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MAMMALIAN ZINC TRANSPORTERS

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■ **Abstract** New insights into mammalian zinc metabolism have been acquired through the identification and characterization of zinc transporters. These proteins all have transmembrane domains, and are encoded by two solute-linked carrier (SLC) gene families: *ZnT* (*SLC30*) and *Zip* (*SLC39*). There are at least 9 ZnT and 15 Zip transporters in human cells. They appear to have opposite roles in cellular zinc homeostasis. ZnT transporters reduce intracellular zinc availability by promoting zinc efflux from cells or into intracellular vesicles, while Zip transporters increase intracellular zinc availability by promoting extracellular zinc uptake and, perhaps, vesicular zinc release into the cytoplasm. Both the ZnT and Zip transporter families exhibit unique tissue-specific expression, differential responsiveness to dietary zinc deficiency and excess, and differential responsiveness to physiologic stimuli via hormones and cytokines.

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INTRODUCTION

Zinc metabolism has been exquisitely delineated in humans and rodents. Experiments with radioisotopes and stable isotopes have identified, through kinetic analysis and metabolic modeling, major pathways by which dietary zinc is processed and how organs in concert produce a fairly effective homeostatic control over absorption and excretion (44, 84). Evidence shows reduction in dietary zinc content produces a marked increase in intestinal absorption and decrease in intestinal zinc losses, with urinary losses that are low and refractory to zinc intake (2, 32, 35, 53, 63, 74, 97, 98, 107). The current dietary recommendations for humans have been based on calculations where zinc intake must balance losses (84). Metabolic studies have identified those organ systems where zinc metabolism is influenced by hormones, cytokines, and growth factors (18, 25, 46, 58). These physiologic stimuli have been investigated for acute effects, but conditions that become chronic, e.g., inflammatory bowel disease, may extend the influence of these mediators on zinc metabolism.

At the organ and cellular levels, kinetic studies have shown zinc uptake kinetics exhibit mediated and nonmediated components (74, 85, 87, 89, 97). Mediated uptake suggests a saturable mechanism and involvement of a transporter(s). Until recently, understanding of the molecular mechanisms responsible for these physiologic characteristics was not delineated. Our understanding of zinc transport at the molecular level began with the cloning and the discovery of the first functional evidence showing actual zinc transport activity of ZnT1, the first mammalian zinc transporter (82). Rapid progress has been made subsequently. Mammalian zinc transporter genomics evolved from experiments with bacteria, plants, and, particularly, yeast (26, 41, 78).

Functional evidence accumulated to date shows at least 14 specific transporters are responsible for either zinc influx or efflux in mammalian cells. At the current juncture, evidence on zinc transporters has been obtained principally from experiments at the level of isolated cells. However, the limited evidence accumulated from integrative systems is congruent with observations made with rodent and human cell models. This review describes the two families of zinc transporters in the numerical sequence in which they were identified and summarizes our current understanding of their integrative physiology and nutritional significance.

ZINC TRANSPORTER CHARACTERIZATION

Mammalian zinc transporters are within two gene families: (*a*) the ZnT proteins [solute-linked carrier 30 (SLC30)] and (*b*) the Zip (Zrt- and Irt-like proteins) family [solute-linked carrier 39 (SLC39)]. Identification of these transporters was

obtained by various means, including expressed sequence tag (EST) database screening, positional cloning, and screening cDNA libraries for genes that confer cell resistance to high zinc levels in culture media. Human transporters are designated as SLC30A1 or SLC39A1, etc., whereas rodent transporters have the Slc30a1 or Slc39a1, etc., designation. Species designations are not used in this review. Current terminology and accession numbers can be obtained through the GenBank database.

ZnT and Zip proteins appear to have opposite roles in cellular zinc homeostasis, where ZnT transporters reduce intracellular cytoplasmic zinc by promoting zinc efflux from cells or into intracellular vesicles, while Zip transporters increase intracellular cytoplasmic zinc by promoting extracellular and, perhaps, vesicular zinc transport into cytoplasm (Figure 1). Involvement of some of these proteins in zinc transport has been proved by overexpression studies in different systems including yeast, mammalian cells, and *Xenopus* oocytes. However, mechanisms of transport by these proteins are still not well characterized. Sequence analyses show no evidence of Walker motifs, suggesting ATP-dependency is not a factor for zinc transport. Rather, these transmembrane proteins are believed to function by facilitated diffusion mechanisms, secondary active transport, or as symporters.

ZnT (SLC30/CDF) FAMILY

The more than 100 members of the SLC30 family are found in organisms at all phylogenetic levels. Evolutionary and basic sequence relationships among the ZnT genes have been reviewed (83). This family is divided into three subfamilies (37).

Subfamily I contains a large proportion of prokaryotic members, whereas subfamilies II and III contain eukaryotic and prokaryotic members in a similar proportion. Most ZnT proteins have six transmembrane domains (TMDs). In some cases, e.g., ZnT5 or the yeast MSC2, ZnT proteins have 12 or more TMDs. Most ZnT proteins are predicted to have cytoplasmic amino and carboxy termini (Figure 1). Predicted topology may vary according to the program used. For this review, the TMPRED software was used. In addition, ZnT proteins have a long histidine-rich loop between TMDs IV and V, $(HX)_n$ ($n = 3$ to 6), which could represent a metal-binding domain. Highly amphiphatic TMDs I, II, and V are well conserved. Deletion of TMDs I and II or the C terminus of ZnT1 disrupts transporter activity; therefore these domains appear critical for zinc transport (82). It is possible that ZnT proteins act as dimers or trimers (6, 72).

ZnT1

ZnT1 (*Slc30a1*) was the first mammalian zinc transporter discovered. It was mapped to chromosome 1 in humans and mice (82). With two exons, this gene has the simplest structure of the *ZnT* family. *ZnT1* (NM_021194) cDNA predicts a 507 amino

acid protein with six TMDs, with intracellular amino and carboxy termini according to the "positive inside rule" (104). *ZnT1* was discovered by virtue of its ability to confer resistance to high levels of extracellular zinc in the zinc-sensitive cell line, baby hamster kidney (BHK), when transfected with a cDNA-encoding rat kidney *ZnT1* (*rZnT1*). *ZnT1* overexpression in these cells increased ^{65}Zn efflux and reduced the intracellular steady state zinc concentration. A shift in sensitivity of the induction of a reporter gene regulated by a metal responsive element (MRE)-containing promoter was also observed in ZnT1-expressing cells, which suggests ZnT1 altered functionally relevant intracellular zinc pools. Moreover, expression of ZnT1 as a green fluorescent fusion protein allowed its localization to the BHK cell plasma membrane (82).

More recently, neuronal cells (PC12) transfected with either *rZnT1* cDNA or *rZnT1* cDNA with an 83 bp fragment deletion [negative dominant (ND) r*ZnT1*] were used to investigate a role for *ZnT1* expression in the neurotoxic effects of zinc (57). An increased zinc efflux and zinc resistance in cells overexpressing *rZnT1* was found, while the opposite effect was observed for cells expressing ND *rZnT1*. ZnT1 immunofluorescence was primarily at the plasma membrane, with some punctate staining throughout these neuronal cells. The results suggest control of apoptosis induced by zinc in neurons is influenced by cellular zinc balance through regulated *ZnT1* expression. Similarly, forebrain ischemia had been shown earlier to induce *ZnT1* mRNA in pyramidal neurons after initially identifying the mRNA by differential display methodology (102). Reduction in neuronal death correlated with decreased zinc uptake produced through chelation or enhanced cellular efflux, perhaps through increased ZnT1 transport activity. In mice, ZnT1 expression in the brain is high in areas rich in synaptic zinc, and a protective role for the transporter in the nervous system has been proposed (92). *ZnT1* also shows a developmental increase in the postnatal brain (79). These results support the notion that ZnT1 is a cellular zinc exporter. Curiously, ZnT1 is the only mammalian zinc transporter shown to play such a role.

ZnT1 mRNA expression has a wide tissue distribution (67, 81); however, it is more highly expressed in tissues involved in zinc acquisition, recycling, or transfer, like small intestine (villus cells), renal tubular epithelium, and placenta (67).

Immunolocalization studies have shown that, in growing male rats, ZnT1 is most abundant along basolateral membranes of enterocytes where it could participate in zinc transfer to the circulation (19, 70, 71). Immunofluorescence from ZnT1 also appears as punctate fluorescence throughout these cells, which suggests intracellular compartmentalization. Of relevance to function is that there is no fluorescence in goblet cells or immune cells of the lamina propria (71). ZnT1 distribution can vary within enterocytes. For example, in rat dams at day 1 of lactation, intestinal ZnT1 appears in vesicles at the apical surface, while at day 14 of lactation, ZnT1-containing vesicles are equally distributed at apical and basolateral regions of enterocytes (68). This differential localization is most likely a reflection of transporter-mediated zinc trafficking in response to differential physiologic stimuli or eating patterns during pregnancy and lactation. Western analysis

revealed ZnT1 was more abundant in villus than in crypt cells, and abundance was highest in proximal small intestine (71).

In the kidney, a basolateral orientation of ZnT1 was most abundant in cells lining the thick ascending and distal convoluted tubules (19). Orientation to the basolateral surface suggests a role of ZnT1 in zinc recovery from the glomerular filtrate. Such a role could explain the high reabsorptive capacity of the kidney over a wide range of zinc intakes (2). Systems having a similar orientation are responsible for sodium and glucose reabsorption.

Abundant ZnT1 expression was found in the placenta, specifically in the villous yolk sac membrane (19, 62, 68). This suggests ZnT1 participates in the exchange of zinc between maternal supplies and the fetus as other nutrient transporters are localized in this region of the placenta. Of particular note is that null mutation of *ZnT1* produces embryonic lethality in mouse (62). This evidence, coupled with the immunolocalization to the villous yolk sac, provides strong support for a critical role for ZnT1 in fetal development.

ZnT1 is regulated by dietary zinc and development in a tissue-specific fashion. Zinc supplementation of the diet of rats for one or two weeks increased *ZnT1* mRNA levels in the small intestine and kidney, but not in the liver (67). Marked upregulation of *ZnT1* mRNA occurs in the small intestine, kidney, and liver of rats given an acute oral dose of zinc to mimic the effect of consuming a zinc supplement. Consumption of a zinc-deficient diet only caused a significant reduction of the mRNA in the kidney. Analyses of ZnT1 protein levels in the small intestine and liver showed a similar trend (71). Major changes in *ZnT1* mRNA are observed in maternal tissues and those of the fetus and neonate during pregnancy and lactation (68). *ZnT1* expression is low in the mammary gland (68), and is further reduced by dietary zinc restriction (56).

Low levels of *ZnT1* mRNA have been observed in mammary gland tumor cells (65). These cells also have a higher than normal concentration of zinc, which suggests misregulation of ZnT1 decreases zinc efflux in these proliferating tumor cells. *ZnT1* mRNA is very abundant in the rat pancreas, which seems to upregulate in neonates (11). Placental visceral yolk sac *ZnT1* mRNA levels were also reduced when mice were fed a zinc-deficient diet (62).

A mechanism for *ZnT1* expression differential response among tissues to zinc supplementation or depletion is not clear. Zinc supplementation results in *ZnT1* mRNA induction in models that have been tested, including human mononuclear (THP1) cells (16, 17), hepatoma (Hepa) cells (62), and human erythroleukemia (K562) cells (J.P. Liuzzi and R.J. Cousins, unpublished results). In contrast, decreasing available intracellular zinc using a cell-permeable chelator such as TPEN markedly reduced *ZnT1* mRNA (Figure 2). This mode of zinc responsiveness is identical to that of the *metallothionein* gene, albeit the magnitudes of change are less for *ZnT1* (17). If this interpretation is correct, regulation of *ZnT1* will involve MREs in the *ZnT1* promoter, and their activation of transcription through the metal responsive transcription factor (MTF) 1 (62). Interestingly, mice with a null mutation in the *metallothionein* gene, and transgenic mice overexpressing the protein,

exhibit normal *ZnT1* expression (22). These results imply that changes in expression of *ZnT1*, a zinc exporter, are not dependent on metallothionein-influenced zinc trafficking. Evidence suggesting *ZnT1* expression by zinc could be mediated by MTF1 has been obtained. MTF1 can bind to two atypical MREs at –87 bp and –116 bp from the potential transcription start point of *ZnT1* (62). They showed a lower *ZnT1* expression in the visceral yolk sac of homozygous MTF1 knockout mice; however, the *MTF1* null mutation did not completely abate *ZnT1* expression. Therefore, it is likely that ZnT1 basal expression could rely on other transcription factors, perhaps activated by other physiologic stimuli.

Interestingly, *ZnT1* mRNA responsiveness to low zinc levels produced in K562 cells by TPEN treatment is rather slow compared to other zinc transporters. After TPEN treatment, expression of the other zinc transporter genes return to normal, but *ZnT1* expression remains low in these erythroid cells (J.P. Liuzzi and R.J. Cousins, unpublished results). Therefore, it is reasonable to hypothesize that the most convenient long-term cellular adaptation mechanism to low zinc availability is to reduce ZnT1 levels and hence efflux, rather than maintain, high levels of zinc importer expression.

ZnT2

ZnT2 (*Slc30a2*) was identified with the same screening process of mutagenesis and zinc resistance used to discover *ZnT1* (80, 82). *ZnT2* is also located on human chromosome 1. Human *ZnT2* produces, by alternative splicing, two different transcript sizes (a and b). The "a" variant has eight exons and encodes a protein of 383 amino acids; the "b" variant has seven exons and encodes a protein of 334 amino acids. The reference sequence (NM_032513) with 323 amino acids of *ZnT2* was derived from the "b" variant. Both variants have a long 3′ untranslated region (UTR) of 1908 bp. This human ZnT protein shares only 67% homology with its mouse ZnT homolog.

As was shown for *ZnT1*, *ZnT2* can confer zinc resistance to BHK cells when it is overexpressed (80). However, in this case, cell viability in a high-zinc medium was increased because of zinc accumulation in vesicles similar to late endosomes rather than through increased cellular zinc efflux as shown for ZnT1. In addition, a ZnT2 fluorescent protein fusion product was colocalized with the zinc fluorescent probe, zinquin, in vesicles. *ZnT2* mRNA has been detected in only a few tissues of rodents: small intestine, kidney, placenta, pancreas, testis, seminal vesicles, and mammary gland (11, 56, 67, 68, 81). This transporter has been localized by immunohistochemistry to vesicles very close to the apical surface of the enterocyte (68) and vesicles distal to the nucleus in mammary tissue (56). The relatively high *ZnT2* mRNA levels in lateral and ventral rat prostate correlate with the high zinc concentrations in those tissues (51). Other zinc transporters in prostate tissue were not similarly correlated. Castration has no effect on *ZnT2* expression, but lowers prostate zinc; therefore high zinc uptake, rather than a high extent of sequestration, may account for the exceptionally high prostate zinc content.

ZnT2 expression in the rat small intestine and kidney is highly responsive to zinc (67). Zinc deficiency reduced *ZnT2* mRNA to nearly undetectable levels. In contrast, zinc supplementation (two weeks of diet or a single zinc oral dose) produced a marked induction of *ZnT2* mRNA (Figure 2). Interestingly, *ZnT2* mRNA, while not normally detected in rat liver, shows high expression after an acute oral dose of zinc. Although it is not known if ZnT2 protein follows the same trend, available evidence suggests that protein and mRNA expression are closely linked (56, 68). During gestation and lactation in rats, *ZnT2* mRNA in both maternal and fetal/neonatal small intestine exhibits periods of high and low expression. ZnT2 localization to the enterocytes of the villus tip directly adjacent to the microvilli at the apical surface of neonates suggests a role in the accelerated zinc absorption that occurs in rat pups (68, 74). Its specific role could be either to store zinc or downregulate absorption.

Collectively, the observations during development, which showed transient upregulation and vesicular localization, plus the protective effects of *ZnT2* overexpression and zinc sequestration into vesicles, suggest the role of ZnT2 may be limited to conditions under which intracellular zinc concentrations increase rapidly. Such a response to high zinc has led to the hypothesis that ZnT2 participates in a mechanism to regulate the involvement of zinc in apoptosis (8).

ZnT3

ZnT3 (*Slc30a3*) was cloned when a mouse genomic λ library was screened with rat *ZnT2* cDNA (81). The gene was mapped to mouse chromosome 5 and to human chromosome 7 (81). The reference human *ZnT3* (NM_003459) encodes a protein of 388 amino acids that has at least six TMDs, with both C and N termini on the cytosolic side.

ZnT3 mRNA has been detected in the rodent brain and testis and in the human breast epithelial cell line (PMC42) (72, 81) and, recently, in mouse thymus (R.K. Blanchard & R.J. Cousins, unpublished observations), whereas ZnT3 protein has been detected in the hippocampus, the cerebral cortex, and the ependyma of mouse spinal cord (21, 81). Expression patterns for the transporter in the adult mouse brain coincide with glutamatergic neurons that contain zinc within synaptic vesicles mainly in the hippocampal mossy fibers. In early postnatal development, the expression pattern of cerebral and cerebellar *ZnT3* mRNA does not match completely the pattern of vesicular zinc distribution, since some regions with chelatable vesicular zinc are devoid of *ZnT3* expression (103).

ZnT3 overexpression did not change the phenotype of BHK cells (81); however, this could be due to the lack of chaperone proteins in that cell model. *Mocha* mice, which lack the δ-subunit of AP-3 vesicular chaperone complex, have a reduced immunoreactivity for ZnT3 and reduced Timm staining for zinc in hippocampal mossy fibers (55); therefore AP-3 could be key to the incorporation of ZnT3 into vesicle membranes (13). A *ZnT3* null mutation causes lack of vesicular zinc in mouse neurons, but does not appear to affect vesicular zinc in pancreatic β-islet

cells or testis (13). Unexpectedly, these mice appear to be normal, although they are more sensitive to kainite-induced seizures (12).

A substantial literature base relates to localization of zinc in presynaptic vesicles and how this ionic zinc plays a role in synaptic transmission (34). Despite this knowledge, relatively little is known about neuronal zinc transport (14). The toxicity of zinc to neurons (10), its relationship to degenerative neurological diseases such as Alzheimer's (50), and its role in altered eating behavior, i.e., anorexia associated with zinc deficiency (93), make an understanding of ZnT3 and other transporters that influence zinc metabolism in the nervous system a prime research area. *ZnT3* mRNA levels in mouse thymus appear to be unresponsive to moderate zinc deficiency (R.K. Blanchard & R.C. Cousins, unpublished observations).

ZnT4

ZnT4 (*Slc30a4*) was identified by investigators using positional cloning to search for the mutant mouse gene *pallid* (48). *ZnT4* is located on mouse chromosome 2 and on human chromosome 15. *SLC30A4* sequence (NM_013309) encodes a 429 amino acid protein, which has a leucine zipper domain in addition to the cation efflux motif and the typical ZnT topology. The rat homologue of *ZnT4* was cloned in 1994 as *Dri 27*, a gene that is differentially expressed during intestinal development (3), but was not functionally characterized. The function of ZnT4 as a zinc transporter, or at least as a protein involved in zinc transport, was demonstrated when its overexpression rescued the growth in a high-zinc medium of the yeast mutant Δ*zrc1*, which has a defective zinc vacuolar transporter ZRC1 (48).

A single point mutation in *ZnT4* (C→T substitution) at base 934 results in a premature translation termination codon. Chromosomal mapping suggested this mutation in *ZnT4* was the basis for the lethal milk (*lm*) disorder in mice. Sequencing of genomic *lm* mouse DNA showed the mutation was in the *ZnT4* gene (48). Dams of the *lm* genotype secrete milk of low zinc content (1, 64), which suggests ZnT4 is important for the incorporation of zinc into milk. Since *lm* mice dams produce milk with inadequate zinc, their pups die of postnatal zinc deficiency unless they are administered zinc parenterally or are fostered by normal dams (86). Zinc absorption and metabolism in young adult *lm* mice appear to be normal; however, by 8 months of age, *lm* mice start to exhibit clinical signs of zinc deficiency, including alopecia, dermatitis, and skin lesions, as well as overproduction of metallothionein (30, 40). An inherited human disorder resulting in low zinc content of milk, presumably the result of defective transport, has been reported (74). Originally thought to be analogous to murine *lm*, the disorder in humans does not appear to involve *ZnT4* (73). Similarly, *ZnT4* was excluded as a candidate gene for acrodermatitis enteropathica (AE) (5, 60, 77), as discussed below (Zip4 section).

Consistent with its possible role in zinc transport to milk, *ZnT4* mRNA is very abundant in the mammary gland, but the gene is also highly expressed in the brain and small intestine in rodents (48, 67). ZnT4 protein has been detected by western blotting in these tissues and in kidney and human PMC42 cells (59, 68, 72, 88).

This transporter has been localized to intracellular vesicles and in the transgolgi network of normal rat kidney (NRK) cells (49). ZnT4 was found in alveolar cells of lactating rat mammary gland as diffuse vesicles predominantly near the basolateral membrane (68). A similar localization was reported for PMC42 cells (72). The ZnT4 distribution pattern in these cells during lactation is similar to that of ß-casein and lactoferrin, which suggests ZnT4 is associated with milk-containing vesicles. Curiously, the ZnT4 expression pattern in PMC42 and Madin-Darbi canine kidney cells does not overlap with zinquin labeling (72, 88), which indicates that ZnT4 may not be associated in vesicles with free or labile zinc.

ZnT4 is highly expressed in rat small intestine, especially in villus cells (67), which suggests that this transporter may play a role in zinc absorption. However, the normal zinc absorption in young *lm* mice with defective ZnT4 suggests other transporters participate in intestinal zinc absorption in mice (see Zip4 section, below).

ZnT4 is located in enterocytes, but not in goblet cells or in lamina propria (68). Early in lactation in rat dams, ZnT4 is distributed evenly in enterocyte cytoplasm as vesicles, which become localized at apical and basolateral surfaces at the end of lactation. In suckling rats, ZnT4 was located close to the apical membrane (68), but in older rats this transporter is primarily basolateral (76).

Regulation of *ZnT4* has not been widely examined. In rat liver, intestine, and kidney tissues *ZnT4* mRNA levels are refractory to changes in dietary zinc intake (68). However, zinc restriction increases mammary gland *ZnT4* expression (56). On the other hand, ZnT4 localization in NRK cells varies from the perinuclear region to the cytoplasm when these cells are incubated in a high-zinc medium (49).

ZnT5

ZnT5 (*SLC30A5*), also named *hZTL1*, was reported almost simultaneously by two different groups (20, 54). It was identified through a search of EST databases for homologs of *ZnT1* and *ZRC1*. *ZnT5* is located on human chromosome 5. One reported sequence (54) encodes a 765 amino acid protein of 84 kDa that has 15 TMDs, whereas another sequence reported from a smaller mRNA encodes a 523 amino acid protein of 57 kDa that has 12 TMDs (20). No signal peptide was found in any sequence (by iPSORT analysis). Interestingly, a monoclonal antibody against hZnT5 recognizes, by immunoblotting, a 55-kDa protein in membrane fractions from human cervix (HeLa) and human placental (JAR) cells.

ZnT5 mRNA is highly expressed in human endocrine pancreas, ovary, prostate, and testis tissues (52, 54). Another study with mouse tissues placed kidney with the highest ZnT5 mRNA expression, followed by brain and small intestine; however, this report did not consider the human tissues mentioned above (20).

Functional studies on ZnT5 show diverse findings. *ZnT5*-mediated ^{65}Zn uptake was observed with *Xenopus* oocytes (20) and golgi-enriched vesicles derived from transfected human cervix cells (54). It is not clear if this transporter acts like other

ZnT proteins to lower intracellular zinc. Transfection of *ZnT5* in yeast increased zinc toxicity, and *Xenopus* oocytes expressing this protein exhibited increased ^{65}Zn uptake (54).

ZnT5 protein was found in insulin-containing β-cells of the human pancreas; therefore ZnT5 was proposed as a transporter of zinc into the zinc-rich insulin storage vesicles (54). *ZnT5* null mice exhibit poor growth and osteopenia from defective osteoblast maturation. Males of this genotype experience fatal arrhythmias, perhaps as the result of downregulation of immediate-early response and heat-shock protein genes (52).

ZnT5 is upregulated by 100 μM zinc in Caco-2 cells, where it localizes to the apical membrane (20). Zinc responsiveness of *ZnT5* was also shown with downregulation in zinc-deficient THP1 cells (17) (Figure 2). Zinc responsiveness could be mediated by MTF1, since five MREs were located in the potential promoter region of ZnT5 (20).

ZnT6

ZnT6 (*Slc30a6*) was identified by investigators searching an EST database with the mouse ZnT4 amino acid sequence (49). Located on human chromosome 6, the SLC3OA6 reference (NM_017964) protein with 461 amino acids contains at least six TMDs; however, unlike other members of the ZnT family, ZnT6 does not have a histidine-rich loop, but has a serine-rich region instead.

Of the tissues examined, the liver, brain, and small intestine have the highest levels of *ZnT6* mRNA (49). Steady-state levels of *ZnT6* mRNA and ZnT6 protein detected by western analysis do not agree, which suggests some undefined processing step occurs. Of considerable interest is that the intracellular location of immunologically detectable ZnT6 shows trafficking from the golgi network to the cell periphery and punctate staining upon addition of high zinc concentrations (49). As was observed with ZnT1, the intracellular location of ZnT6 is not uniform. *ZnT6* expression in NRK cells did not change with high levels of zinc (200 μM). In THP1 cells, small reductions in *ZnT6* mRNA occur when the cells are zinc-depleted or given excess zinc for short periods (17). ZnT6 is upregulated in the liver of lipopolysaccharide (LPS)-treated mice (Figure 2), which suggests a role for this transporter in zinc trafficking during acute immune responses (J.P. Liuzzi and R.J. Cousins, unpublished observations).

ZnT7

ZnT7 (*Slc30a7*) was identified in a search of a mouse EST database with the mouse ZnT1 protein sequence (59). Human *ZnT7* (NM_133496) is located on chromosome 1. The hZnT7 protein has 376 amino acids and shares 95% identity with mZnT7, the highest for any ZnT. ZnT7 also has the typical topology of the ZnT family.

ZnT7 mRNA is expressed at high levels in mouse small intestine and liver, followed in decreasing abundance in spleen, kidney, and lung (59). As observed with ZnT6, steady-state protein levels and mRNA for *ZnT7* do not always correspond. Western analysis also shows multiple sizes for the protein. These multiple molecular mass measurements could be the result of posttranslational modifications including glycosylation or, possibly, ubiquitination. The latter has been suggested as an explanation for the larger-than-expected size for the iron efflux transporter, ferroportin (108).

ZnT7 was localized in perinuclear vesicles, possibly associated with the Golgi apparatus in various cell types (59). Overexpression of *ZnT7* in Chinese hamster ovary (CHO) cells leads to accumulation of vesicular zinc in the perinuclear region when zinc in the medium is increased, which suggests that ZnT7 could transport zinc into vesicles to reduce intracellular cytoplasmic zinc (59).

ZnT7 mRNA levels showed a positive responsiveness to zinc, i.e., reduction in zinc deficiency and increase in zinc supplementation of THP1 cells (17) (Figure 2). In that regard, the response of the *ZnT7* gene to zinc is comparable to that for *metallothionein* and *ZnT1* genes, which suggests an MRE/MTF1 mode of transcriptional regulation.

ZnT8

A human *ZnT8* (*SLC30A8*) sequence has been reported (GenBank NM_173851). *ZnT8* is located on chromosome 8. The reference sequence encodes a 369 amino acid protein, which has at least six TMDs and a cation efflux motif. *ZnT8* transcripts have not been found in mouse small intestine, bone marrow, or spleen; however, it was detected in pancreas and liver (J.P. Liuzzi and R.J. Cousins, unpublished data). ZnT8 has not been characterized, and no evidence of its physiological function as a putative zinc transporter exists; however, ZnT8 shares a high (42%) homology with ZnT3.

ZnT9

A gene named *Huel* was isolated from human embryonic lung cells (MRC-5) after a portion of its sequence was amplified with primers for the ligand-binding domain of the nuclear receptor c-erb Ab (94). The gene is located on human chromosome 4. The putative ZnT9 reference sequence (NM_006345) encodes a 569 amino acid protein, which has a cation efflux motif, a DNA excision repair motif, and a nuclear receptor interaction consensus sequence LXXLL. In addition, this protein could have six TMDs; however, this putative transporter does not seem to be associated with plasma membrane or microsomes; instead it has been found in both cytoplasm and nuclear fractions of MRC-5 cells (94). Evidence suggests the protein is translocated from cytoplasm to the nucleus during S phase, and

interacts with several mitotic proteins (95). A zinc transporter role has not been established, and there is no evidence that the gene is nutritionally regulated.

ZIP (SLC39A) FAMILY

There are at least 86 members of the Zip family. Evolutionary and basic sequence relationships of the Zip genes have been reviewed in detail (27). Zip proteins have been divided into two subfamilies: subfamily I consists mostly of fungal and plant sequences, and subfamily II is composed of insect, nematode, and mammalian sequences (42). Subsequently, two additional subfamilies, gufA and the LIV-1 or LIV-1 subfamily of ZIP transporters (LZT), have been identified by PSI-BLAST (position-specific iterative–basic local alignment search tool) analysis (37). Finally, the KE4 subfamily is within the LZT protein subfamily (101).

Most Zip proteins are predicted to have eight TMDs with extracellular (or intravesicular) amino and carboxy termini (Figure 1). A common feature among ZIP proteins is a long loop region between TMDs III and IV and a very short C terminus. The length and sequence of this loop region is not well conserved but has a histidine-rich domain $(HX)_n$ ($n = 3$ to 6) (101). The greatest degree of conservation in the Zip family is found in TMD IV to VIII (37). The LZT subfamily has a long extracellular N terminus with 2 to 34 histidine residues and a short C terminus, and the novel metalloprotease motif (HEXPHE) situated in TMD V, which could impart an alternative function (101). The aqueous cavity in Zip proteins, through which cation substrate passes, has been predicted between TMDs IV and V (90). The Zip transporter family is not related to the zeta interacting proteins (ZIP) proteins, which bind to gamma aminobutyric acid (GABA) receptors and protein kinase C, or the basic zipper (bZip) domain transcription factor family.

Zip1

Zip1 (*SLC39A1*) was found in a human EST database by its homology with *Arabidopsis* Zip1 transporter (15). *Zip1* (NM_014437) is located on human chromosome 1. The protein sequence of 324 amino acids has a predicted topology of eight TMDs and a leucine zipper DNA-binding motif.

Overexpression of human or mouse *ZIP1* has demonstrated the ^{65}Zn uptake-enhancing properties of Zip1 (23, 33, 38). Km values found in these studies ranged from 1.7 to 7 μM, higher than the intracellular concentration of free Zn^{2+} in plasma or in cells, which is estimated to be in the low nM range or less. Nevertheless, it is possible that this transporter as well as other zinc transporters use zinc complexes as substrates. This possibility was shown when ^{65}Zn uptake by human prostate adenocarcinoma (PC3) cells was not affected when free Zn^{2+} in the medium was reduced by citrate (33). hZip1 specificity for zinc is reported to be lower than that of mZip1 (23, 38).

Classical studies with inhibitors showed hZip1-influenced uptake was energy-independent in K562 cells (38). However, zinc uptake by rat liver parenchymal cells was partially decreased by inhibitors of oxidative metabolism (31). The use of antisense oligonucleotides to stimulate *hZIP1* mRNA degradation resulted in downregulation of ^{65}Zn uptake in both K562 and PC3 cells. This suggests that hZIP1 transport activity is key for zinc uptake by these cells. In agreement with this, saturable ^{65}Zn uptake kinetics were also shown to correlate with *Zip1* mRNA induction produced by prolactin in PC3 cells and by both prolactin and testosterone in human malignant prostate (LNCaP) cells (15) (Figure 2). Glucocorticoids also can increase zinc uptake by PC3 cells through a mechanism inhibited by actinomycin D (39). However, the effect of glucocorticoid hormone on *ZIP1* mRNA has not been studied.

A screen of numerous tissues showed wide *hZip1* expression in organs and glands, including the small intestine and pancreas (38). In situ hybridization in murine tissues revealed that *mZIP1* appears in late embryonic development (66). A Zip1-fusion protein was localized to the plasma membrane of K562 cells (38, 75); however, in COS and PC3 cells the native hZIP1 protein was located in intracellular vesicles (75). In pregnant mice the *mZIP1* mRNA expression in the small intestine and visceral yolk does not seem to be affected by zinc deficiency (23). *Zip1* is also expressed in THP1 cells, and showed slight upregulation in acute zinc depletion and downregulation with zinc supplementation (17).

Zip2

Zip2 (*SLC39A2*) was isolated by its homology to other members of the Zip family (36). *hZIP2* is located on human chromosome 14. The reference sequence (NM_014579) encodes a protein of 309 amino acids, includes an amino terminal signal peptide of 26 amino acids, and has the predicted topology of Zip proteins.

The human or mouse *Zip2* overexpression in transfected K562 and human embryonic kidney (HEK293) cells, respectively, increased ^{65}Zn uptake in these cells (23, 36). *hZIP2*-induced ^{65}Zn uptake was shown to be highly inhibited by Mn^{2+} and Co^{2+}, metals that did not substantially affect *hZIP1*-, *mZIP1*-, or *mZIP2*-induced ^{65}Zn uptake (23, 38). ^{65}Zn uptake mediated by hZIP2 appears to be stimulated by HCO_3^-, non-ATP dependent, but sensitive to N-ethylmaleimide, a sulfhydryl-reactive agent (36). These observations are similar to the rapid zinc uptake/accumulation kinetics observed with hepatocytes (85). The energy dependency of longer-term zinc accumulation by hepatocytes may occur through energy needs of intracellular trafficking rather than transport (31). Transfection experiments and epitope tagging localized Zip2 to the plasma membrane of K562 cells, which suggests Zip2 has a facilitator role in zinc uptake.

Evidence of a role for hZip2 in zinc homeostasis was strengthened with the demonstration that zinc depletion of THP1 and human peripheral blood mononuclear cells resulted in marked upregulation of *Zip2* expression (7). Furthermore, global cDNA array analysis of zinc-regulated human genes showed, of 22,000

genes screened, *Zip2*, at a 27-fold increase, was the gene most responsive to zinc depletion (17). In addition, the downregulation of *Zip2* with zinc supplementation suggests a role for this transporter in zinc homeostasis (Figure 2) (17). In this regard, hZip2 may partner as a zinc importer in mononuclear cells with ZnT1 (a zinc exporter), which has the opposite mode of zinc responsiveness (17). Such a reciprocal expression of these and other transporters could explain the strong homeostatic control of zinc metabolism (7, 17). Furthermore, the reciprocal expression of *Zip2* and *ZnT1* in cells obtainable by venipuncture suggests the responsiveness of these genes to zinc could render them important markers for the assessment of zinc nurtriture.

Expression of *ZIP2* mRNA in mouse liver is much higher than in the spleen, followed by the small intestine and bone marrow (J.P. Liuzzi and R.J. Cousins, unpublished observations).

Zip3

hZip3 (*SLC39A3*) is located on the human chromosome 19 (36). The reference sequence (NM_144564) encodes a 314 amino acid protein that has an N-terminal signal peptide. Overexpression of the *mZIP3* homolog in HEK293 cells increased ^{65}Zn uptake in similar fashion to *mZIP1* and *mZIP2*; however, mZIP3 specificity for zinc could be less than reported for the two other Zip transporters (23).

Zinc-depleted THP1 cells have downregulated *Zip3* expression (17). *mZIP3* is highly expressed in bone marrow and spleen, with lower expression in small intestine and liver (J.P. Liuzzi and R.J. Cousins, unpublished observations). Zinc deficiency does not affect mRNA expression of this transporter in the small intestine or visceral yolk of pregnant mice (23).

Zip4

hZip4 (*SLC39A4*) was localized to the same chromosomal region (8q24.3) where AE has been mapped (106). hZip4 belongs to the LZT subfamily (101). There are two hZIP4 isoforms, which only differ in their amino and carboxy termini. The largest isoform (NM_130849) encodes a 647 amino acid protein with an N-terminal signal peptide, whereas the shorter isoform (NM_017767) has only 626 amino acids.

After initial inquiries regarding *hZnT4* as a candidate for AE (5, 60, 77), *hZip4* was identified as the gene responsible for this human zinc malabsorption disorder. Several mutations, including missense mutations and a premature termination codon, were found in this gene in genomic DNA from AE patients (61, 105, 106). This provides a definitive relationship between defective zinc transport and pathophysiology. The administration of oral zinc can overcome the zinc deficiency in AE patients (74). Perhaps in such situations, zinc absorption occurs either through activity of low affinity zinc transporters or by a paracellular route.

hZip4 expression in human tissues is limited to small intestine and kidney (106). In a screen of tissues from adult mice, only small intestine showed pronounced *Zip4* mRNA levels, with lesser amounts in stomach and liver, but none in kidney (24). This difference from humans may reflect dietary, physiological, or species differences. Occurrence of *Zip4* transcripts in visceral yolk sac points to a role in fetal/maternal zinc transport. *mZip4* gene transfection into HEK293 increased ^{65}Zn accumulation by a mechanism exhibiting saturable kinetics and high specificity (24).

Of major interest was the upregulation of *mZip4* in maternal zinc deficiency, as shown in both maternal intestine and visceral yolk sac (24). The expression of this transporter was also shown to increase in the small intestine of adult nonpregnant mice fed a zinc-deficient diet, and to decrease after zinc supplementation (Figure 2) (24). These results suggest *mZIP4* expression regulation in organs essential to zinc acquisition plays a role in body zinc homeostasis, i.e., increasing when zinc supply is low and decreasing during zinc excess. Furthermore, saturable uptake kinetics demonstrated in *Zip4*-transfected cells (24) agree with the upregulation of the saturable component of intestinal zinc absorption in zinc-deficient rats (47).

Although *Zip4* is sensitive to zinc deficiency, LPS has no acute affect on intestinal *Zip4* mRNA expression in mice (J.P. Liuzzi and R.J. Cousins, unpublished observations). *Zip4* is not expressed in human peripheral blood mononuclear cells (17). Because both *Zip2* (7, 17) and *Zip4* (24) are upregulated by zinc deficiency, a common mechanism likely is responsible for their zinc responsiveness. Given the likely involvement of ZIP4 in intestinal zinc absorption, the screening for factors that regulate this gene is warranted.

OTHER ZIP TRANSPORTERS

Structural evidence suggests a number of other mammalian genes encode proteins of the *Zip* family. Of particular interest for this review are the *LZT* genes *LIV-1*, *BIGM103*, and *hKE4*, because overexpression of these genes in CHO cells results in increased zinc accumulation (4, 99, 100).

HLIV-1 or *Zip6* (*SLC39A6*) was disclosed as a gene whose expression is stimulated by estrogen (Figure 2) in breast cancer cells (69). This gene (NM_012319) encodes a 749 amino acid protein with eight predicted TMDs, with an N-terminal signal peptide. It is highly expressed in prostate, placenta, and HeLa cells (99), in addition to mammary gland cells. Its expression in breast cancer makes it a potential disease progression marker. *LIV-1* expression in cells could also be induced by insulin, IGF-1, TGFα, and EGF (28, 29). LPS acutely upregulates LIV-1 mRNA in mouse liver (J.P. Liuzzi and R.J. Cousins, unpublished observations). This protein was located in the plasma membrane of CHO cells, especially in the lamellipodiae (99), a location similar to that of some matrix metalloproteinases (9). Having a metalloproteinase motif and localization on the plasma membrane suggests LIV-1

could act as a metalloproteinase; however, this is yet to be determined. Metal metalloproteinases cleave the protein of extracellular matrix and have been implicated in tumor development and metastasis (96).

BIGM103 (NM_022154) encodes a protein of 460 amino acids that predicts eight TMDs with an N-terminal signal peptide and a leucine zipper DNA-binding motif. The gene is highly expressed in the human pancreas, lung, liver, and thymus. In monocytes, BIGM103 expression is induced by *Mycobacterium bovis* bacterial cell wall, LPS, and TNFα (Figure 2) (4). This protein appears to have an intracellular location, including lysosomes and endosomes (4).

hKE4 (NM_006979) is the human homologue of *mKE4*, which was found in the H2-K region of the mouse major histocompatibility complex (45). This 429 amino acid protein has six predicted TMDs, and was found in the endoplasmic reticulum of CHO-transfected cells (100). It has a wide tissue distribution, with a prominent expression in placenta, liver, pituitary gland, pancreas, salivary gland, kidney, and prostate tissues (100).

DCT1/DMT1/NRAMP2 (SLC11A2)

DCT1/DMT1 belongs to the Nramp family (43), which is unrelated to Zip or ZnT proteins. DCT1/DMT1 was proposed as a potential zinc transporter because Zn^{2+}, as well as other cations, was able to generate an inward current in *Xenopus* oocytes expressing this protein. Further characterization showed that Zn^{2+} induces a proton leak in *Xenopus* oocytes expressing DCT1/DMT1, without zinc transport activity, which suggests this iron transporter does not transport zinc (91).

CONCLUSIONS

The research results reviewed here show the rapid progress that has been made in understanding some basic aspects of the two zinc transporter families currently known. The unique structure of these proteins, their mechanism of transporter activity, their tissue-specific expression, and their modes of regulation provide the basis for a fertile field of future investigation. Furthermore, most *ZnT* and *Zip* families show evidence of polymorphisms, which could produce structurally different proteins and, hence, transporter activity and/or specificity for zinc. Such polymorphisms could influence dietary zinc requirements and zinc metabolism.

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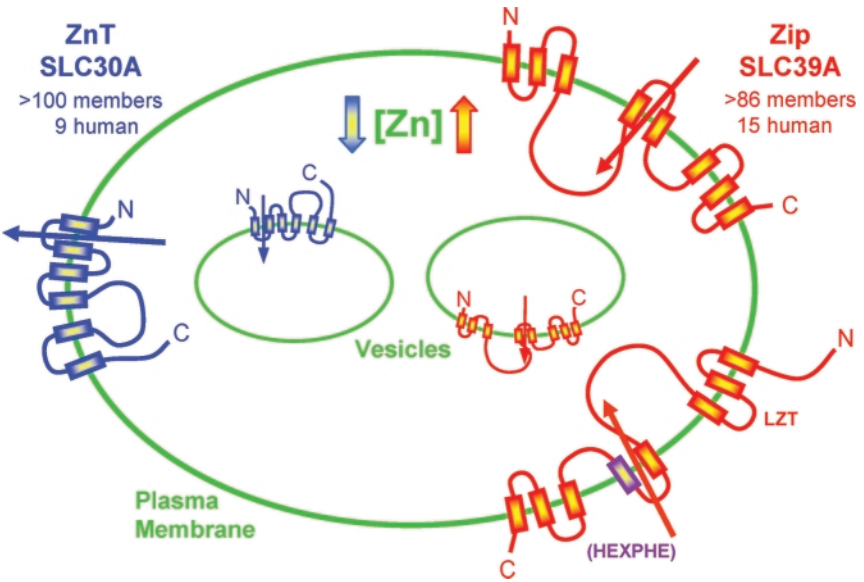

Figure 1 The representative cell shows generalized representations of the ZnT and Zip zinc transporter protein families. Transmembrane domains (ϒ) and the proposed direction of solute transport (↑) are shown. The metalloprotease sequence (HEXPHE) of the LZT subfamily of Zip transporters is shown. The association of ZnT or Zip proteins with either the plasma membrane or vesicular membranes is indicated. The influence of the opposing actions of the ZnT and Zip transporters on cellular zinc is shown.

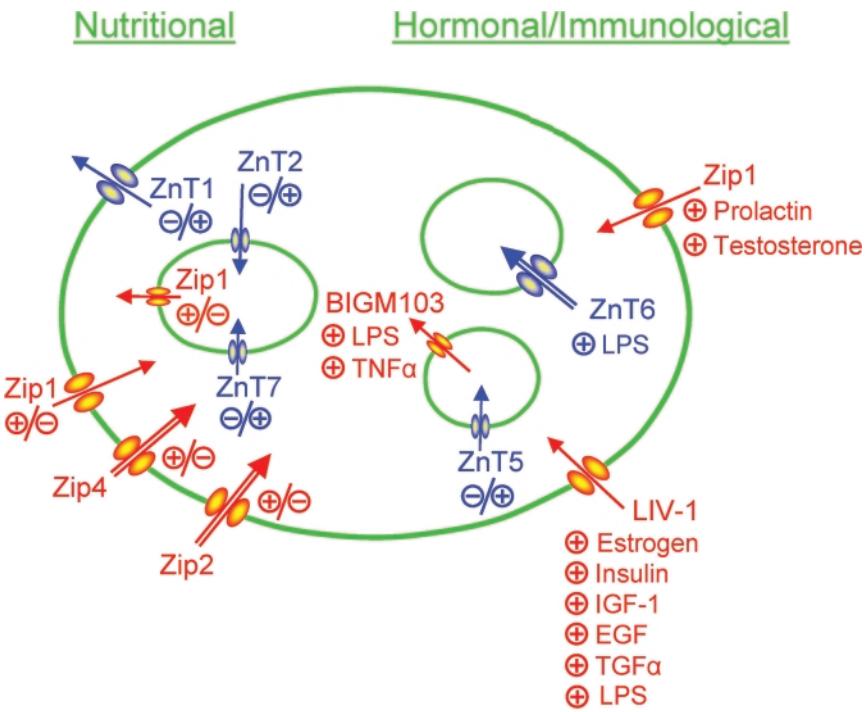

Figure 2 The representative cell shows some of the observed nutritional and physiological inputs that influence expression of zinc transporter genes. At this juncture, it is not possible to define most sites of action for individual transporters. Nutritional regulation is shown as the direction of response to zinc depletion/zinc supplementation (i.e., –/+ or +/–). Physiologic regulation shows responses in integrative or isolated cell systems, and is illustrated only as a positive response. Tissue-specific regulation has not been considered in this composite representation. Abbreviations: EGF, epidermal growth factor; IGF, insulin-like growth factor; TGFα, transforming growth factor.