a fetal restricted expression. It is therefore possible to speculate that ZnT10 plays an important role in zinc homeostasis during fetal development. However, no studies have been conducted to analyze the function of ZnT10.

THE ZIP FAMILY (SLC39)

The human SLC39 (Slc39 nomenclature for the mouse) transporters are members of the ZIP family of metal ion transporters (41, 47). The first members to be identified were Zrt1 and Zrt2, the primary zinc uptake transporters in the yeast *Saccharomyces cerevisiae*, and Irt, the major iron uptake transporter in roots of *Arabidopsis thaliana;* hence, the designation ZIP for Zrt, Irt-like protein (reviewed in 37). Most ZIP proteins have eight predicted transmembrane domains (TMDs) and similar predicted membrane topologies with the N- and C-termini of the protein located along the extracellular surface of the membrane. Many members also have a long loop region located between TMDs 3 and 4 and a histidine-rich domain with the sequence (HX)n where n generally ranges from 3 to 5. Due to their sequence conservation and amphipathic nature, TMDs IV and V are predicted to form a cavity through which metals may pass (38).

Zip1 (SLC39A1)

Zip1/ZIRTL was identified through homology with the *Arabidopsis thaliana* ZIP1 transporter and is expressed in a wide variety of tissues and cell types (47, 88). ZIP1 is localized to different areas of the cell in a cell-type-specific manner. In K562 cells, hZip1 localizes to the plasma membrane, where it allows energy-independent zinc uptake (47). In cell types such as COS-7 or PC3, hZip1 is localized mainly in the endoplasmic reticulum (103). Transport of zinc into K562 cells by hZIP1 was indistinguishable from endogenous uptake and could be abolished by antisense RNA directed against hZip1, indicating a requirement for ZIP1-mediated zinc transport in these cells (47). Moreover, saturable ^{65}Zn uptake kinetics correlate with increased Zip1 mRNA abundance after exposure to prolactin and testosterone (reviewed in 22, 91).

The murine orthologue, mZip1, is present in all tissues except for the pancreas, and the abundance of Zip1 mRNA is not regulated by dietary zinc in the intestine or visceral endoderm, tissues involved in nutrient absorption (33). Furthermore, studies of transfected cells revealed that ZIP1 is mainly present in intracellular organelles in cells cultured in zinc-adequate medium but is recruited to the cell surface when zinc is limiting, suggesting a post-transcriptional regulatory mechanism (147). Recently, a protein chimera of Zip1 was created, demonstrating that a di-leucine sorting signal of ZIP1 was required and sufficient for endocytosis of the protein (60).

Additionally, homozygous knockout of mZip1 produces no phenotype when dietary zinc intake is normal but can adversely affect embryo survival during pregnancy, when intake of zinc is limiting (32).

Zip2 (SLC39A2)

The human zinc transporter, hZip2, was identified by similarity of the protein-coding sequence to zinc transporters characterized in fungi and plants (46). Similarly, the murine orthologue mZip2 was identified by sequence similarity with hZip2 (33). Expression of hZip2 is low and appears to be limited to the prostate, uterus, cervical epithelium, optic nerve, and

monocytes (reviewed in 91). Expression of mZip2 also appears to be tissue-restricted, with the highest levels detected in the skin, liver, ovary, and visceral yolk sac (33). Both hZip2 and mZip2, when transfected into K562 or HEK293 cells, allow zinc uptake activity (33, 46).

Although the human and murine orthologues share 78% sequence similarity, they appear to be regulated differently and in a cell-type/tissue-specific manner. Treatment of the THP-1 monocytic cell line, or human peripheral blood mononuclear cells with TPEN, a cell-permeable zinc chelator, resulted in a large increase in hZip2 mRNA levels, suggesting that zinc regulated expression (13). However, mZip2 was unresponsive to dietary zinc restriction in the intestine and visceral yolk sac (33). $Zip2^{-/-}$ mice demonstrate no overt phenotype but are more sensitive to dietary zinc deficiency during pregnancy (117).

Zip3 (SLC39A3)

Similar to hZip1 and hZip2, hZip3 was identified by comparison of fungal and plant Zip members with mammalian ESTs (46). The mouse orthologue, mZIP3, was again identified through protein homology with its human counterpart (33). Low levels of Zip3 expression can be detected in many tissues, with the highest levels in the testes (33). Additionally, Zip3 mRNA is not regulated by dietary zinc in the intestine or visceral endoderm, tissues involved in nutrient absorption. ZIP3 is capable of zinc uptake when transfected into HEK293 cells. However, zinc uptake could be inhibited by various metals, suggesting that ZIP3-mediated metal transport is not specific for zinc (33). Cell transfection of Zip3 revealed the presence of the protein in intracellular organelles in zinc-replete medium but recruitment to the cell surface when zinc is limiting (147).

Although mammary epithelial cells were shown to have a requirement for ZIP3-mediated zinc import (74), mice lacking the Zip3 transporter exhibited no obvious phenotypic abnormalities under normal growth conditions and were only slightly more susceptible to the effects of dietary zinc deficiency (32). These findings are somewhat disappointing considering a substantial amount of Zn^{2+} is transferred by the mammary gland from the maternal circulation into milk, supplying zinc to the suckling neonate. However, creation of ZIP1, ZIP3 double-knockout mice showed that these proteins were essential for normal embryo development during zinc deficiency (32). Moreover, the Zip1, Zip2, and Zip3 triple-knockout mouse was indistinguishable from its wild-type littermates when zinc was adequate but displayed a similar zinc-deficiency-sensitive phenotype (71).

Zip4 (SLC39A4)

Zinc deficiency leads to growth retardation, immune-system dysfunction, alopecia, severe dermatitis, diarrhea, and, occasionally, mental disorders. This pathophysiology is seen in the rare, autosomal recessively inherited disease of intestinal zinc malabsorption, acrodermatitis enteropathica (AE). The genetic origin of the disease is a telomeric region of 3.5 cM on chromosome 8q24.3 and identified as the AE susceptibility gene region (149). Through screening of potential gene targets, a genomic sequence predicted to produce a protein with the capability of zinc binding and shown to be homologous to other ZIP proteins was identified and named hZip4 (150). Abundant expression of hZIP4 was identified in tissues involved in zinc absorption/reabsorption, such as the small intestine, stomach, and colon, as well as in the kidney (150). Several mutations in hZip4, ranging from missense mutations, splicing defects, and transcription-inactivating upstream deletions, were discovered in patients with AE (84, 150). Although hZip4 mutations are critical to the etiology of AE, they can be overcome through dietary zinc supplementation (150). Therefore, other mechanisms of intestinal zinc absorption must be present.

The study of the murine orthologue, mZip4, has provided most of the information regarding the structure, function, and regulation of Zip4 (reviewed in 5). The mouse and human Zip4

proteins are well conserved and share 76% homology (34). Although ZIP4 functions as a zinc transporter in transfected cells, several AE mutations appear to abolish its activity by causing retention in the endoplasmic reticulum, and others apparently diminish its zinc uptake activity (152).

Expression of Zip4 appears to be regulated by both transcriptional and post-transcriptional mechanisms in response to zinc availability. The abundance of Zip4 mRNA, cellular localization, and turnover of this protein are regulated by zinc availability in the intestine and visceral yolk sac (33, 34, 77, 93, 99, 153). By using RNA and protein synthesis inhibitors and run-on transcription assays, increased expression of Zip4 during zinc deficiency was shown to be due to stabilization of Zip4 mRNA, not transcription (153). Recently, however, the transcription factor Krüppel-like factor 4 (KLF4), which is induced during zinc restriction, was identified as a component of the mechanism responsible for the transcriptional up-regulation of ZIP4 (23). During dietary zinc deficiency, ZIP4 localizes to the apical membranes of enterocytes in the intestine and visceral endoderm cells in the embryonic visceral yolk sac (34, 35, 93). However, zinc repletion was reported to cause mRNA degradation and rapid endocytosis of ZIP4 (153). Interestingly, a histidine-rich region within the large intracellular loop between putative transmembrane domains III and IV may play a role in the response of ZIP4 to zinc by regulating endocytosis and ubiquitination (99). Moreover, dietary zinc restriction affects proteolytic processing of the protein, resulting in removal of the extracellular amino-terminal ectodomain, and leaves a 37-kDa peptide of ZIP4 as the primary protein found (70, 153). Furthermore, certain AE mutations can inhibit this cleavage, suggesting an important role of proteolytic cleavage in regulation of ZIP4 (70).

In mice, homozygous knockout of Zip4 is embryonic lethal (35). In humans, however, complete loss of ZIP4 function, as in AE patients, is not lethal but left untreated postnatally results in morbidity that can be relieved with supplemental zinc (reviewed in 122). On the contrary, Zip4 knockout embryos could not be saved by providing excess zinc orally and/or by intraperitoneal injection to the mother (35). This difference between species could be due to the lack of an alternate transport system to supply zinc to the developing mouse embryo from the dam. Furthermore, heterozygous Zip4-knockout mouse embryos are more hypersensitive to zinc deficiency relative to their wild-type littermates and display growth retardation and morphologic abnormalities (35).

Zip5 (SLC39A5)

Although several AE mutations in the Zip4 gene are likely to abolish the transport function of the Zip4 protein (84, 85, 149, 150), the symptoms of AE can be alleviated through supplemental dietary zinc. Furthermore, some AE mutations fail to map to the same chromosomal region as SLC39A4 (150). Therefore, an additional ZIP protein was thought to be associated with the disease (150; reviewed in 37), which initially was named "hORF1" and is now designated "Zip5."

The Zip4 and Zip5 proteins share 30% homology, and mouse and human Zip5 are very similar in sequence, sharing 84% identity (152). Human Zip5 displays a similar pattern of tissue-specific expression as seen in mouse and human Zip4, with high expression in the liver, kidney, pancreas, and throughout the small intestine and colon (152). Unlike ZIP4, ZIP5 is localized to the basolateral surface of these cell types under zinc-replete conditions but is internalized during periods of dietary zinc deficiency (36). In transfected cells, mZIP5 does function in zinc uptake and is specific for zinc as a substrate (148). Zip5 mRNA abundance is irresponsive to zinc, but the translation of this mRNA was found to be zinc responsive. During zinc deficiency, Zip5 mRNA remains associated with polysomes, while the protein is internalized and degraded in enterocytes, acinar cells, and endoderm cells (153). Zinc-gavage induces rapid resynthesis of ZIP5, where it is then targeted to the basolateral membranes of these cell

types. These results suggest that ZIP5 may oppose ZIP4 and may be involved in enterocyte sensing of body zinc status through serosal-to-mucosal transport of zinc (36, 148).

Zip6 (SLC39A6)

ZIP6 (LIV-1) was identified as a novel gene whose expression is stimulated by estrogen treatment of MCF-7 and ZR-75 breast cancer cells (98). ZIP6 is known as the founding member of the LZT (LIV-1 subfamily of ZIP zinc transporters) subfamily of ZIP transporters. The LIV-1 subfamily is a highly conserved group of eight transmembrane domain proteins that are mainly situated on the plasma membrane and transport zinc into cells. All nine of the LIV-1 family members contain the common histidine-rich domain between TMDs III and IV, which is a hallmark of all ZIP proteins, as well as a unique, highly conserved putative metalloprotease motif (HEXPHEXGD), which closely resembles the active site motif of matrix metalloproteases, located in transmembrane domain V, and considerably increased histidine residues on the N-terminus and extracellular loop between TMD II and III (reviewed in 91, 136, 140).

Functional analysis of cells transfected with Zip6 indicate that this protein does act as a zinc importer that is localized to the plasma membrane of certain cell types (140). Elevated expression of Zip6 is observed in tissues sensitive to steroid hormones such as the placenta, mammary gland, and prostate (140). Furthermore, the association of abundant Zip6 expression in HeLa and lung carcinoma cell lines, as well as in breast cancer cells with metastatic ability, suggests a role for LIV-1 in breast cancer progression (100, 140).

Recently, investigations of breast cancer specimens have substantiated an association of ZIP6 with estrogen receptors. These studies are fascinating in the fact that Zip6 is considered a reliable marker of estrogen-receptor-positive cancers (125, 143) and, moreover, that it is one of the genes used routinely to distinguish the luminal A type of clinical breast cancer (reviewed in 20, 116, 138). Furthermore, the transcription factor STAT3 was shown to activate Zip6, which implies a link to cancer development. Additionally, nuclear localization of the transcription factor Snail, which plays a major role in the epithelial-to-mesenchymal transition (EMT) because of its ability to downregulate the expression of genes critical to cell adhesion, was dependent on ZIP6 expression (159).

Downregulation of ZIP6 after LPS exposure is associated with decreased intracellular zinc, increased surface expression of MHC class II molecules, and therefore maturation of splenic $CD11c^+$ dendritic cells (81). The addition of TPEN, a cell-permeable zinc chelator, increased the surface expression of MHC class II and costimulatory molecules on DCs, just as LPS did, and zinc supplementation or overexpression of ZIP6 inhibited the LPS-induced up-regulation of MHC class II and costimulatory molecules (81). These results suggest that zinc homeostasis through regulation of zinc transporters, specifically ZIP6, is crucial to host immune response.

Zip7 (SLC39A7)

Originally identified through homology to the mouse KE4 gene, which has been mapped to the H2-K region of the mouse major histocompatability complex on chromosome 17 (3), the human HKE4 gene was similarly mapped to the centromeric side of the HLA class II region of chromosome 6 (3). Subsequently, both human and mouse sequences were aligned with the ZIP family of proteins as unknown open reading frame sequences (ORFs) and were shown to exhibit similarity to the consensus sequence for ZIP transporters (41). HKE4, therefore, is now known as ZIP7.

ZIP7 appears to be ubiquitously expressed (136). Transfection of Zip7 into cells causes an increase in intracellular zinc, as would be expected for a zinc importer (136). However, ZIP7 localizes to the Golgi apparatus, not the plasma membrane, suggesting the increase in intracellular zinc is of vesicular

origin (59, 136). Moreover, by using a mutant strain of yeast that was defective in the ZIP7 orthologue, zrt3, which controls release of stored zinc from vacuoles, complementation studies showed that ZIP7 was able to decrease the level of accumulated zinc in these yeast vacuoles and concomitantly increase the nuclear/cytoplasmic labile zinc level in the ZIP7-expressing zrt3 mutant (59). Additionally, whereas Zip7 gene expression and protein localization remains unchanged by zinc status, the protein abundance of ZIP7 is repressed by supplemental zinc.

Another interesting aspect of ZIP7 function is the possibility that this protein may be involved in breast cancer progression (137). Recent studies in the human breast cancer cell line MCF-7 and tamoxifen-resistant (TamR) MCF-7 cells indicate that ZIP7 is required for increasing intracellular zinc levels, leading to activation of EGFR, Src, and IGF-1R-signaling molecules as well as increases in growth and invasion (137), which are hallmarks of the aggressive phenotype of TamR cells (138).

Zip8 (SLC39A8)

Resistance to Cd-induced testicular toxicity is a trait seen in a few inbred mouse strains (97). The resistance phenotype is autosomal recessive, and the gene responsible for the trait was named *Cdm* (139). Refinement of the *Cdm* gene locus (29) allowed for identification of the *Cdm* gene as the eighth member of the Zip family, Zip8 (28).

Cd is presumably transported inadvertently into the vascular endothelial cells of the testis, resulting in increased cellular accumulation and toxicity (28). Moreover, transgenic mice (BTZIP8-3) created with three copies of the 129/SvJ Slc39a8 gene inserted into the Cd-resistant C57BL/6J genome (already containing two copies of the Slc39a8 gene), showed that Cd treatment reversed Cd resistance (seen in nontransgenic littermates) to Cd sensitivity in BTZIP8-3 mice (146).

ZIP8 expression is found in lung, kidney, testis, liver, brain, small intestine, and the membrane fraction of mature RBCs (121, 146). MDCK cultures transfected with Zip8 reveal a plasma membrane localization for this transporter during ZnD conditions but internalization during ZnA conditions (146). In contrast, no difference in plasma membrane–bound ZIP8 could be detected in mature RBCs after dietary zinc deficiency (121). These may be cell-type differences, or in vivo versus in vitro effects. ZIP8 functions as a divalent cation transporter for Mn, Zn, and Cd in mouse fetal fibroblast (MFF) cultures (28, 54). However, specificity of Zn transport could not be shown until studies of inhibition of Cd influx were conducted in ZIP8 cRNA-injected *Xenopus* oocytes (89). Additionally, electrogenic experiments in *Xenopus* oocytes revealed that ZIP8-mediated divalent cation movement across the membrane occurs as the $Cd^{2+}/[HCO_3^-]_2$ and $Zn^{2+}/[HCO_3^-]_2$ electroneutral complexes (89).

Human Zip8 was originally named *Bacillus* calmette-guerin-induced gene in monocyte clone 103 (BIGM103) because the gene was induced in primary human monocytes following exposure to the *Bacillus* calmette-guerin cell-wall skeleton (6). Interestingly, BIGM103 was not constitutively expressed but could be induced by inflammatory mediators such as LPS and TNF-α in the lung. Furthermore, TNF-α stimulated Zip8 expression in primary human lung epithelia obtained from multiple human donors and BEAS-2B cell cultures (8). In addition, TNF-α induced the expression of glycosylated ZIP8 that translocated to the plasma membrane and mitochondria, resulting in an increase in intracellular zinc content and cell survival. In contrast, Zip8 inhibition reduced cellular zinc content and impaired mitochondrial function in response to TNF-α, resulting in greater cell death (8).

Zip9 (SLC39A9)

The sequences of mouse and human Zip9 were identified by the National Institutes of Health Mammalian Gene Collection (MGC) Program to identify and sequence a cDNA clone containing a complete ORF for each human and

mouse gene (133). Sequence homology places ZIP9 in the ZIP family; however, it is the lone mammalian member of ZIP subfamily I (136). There are no other descriptions of structure, function, or regulation of ZIP9 in the literature.

Zip10 (SLC39A10)

Metal-response element-binding transcription factor-1 (MTF-1) is a zinc finger protein that recognizes short *cis*-acting DNA sequences, termed "metal-response elements" (TGCRCNC), which are present in the promoters of metal-responsive genes (127). MTF-1 is conserved throughout evolution, with orthologues having been characterized in the mouse (120), humans (11), *Drosophila* (162), and fish (16). Homozygous disruption of the mouse MTF-1 gene results in lethal liver degeneration on day 14 of gestation (151). However, development of liver-specific MTF-1 conditional knockout mice allowed for identification of a novel MTF-1 regulated gene, Zip10 (158).

Unlike the previously described activation of another zinc transporter, ZnT-1 by MTF-1, Zip10 expression is suppressed by induction of MTF-1 (158). This was the first Zip gene identified as an MTF-1 target, and moreover Zip10 was the first gene repressed by metal induction of MTF-1. Of particular note is the location of the MRE in Zip10. The MRE identified by Wimmer et al. (158) was located +17 bases downstream of the transcription start site (TSS). Furthermore, an additional MRE was found upstream of the TSS in zebrafish that is not conserved in other species (163). Zip10 expression in vivo is suppressed by zinc in both the gill and kidney of zebrafish. Analysis of drZip10 suggested that two transcripts are produced and regulated by two separate promoters located approximately 16kb apart. Interestingly, reporter gene studies utilizing the first promoter (associated with zebrafish gill) that contains 2 MREs flanking the TSS (upstream and downstream) were positively influenced by zinc. In contrast, the second promoter (present in the kidney) contains an additional MRE located in the first intron that was required to repress reporter gene activity (163). These regulatory differences seen in vitro versus in vivo may be due to the physical inability of the promoters to interact through DNA looping in vitro (163). However, other mechanisms are plausible (e.g., steric hinderance of Pol II transcription) and therefore need to be examined further.

Prior to identification of mZip10, a 40-kDa zinc-transport protein in the rat renal brush border was purified (83). Later characterization of the protein, along with its mRNA, revealed sequence homology to known Zip proteins, and the protein was identified as the rat orthologue of ZIP10 (69), but, appears to align more closely with hZip4 (163) and has been subsequently assigned the Zip4-like designation. In transfected cells, rZIP10 localizes to the plasma membrane, where it transports zinc. In contrast to the zinc suppression of Zip10 expression in the mouse and zebrafish, rZip10 increases expression in response to supplemental zinc. Furthermore, rZip10 responds positively to thyroid hormone stimulation (114). These results, along with the lack of MREs in the rZip10 promoter, suggest that rZip10 is regulated in a different manner than the mouse, human, and zebrafish orthologues.

Another interesting aspect of Zip10 function is its possible role in metastatic breast cancer progression. Screening for ZIP10 mRNA expression in breast cancer samples suggested that ZIP10 was significantly associated with the metastasis of breast cancer to the lymph node (68). In addition, the expression of ZIP10 mRNA was higher in the invasive and metastatic breast cancer cell lines. Moreover, migratory activity of metastatic breast cancer cells was inhibited via knockdown of ZIP10, and concomitantly decreased intracellular zinc. These findings demonstrate an intriguing role for zinc and ZIP10 in the migratory activity of highly metastatic breast cancer cells and suggest that ZIP10 (similar to ZIP6 for other forms of breast cancer) may be used as a possible marker for the metastatic phenotype of breast cancer and a novel drug target.

Zip11 (SLC39A11)

The Zip11 protein product is a member of the gufA subfamily of ZIP transporters, named after the *Myxococcus xanthus* gene, which has unknown function. No other structure, function, or regulatory information is available.

Zip12 (SLC39A12)

The schizophrenic brain seems to have a lower concentration of zinc than does a normal brain (78). Screening of a schizophrenia susceptibility locus on chromosome 10p for proteins that may be involved in zinc transport revealed Zip12. An association was made between a missense homozygous mutation in Zip12 and frequency of schizophrenia development in a small group of patients (9). No other structure, function, or regulatory information is available.

Zip13 (SLC39A13)

Patients with Ehlers-Danlos syndrome (EDS) type VI (MIM225400) display a phenotype of progressive kyphoscoliosis, hypermobility of joints, and hyperelasticity of skin combined with severe hypotonia of skeletal muscles (7). The molecular defect in this kyphoscoliotic form of EDS is deficiency of lysyl hydroxylase (LH1; encoded by the PLOD1 gene), the enzyme responsible for conversion of certain lysyl residues in the triple-helical domains of collagen α-chains to hydroxylysine (50). A disorder similar to EDS VI was recognized in six patients from two consanguineous families; however, distinct phenotypic components such as short stature were identified as well (50). This led to clinical characterization of the spondylocheiro dysplastic form of EDS (SCD-EDS). An SCD-EDS-linked region on chromosome 11 was isolated and searched for possible mutations among candidate genes. Genomic and cDNA sequencing of 26 candidates revealed only polymorphic mutations, except those in SLC39A13, in which all six patients were homozygous for the same 9 bp in-frame deletion in exon 4.

SLC39A13 encodes the previously uncharacterized zinc transporter ZIP13, a member of the Liv-1 subfamily of ZIP zinc transporters (136). Phylogenetic analysis revealed a close homology between the Golgi-associated Zip7 and Zip13. This led Giunta et al. (51) to suggest that the defect present in ZIP13 would cause an increase in the concentration of Zn^{2+} in the ER and a competition with Fe^{2+} for binding to lysyl hydroxylase, prolyl 4-hydroxylase, and prolyl 3-hydroxylase, thus impairing hydroxylation of lysyl and prolyl residues. This is entirely possible considering this deletion in Zip13 affects TMD III, which therefore may hinder proper folding of the protein and thus impairs 3D conformation and function of the transporter.

Shortly after identification of the association of Zip13 with SCD-EDS, the homozygous Zip13 knockout mouse was characterized (45). The phenotypic abnormalities associated with the $Zip13^{-/-}$ mice are reduced osteogenesis, abnormal cartilage development, reduced dentin and alveolar bone, and abnormal craniofacial features, as well as decreased dermal and corneal stromal collagen. Of importance is the identified involvement of ZIP13 in BMP/TGF-β signaling pathways in connective tissue forming cells and in nuclear translocation of Smad proteins. Loss of Slc39a13 caused dysregulation of BMP/TGF-β-mediated gene expression, including expression of *Runx2* and *Msx2*, genes critically involved in bone, tooth, and craniofacial development (2, 82, 108, 124, 144). Smad proteins are phosphorylated downstream of BMP or TGF-β receptor complex, followed by nuclear translocation. Among the Smad proteins, all receptor-regulated Smad (R-Smad) and Smad4 possess a Zn-binding motif in the MH1 domain for their DNA binding (14). How ZIP13 affects these signaling pathways is unclear. However, ZIP13 was shown to localize to the Golgi apparatus, and Zn^{2+} accumulation in the Golgi was increased in $Zip13^{-/-}$ cells, indicating that ZIP13 functions as a zinc transporter allowing for efflux of Zn^{2+} from the Golgi into the cytoplasm, where Zn^{2+} may interact with Smad.

Overall, the phenotypic changes observed in SCD-EDS and Zip13$^{-/-}$ mice appear quite similar. Of particular importance, clinical observations such as dwarfism, delayed bone growth, and increased skin fragility are seen in cases of dietary zinc deficiency (52). Therefore, further analysis of these zinc-regulated pathways involved in bone and connective tissue development is needed.

Zip14 (SLC39A14)

The LZT subfamily members are distinguished from other ZIP transporter members by their consensus sequence HEXPHEXGD in TMD V (140). Amino acid sequence analysis of human ZIP14 revealed a slightly altered motif, EEXPHEXGD, similar to that of ZIP8 (141). Curiosity about this difference led to the first study of ZIP14 function. Zinc uptake experiments demonstrated that although the histidine residue in TMD V is critical for transport of zinc by other zinc transporters, the glutamic acid residue substitution in ZIP14 also allows zinc transport (141). This was the first demonstration of zinc influx by a human LZT protein containing an altered signature motif.

The murine orthologue, mZip14, was identified by isolation of genes expressed in the mouse fibroblastic cell-line 3T3-L1 (which mimics adipocyte hyperplasia) during the earliest stages of adipocyte differentiation that positively regulate the differentiation (63, 109). Although ZIP14 expression was elevated during adipogenesis and was highly restricted to the differentiation state of 3T3-L1 cells, the exact role that ZIP14 plays in this process remains to be determined (142).

A very interesting function of ZIP14 lies in the response of this gene to inflammation. Hypoferremia and hypozincemia are among the classical changes observed across species during the acute-phase response (104). The exact reason for a decrease in serum levels of these minerals is unclear, but it may be related to host defense by decreasing iron and zinc availability for pathogenic microorganisms (67). In response to cytokine treatment and inflammation, zinc is redistributed among various tissues, particularly the liver (23). A common model of murine inflammation involves turpentine injection activating a known cytokine cascade of IL-6 and leptin mediated by IL-1β. IL-6 is the main proinflammatory cytokine regulating the response of acute-phase genes (129). Screening of all known ZnT and Zip transcripts from the livers of wild-type and IL-6$^{-/-}$ mice injected with turpentine led to identification of Zip14 as an acute-phase gene (94). These studies demonstrated a clear dependence of liver ZIP14 function on IL-6 production, whereby Zip14 contributes to the hypozincemia of inflammation and infection. Furthermore, for the first time, endogenous ZIP14 was localized to the plasma membrane of hepatocytes, where the abundance of the transporter was increased by IL-6.

Identification of Zip14 regulation by IL-6 led to speculation of other transport activities. The hypoferremia of inflammation is produced by IL-6 through induction of hepcidin synthesis in the liver (106). The mechanism accounting for the reduction in serum iron is through the hepcidin-induced internalization and degradation of ferroportin-1 (*fpn1*) (107). Because of this similarity in the clearance of both metals by IL-6, albeit by different mechanisms, was there a chance that ZIP14 also transported iron? To answer that question, iron metabolism needed to be examined more closely.

Homeostatic mechanisms tightly control the intestinal absorption, systemic transport, cellular uptake, storage, and cellular efflux of iron. Normally, iron in plasma is bound to its transport protein transferrin (Tf). However, during iron overload, the iron-binding capacity of plasma Tf can be exceeded, resulting in the hepatic accumulation of non-Tf-bound iron (NTBI) (56). The NTBI concentrations in the plasma of humans with hereditary hemochromatosis or β-thalassemia usually range from 0.4 to 20 μM (12, 66). Animal studies indicate that the liver is the major target of plasma NTBI (27). Therefore, the question was asked, could ZIP14 transport NTBI? In a study by Liuzzi et al. (96), ZIP14 was indeed shown to mediate both zinc and NTBI uptake into hepatocytes.

Furthermore, patients with hereditary hemochromatosis have significant levels of NTBI in their serum (19, 123). Mutation of a single base pair in the hereditary hemochromatosis gene (HFE) causes iron overload in the liver as well as the heart, pancreas, parathyroid, and pituitary glands, leading to multiorgan dysfunction (42, 56). Functional studies show hepatocytes from $Hfe^{-/-}$ knockout mice can take up more NTBI (19) and accumulate more hepatic iron than can wild-type mice (164). The role of ZIP14 in HFE-mediated iron overload was examined in HepG2 cells (48). Interestingly, expression of HFE in HepG2 cells resulted in a lower abundance of ZIP14, possibly by a post-transcriptional mechanism. Additionally, iron uptake was unaffected by HFE expression after Zip14 knockdown, implying that HFE has a direct effect on Zip14-mediated iron transport. Therefore, the reduction in NTBI uptake by HFE may be mediated by ZIP14 function.

The Zip14 gene is located on mouse chromosome 14, spanning base-pair position 70703274 to 70751231. Multiple studies and tissue array data show that the liver expresses the greatest amount of Zip14, followed by the intestine (49, 94, 142). At least two distinct mRNA transcripts have been identified (49, 93). The reference sequence, NM 144808, is a 4927 bp sequence containing the entire Zip14 coding sequence (CDS 259–1728). This mRNA also contains a 3199 bp 3′ untranslated region (UTR). The second mRNA is a splice variant. This variant, BC021530 or ZIP14B, is a 2174 bp sequence also containing the complete coding sequence for Zip14 (CDS 262–1731). However, the ORFs of the two transcripts are not perfect matches (67% similarity). The reference transcript, named ZIP14A, contains exon 5 located at bps 710–879, whereas the splice variant contains exon 3 at bps 713–882. Furthermore, the variant is missing the extended 3′-UTR that is contained in the reference sequence. Recently, the tissue distribution and transport functions of the splice variants were investigated (49). In the C57BL/6J mouse, ZIP14A expression is highest in liver, duodenum, kidney, and testis, whereas ZIP14B expression is highest in liver, duodenum, brain, and testis. Both variants were found to transport Zn^{2+} in stably retroviral-infected mouse fetal fibroblast cultures and transiently transfected Madin-Darby canine kidney (MDCK) polarized epithelial cells. Transport of Cd^{2+} was also demonstrated to be HCO_3^- dependent. Similar to previous results (94, 142), membrane-bound ZIP14A and ZIP14B transporters localized to the apical surface of MDCK cells and are generally glycosylated. Although transport activities and cellular localization of the variants are similar, it is unclear if there is functional significance to the presence of alternative Zip14 products. However, ZIP14A and ZIP14B may play tissue-specific roles in zinc transport. Furthermore, the critical nature of ZIP14 function during pathophysiologic conditions might lead one to speculate on the importance of this transporter during growth and development. Although the only ZIP transporter shown to be critical for development was Zip4, Zip14 may also prove to be vital to this process.

PERSPECTIVE

The focus of this review is on the relationship(s) between the ZnT and ZIP families as they relate to the metabolism of zinc and functional outcomes of cellular abundance of this micronutrient. A number of research reports have documented that specific members of these two protein families are able to facilitate the transport of other cations. Salient examples are ZIP14 and the transport of NTBI and both ZIP8 and ZIP14 in transport of the nonessential cation, cadmium. In the case of NTBI, cellular uptake is an outcome of disorders of iron metabolism. Consequently, tissues where ZIP14 is highly expressed, e.g., the liver, may be targets for accumulation of excess iron during such conditions. ZnT and ZIP proteins localized to subcellular sites may well participate in transport of iron and perhaps other cations, e.g., manganese. On the other hand, cadmium is an environmentally relevant toxic metal that the body attempts to exclude from normal

pathways for nutrient metals. The physiologic relevance of the transport of cadmium and other toxic metals by ZnT and ZIP proteins in integrative systems remains to be established.

ACKNOWLEDGMENTS

R.J.C acknowledges research grant support from the National Institutes of Health and the Boston Family Endowment of the University of Florida Foundation. L.A.L. acknowledges support from a University of Florida, College of Agricultural and Life Sciences Alumni Award and a Davis Graduate Nutrition Enhancement Award. Graphics were produced by **http://www.AASArts.com**.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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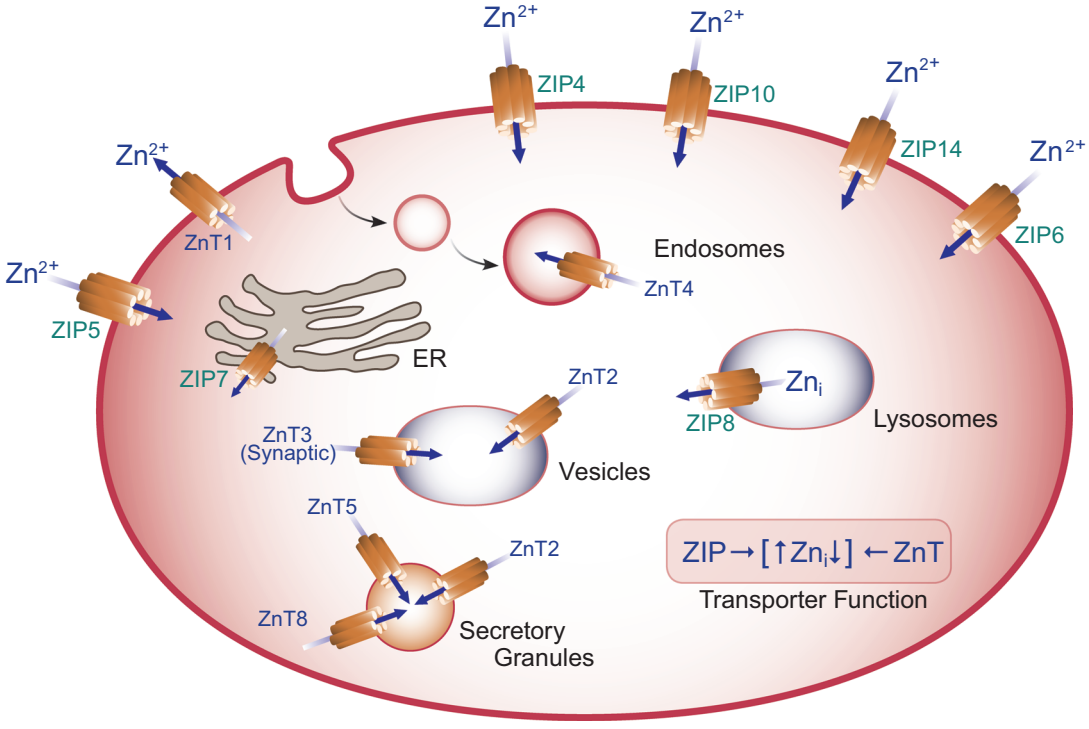

Figure 1

Generalized cell showing locations of some key zinc transporter proteins. The six and eight trans-membrane domains for the majority of ZnT and ZIP proteins, respectively, are shown. Restricted localization specific to the plasma membrane or specific intracellular organelles has yet to be established for other members of both protein families. As shown, the function of the Znt and ZIP transporter families is to reduce and increase the cytoplasmic zinc concentrations, respectively. Such diverse distribution of these proteins suggests individual roles in executing the catalytic, structural, and regulatory roles of zinc. ER, endoplasmic reticulum.

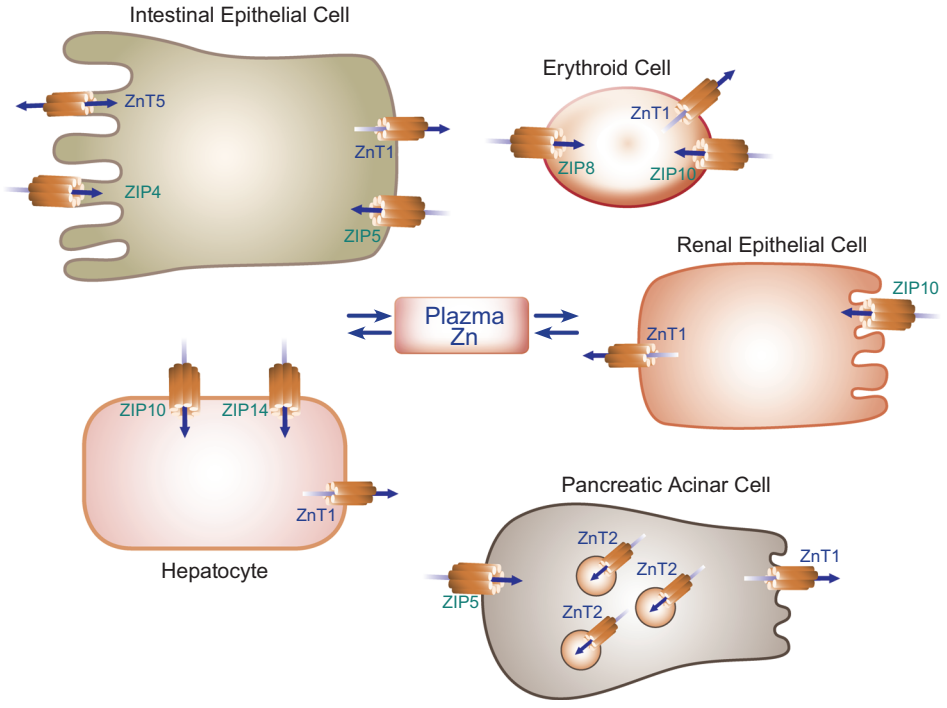

Figure 2

Transporter proteins in major organs predicted or shown to be involved in regulation of interorgan zinc fluxes in animals. Evidence from metabolomic studies on zinc has defined the intestinal epithelium as the site of absorption and the pancreatic secretions as the major sources of endogenous loss. The renal epithelium carries out extensive reabsorption. The liver parenchymal cells and erythroid cells are sites of major metabolic zinc fluxes.

Annual Review of Nutrition
Volume 29, 2009

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Errata

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