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The SLC39 family of metal ion transporters

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Abstract SLC39 proteins are members of the broader ZIP family of metal ion transporters found in organisms at all phylogenetic levels. Most ZIP transporters have eight predicted transmembrane domains and a similar predicted topology. Their biochemical mechanism(s) of substrate transport are not yet known. Where characterized, these proteins have been found to transport metal ions from the cell exterior or lumen of intracellular organelles into the cytoplasm. Furthermore, members of the ZIP family have been implicated in the transport of zinc, iron, and/or manganese indicating that these proteins have diverse functions. There are 14 SLC39-related proteins encoded by the human genome. Studies of SLC39A1, SLC39A2, and SLC39A4, encoding the proteins hZip1, hZip2, and hZip4, have indicated roles in zinc uptake across the plasma membrane of various cell types. Genetic studies have specifically implicated SLC39A4 in the uptake of dietary zinc into intestinal enterocytes. Mutations in SLC39A4 have been identified in patients with acrodermatitis enteropathica, a genetic disease of zinc deficiency.

History of Slc39-related genes

The SLC39 transporters are members of the ZIP family of metal ion transporters [9, 11, 18]. This designation stands for Zrt-, Irt-like protein, and reflects the first members of this transporter family to be identified. Zrt1 and Zrt2 are the primary zinc uptake transporters in the yeast *Saccharomyces cerevisiae* and Irt1 is the major iron uptake transporter in roots of *Arabidopsis thaliana* [6, 20, 35, 37, 41, 42]. Since the initial identification of these proteins, the ZIP family has grown to over 90 members including proteins in bacteria, nematodes, insects, and mammals [11]. Among these, ZupT is a zinc uptake transporter in *Escherichia coli* [16] and several ZIPs have been implicated in zinc, iron, and/or manganese transport in plants [17, 24, 31, 36]. Database analyses have indicated that there are 14 SLC39-related ZIP proteins encoded by the human genome (Table 1). A dendrogram describing the sequence relationships of human SLC39 proteins is shown in Fig. 1.

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Functional characteristics

With no known exceptions, ZIPs transport metal ion substrates across cellular membranes into the cytoplasm. While many members are involved in the uptake of metal ions across the plasma membrane, some efflux metals from intracellular compartments. For example, the yeast Zrt3 protein is responsible for transporting stored zinc out of the lysosome-like vacuole into the cytoplasm for subsequent utilization [27]. Also in yeast, the Atx2 protein appears to transport manganese out of the Golgi [25].

The biochemical mechanisms involved in this transport have not been extensively investigated. In yeast, Zrt1 and Zrt2 are known to be energy dependent [41, 42]. The mechanism of transport used by SLC39 proteins is unclear. Zinc uptake by SLC39A1 and SLC39A2 was found to be energy independent [10, 12]. Zinc uptake by these proteins was not dependent on K^+ or Na^+ gradients but SLC39A2 activity was stimulated by HCO_3^- suggesting a Zn^{2+}/HCO_3^- symport mechanism [10]. Alternatively, zinc uptake by these proteins could be driven simply by the concentration gradient of labile zinc likely to exist across the plasma membrane [30].

Description of human *SLC39* genes

SLC39A1

SLC39A1, encoding hZip1/ZIRTL, is expressed in a wide variety of tissues and cell types [12, 26]. In transfected

Table 1 Summary of SLC39-related genes

Human gene name	Protein name	Aliases	Predominant substrates	Transport type	Tissue distribution and cellular/subcellular expression	Link to disease	Human gene locus	Sequence Accession ID
SLC39A1	hZIP1	ZIRTL	Zinc	Unknown	Widespread/plasma membrane and intracellular vesicles	None known	*1q21*	NM_014437
SLC39A2	hZIP2	6A1	Zinc	Unknown	Prostate, uterus, cervical epithelium, optic nerve, monocytes, plasma membrane[a]	None known	*14q11.1*	NM_014579
SLC39A3	hZIP3		Unknown	Unknown	Widespread[a]	None known	19*p13.3*	NM_144564
SLC39A4	hZIP4		Zinc	Unknown	Small intestine, stomach, colon, cecum, kidney/plasma membrane	Acrodermatitis enteropathica	*8q24.3*	NM_017767
SLC39A5			Unknown	Unknown	Kidney, liver, spleen, colon, stomach, pancreas[a]	None known	*12q13.13*	NM_173596
SLC39A6	LIV-1		Unknown	Unknown	Widespread/unknown[a]	None known	*18q12.1*	NM_012319
SLC39A7	KE4		Manganese	Unknown	Widespread/endoplasmic reticulum[a]	None known	*6p21.3*	NM_006979
SLC39A8	BIGM103		Zinc	Unknown	Widespread/unknown[a]	None known	*4q22-q24*	NM_022154
SLC39A9			Unknown	Unknown	Widespread/unknown[a]	None known	*14q24.1*	NM_018375
SLC39A10			Unknown	Unknown	Widespread/unknown[a]	None known	*2q33.1*	XM_047707
SLC39A11			Unknown	Unknown	Widespread/unknown[a]	None known	*17q25.1*	NM_139177
SLC39A12			Unknown	Unknown	Brain, lung, testis, retina/unknown[a]	None known	*10p12.33*	NM_152725
SLC39A13			Unknown	Unknown	Widespread/unknown[a]	None known	*11p11.12*	NM_152264
SLC39A14			Unknown	Unknown	Widespread/unknown[a]	None known	*8p21.2*	XM_046677

[a] Based in part or in toto on representations in Unigene EST database

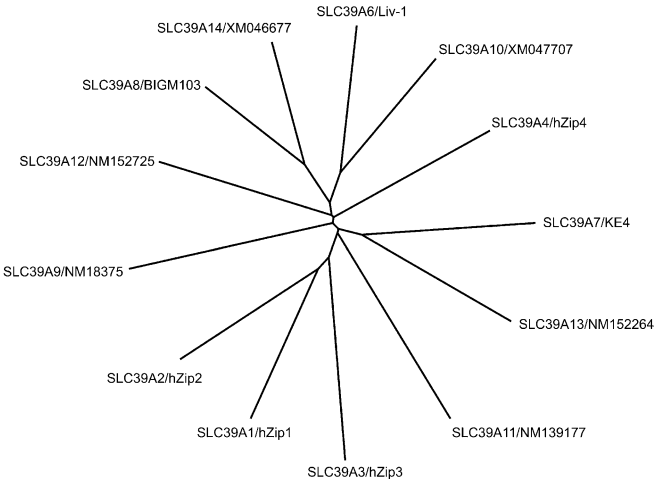

Fig. 1 The amino acid sequence relationships of SLC39-related proteins. The dendrogram was generated from the amino acid sequences using CLUSTALW. Orphan transporters are designated by their sequence accession number as listed in Table 1. Some example sequence identities are hZip1 versus hZip2 (36%), hZip1 versus NM139177 (27%), and hZip1 versus NM152725 (23%)

K562 cells, hZip1 is localized to the plasma membrane where it confers zinc uptake activity [12]. In other cell types (e.g., COS-7, PC3), hZip1 is localized predominantly to the endoplasmic reticulum [29]. Antisense RNA studies have indicated that hZip1 is responsible for most of the zinc uptake activity detectable in K562 cells suggesting a role in zinc uptake in other cells as well [12]. SLC39A1 expression appears to be regulated by zinc status and by hormone treatment; i.e., mRNA levels are repressed by zinc treatment and induced by prolactin treatment of PC-3 and LNCaP prostate-derived cancer cells [5].

SLC39A2

SLC39A2, encoding hZip2, is expressed at low levels and in only a few tissues [2, 10, 38]. Like hZip1, hZip2 also appears to be involved in zinc uptake. hZip2 localizes to the plasma membrane of transfected K562 cells and confers zinc uptake activity on those cells [10]. As was found for SLC39A1, SLC39A2 expression may also be regulated by zinc; treatment of the THP-1 monocytic cell line, or human peripheral blood mononuclear cells with TPEN, a cell-permeable zinc chelator, resulted in approximately fourfold increases in SLC39A2 mRNA levels [2]. Furthermore, Yamaguchi (1995) found that SLC39A2 expression was induced by contact inhibition in cultured cervical epithelial cells [38].

SLC39A4

hZip4, the product of the *SLC39A4* gene, is an important zinc uptake transporter in the human intestine and responsible for absorption of dietary zinc. This conclusion is based on the work of two groups linking SLC39A4 to acrodermatitis enteropathica (AE), a recessive genetic disorder of zinc absorption [23, 39, 40]. Homozygosity mapping using consanguineous families with affected individuals indicated the gene responsible for AE mapped to 8q24.3 [39]. Located in this region was a likely candidate gene, *SLC39A4*. Sequencing of exons from

affected individuals indicated the presence of mutations that were likely to disrupt function of the protein [23, 40]. These mutations were not found in unaffected samples. Moreover, the *SLC39A4* gene is expressed in tissues involved in zinc absorption, the small intestine, colon, and kidney, and the hZip4 protein was localized to the apical plasma membrane of mouse enterocytes [40]. These results strongly suggest an important role of hZip4 in dietary zinc uptake and reabsorption of zinc in the kidney. Approximately 10% of the endogenous zinc lost per day in humans is in urine and this loss is reduced under conditions of zinc deficiency suggesting homeostatic regulation of kidney zinc reabsorption [3]. Based on the EST database, two forms of hZip4 may be expressed that have different amino-termini. The more commonly expressed form has a 329 amino acid segment upstream of TM1 including a signal sequence. The alternative form appears to be expressed at lower levels and has an N-terminal tail of 305 amino acids with no signal sequence. This latter form arises from transcription initiation within intron 1 of the longer transcript [40]. So far, no experiments characterizing the regulation of SLC39A4 expression have been published.

Studies of other *SLC39* genes

Additional *SCL39*-related genes are described in Table 1. hZip3, the product of SLC39A3, has not yet been characterized beyond the sequence level [10]. The "LIV-1" gene was cloned because its expression is induced by estrogen in breast cancer cells [28]. LIV-1 expression is also increased by treatment with epidermal growth factor, TGFα, insulin, or IGF-1 [7, 8]. The gene referred to as "BIGM103" also encodes an SLC39-related protein and its expression is induced in monocytes treated with *Mycobacterium* cell wall and lipopolysaccharide preparations [1]. Moreover, expression of BIGM103 in CHO cells increased zinc accumulation consistent with a potential role in zinc uptake. The "Ke4" gene was identified during the characterization of genes near the major histocompatibility complex on chromosome 6 [19, 21]. Ke4 may share common promoter elements with the retinoid X receptor-β (RXR β), the adjacent divergently transcribed gene, and be repressed by tumor necrosis factor (TNF)-α [33]. Expression of mouse Ke4 in *Arabidopsis* indicated that this protein could functionally substitute for IAR1, a potential Mn transporter [24]. Finally, mouse Ke4 was localized to ER membranes when expressed in transfected cells [34]. Thus, Ke4 may be an intracellular Mn transporter in mammals.

Biochemical and structural information

Most ZIP proteins have eight transmembrane (TM) domains and similar predicted topologies (Fig. 2). Aspects of this topology model have been confirmed for yeast [13, 14] and some mammalian ZIP transporters [10, 12]. While most loops between TM domains are quite short, a longer loop region is frequently found between TM domains III and IV. This region often contains a histidine-rich domain with the sequence $(HX)_n$ where n generally ranges from 3 to 5. The function of this domain is not yet clear. Determinants of substrate specificity have been mapped to the extracellular loop between TM II and III in Irt1 [32]. For some SCL39 proteins (e.g., Liv-1), this regions is also rich in histidine residues. Finally, some SLC39 proteins (e.g., SLC39A4) have long N-terminal domains. Transmembrane domains IV and V are particularly amphipathic and contain conserved histidine residues frequently with adjacent polar or charged amino acids. Given their sequence conservation and amphipathic nature, TM IV and V are predicted to line a cavity in the transporter through which the substrate passes. Consistent with this hypothesis, conserved residues in these regions are essential for function [32].

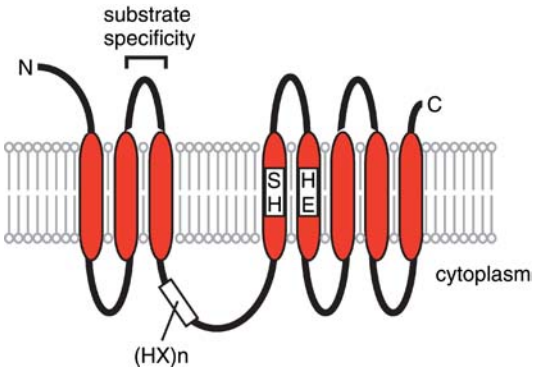

Fig. 2 Topological model of SLC39 proteins. Transmembrane (TM) domains are indicated in *red*. The histidine-rich region in the cytoplasmic loop between TM III and IV, conserved histidines and charged/polar residues in TM IV and V, and the location of substrate specificity determinants mapped in Irt1 are also shown

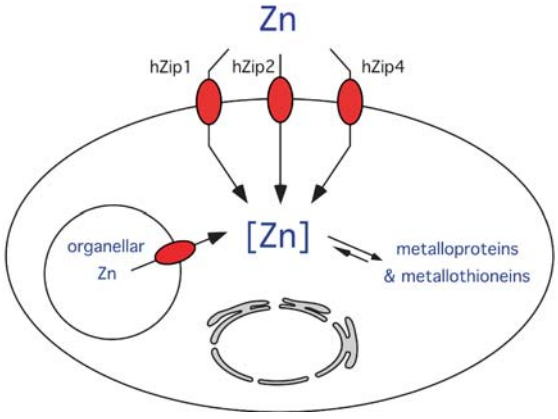

Fig. 3 Roles of SLC39 proteins in metal metabolism. Available evidence indicates that hZip1, hZip2, and hZip4 are responsible for zinc uptake in cells where they are expressed. Based on their similarity to yeast ZIP proteins, it is possible that human SLC39 proteins transport metal ions out of intracellular compartments. Furthermore, some SLC39 proteins may transport substrates other than zinc (e.g., Fe or Mn)

Physiological and pathological implications

Like other ZIP transporters, SLC39-related proteins are likely to play diverse roles in cell and organismal physiology (Fig. 3). The products of some of these genes, e.g., *SLC39A1*, *SLC39A2*, and *SLC39A4*, are or appear to be involved in zinc uptake across the plasma membrane. Other members may function in the uptake of other metal ions (e.g., Fe and Mn) or may transport metal ion substrates across organellar membranes. In this latter role, SLC39-related proteins may be involved in metal ion homeostasis within the compartment or, perhaps, play a role in mobilizing stored metal ions for use under deficiency conditions. Finally, some ZIP transporters (e.g., Zrt1 and Irt1) have been shown to be capable of cadmium transport [4, 15, 22]. Therefore, SLC39 proteins may play a role in cadmium uptake into the intestine and into other tissues of the body. Consistent with this hypothesis, zinc uptake by SLC39A1 and SLC39A2 is strongly inhibited by cadmium suggesting that this toxic metal may also be a substrate for these transporters [10, 12].

Given the importance of zinc and other metal ions, it is likely that SLC39-related proteins will have far-reaching pathological implications. The only case where some of these implications are known is *SLC39A4*, the gene responsible for AE. If left untreated, AE patients can suffer severe zinc deficiency that results in growth impairment, immune system dysfunction, alopecia, severe dermatitis, and mental disorders. Successful treatment of AE can be achieved by supplementing the diet of these patients with zinc. This suggests that additional zinc transporters are present in the intestine for dietary zinc absorption.

The SLC39 transporters are not the only proteins responsible for zinc transport in humans. DMT1/SLC11 may also play a role in dietary zinc uptake in the intestine and into various tissues. Moreover, the activity of SLC39 proteins is closely integrated with the activity of members of the SLC30 family. While SLC39 proteins transport metal ions into the cytosol, SLC30 proteins efflux that zinc either out of the cell or into organelles. For example, dietary zinc taken up into enterocytes of the intestine via SLC39A4 may be exported across the basolateral membrane into the bloodstream by Znt-1/SLC30A1 and/or ZnT-4/SLC30A4. Similarly, zinc taken up into cells of other tissues by SLC39 transporters (e.g., SLC39A1) may be transported into the Golgi by Znt-5/SLC30A5. Thus, zinc homeostasis and utilization in humans involves the coordinated activity of members of several zinc transporter families.

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