THE PHYSIOLOGICAL, BIOCHEMICAL, AND MOLECULAR ROLES OF ZINC TRANSPORTERS IN ZINC HOMEOSTASIS AND METABOLISM

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Kambe T, Tsuji T, Hashimoto A, Itsumura N. The Physiological, Biochemical, and Molecular Roles of Zinc Transporters in Zinc Homeostasis and Metabolism. Physiol Rev 95: 749–784, 2015; Published June 17, 2015; doi:10.1152/physrev.00035.2014.—Zinc is involved in a variety of biological processes, as a structural, catalytic, and intracellular and intercellular signaling component. Thus zinc homeostasis is tightly controlled at the whole body, tissue, cellular, and subcellular levels by a number of proteins, with zinc transporters being particularly important. In metazoan, two zinc transporter families, Zn transporters (ZnT) and Zrt-, Irt-related proteins (ZIP) function in zinc mobilization of influx, efflux, and compartmentalization/sequestration across biological membranes. During the last two decades, significant progress has been made in understanding the molecular properties, expression, regulation, and cellular and physiological roles of ZnT and ZIP transporters, which underpin the multifarious functions of zinc. Moreover, growing evidence indicates that malfunctioning zinc homeostasis due to zinc transporter dysfunction results in the onset and progression of a variety of diseases. This review summarizes current progress in our understanding of each ZnT and ZIP transporter from the perspective of zinc physiology and pathogenesis, discussing challenging issues in their structure and zinc transport mechanisms.

I. INTRODUCTION

Zinc is the second most abundant trace element (after iron) essential for all living organisms. Zinc exists as a divalent cation (Zn$^{2+}$) and is not redox active under physiological conditions, which explains why zinc performs multifarious physiological roles in a variety of biological processes (TABLE 1). This feature of Zn$^{2+}$ hampered its detection, and clarification of the dynamic state of zinc was a challenge until recent improvements in zinc-imaging techniques became available, including small fluorescent sensor molecules, FRET molecules designed to sense zinc ions, X-ray fluorescence microscopy, and laser ablation inductively coupled, plasma mass spectrometry (99, 174, 335, 338, 412, 425).

For zinc to perform its diverse bioactive roles, a number of specific systems to transport zinc across the biological membrane are required. Thus zinc transport proteins are indispensable for the physiology of zinc. In particular, Zn transporter (ZnT) and Zrt-, Irt-related proteins (ZIP) contribute to a wide array of physiological and cellular functions (e.g., immune, endocrine, reproductive, skeletal, and neuronal) by tightly controlling zinc homeostasis. The physiological importance of this homeostasis in humans is illustrated by the deleterious consequences of inherited diseases (reviewed in Refs. 110, 111, 191, 194).

In this review, recent progress in our understanding of the physiological and cellular roles of ZnT and ZIP transporters is summarized. Molecular links between the dysfunction of these transporters with diseases are also discussed, along with brief descriptions of phenotypes of knockout and mutant model organisms. Besides ZnT and ZIP transporters, a number of other membrane transport proteins such as calcium channels have been shown to be involved in zinc homeostasis (40), but these are not discussed here. Some literature refers to “ZIP” as a “channel” (243, 401); however, in this review, “ZIP” is described as a “transporter.”

A. The Essentiality of Zinc for Life and in Human Physiology

Zinc is a trace nutrient indispensable for life. The importance of zinc for living organisms was first recognized in 1869 in Aspergillus niger (344). Subsequently, zinc was found to be essential for the normal development of plants (277) and for normal growth of rats (411) and birds (301).
However, not until 1961 was zinc identified as an essential micronutrient for humans, with symptoms of severe anemia, growth retardation, hypogonadism, skin abnormalities, and mental lethargy attributed to nutritional zinc deficiencies (332). After this seminal finding, many symptoms caused by zinc deficiency have been described, including persistent diarrhea, alopecia, taste disorders, immune insufficiency, brain dysfunctions, impairment of wound healing, loss of appetite, chronic inflammation, liver disease, and neuropsychological changes such as emotional instability, irritability, and depression (reviewed in Refs. 80, 147, 267, 329, 356, 395, 421).

Zinc deficiency still remains a substantial global public health problem (147, 330, 357). Zinc deficiency in developing countries is estimated to be responsible for 4% of the global morbidity and mortality of young children (314). In contrast, an increase in marginal zinc deficiency is evident in older people in industrialized countries. Zinc supplementation in humans has the potential to decrease diarrhea mortality in children as well as the incidence of infections and to improve immune functions (142, 314, 331, 416). Moreover, the efficacy of zinc supplements in boosting health and well-being is revealed in various cases: in growth and body weight gain of children in the meta-analysis (43) and in decreasing incidences of blindness and the risk of developing age-related macular degeneration (2). Zinc has low toxicity and is generally considered to be safe; however, excessive amounts of zinc can be toxic, e.g., leading to inadequate absorption of copper (42). The roles of zinc in human health have been extensively reviewed previously (347).

### Table 1. Representative examples of a wide variety of zinc functions

<table>
<thead>
<tr>
<th>Zinc Functions</th>
<th>Comments</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural component</td>
<td>Zinc fingers</td>
<td>6, 119, 222, 263, 421</td>
</tr>
<tr>
<td></td>
<td>C2H2-like finger; classical zinc finger motif, transcription factor TFIIIA, 20–30 amino acid sequence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zinc ribbon; many transcription factors, ribosomal proteins, RanBP</td>
<td></td>
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<tr>
<td></td>
<td>Treble clefs; RING finger domain, ArfGAP domain, LIM domain, FYVE domain, PHD domain, MYND domain, nuclear receptor DNA-binding domain, GATA, PKC</td>
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</tr>
<tr>
<td></td>
<td>Zinc necklaces; TAZ domain in transcriptional adaptor protein CBP/p300</td>
<td></td>
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<tr>
<td></td>
<td>Interprotein binding mediator [e.g., zinc hock motif]</td>
<td>165, 361</td>
</tr>
<tr>
<td></td>
<td>Crystallization of peptides such as insulin</td>
<td>364</td>
</tr>
<tr>
<td>Catalytic factor</td>
<td>Enzyme cofactors in six main enzyme classes</td>
<td>5, 420, 421</td>
</tr>
<tr>
<td></td>
<td>Oxidoreductase; alcohol dehydrogenase, sorbitol dehydrogenase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transferase; the major function of zinc is not catalytic in this class (only 34% are catalytic)*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Hydrolase; carboxypeptidases, alkaline phosphatases, angiotensin-converting enzyme</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lyase; carbonic anhydrase, 6-aminolevulinic acid dehydratase</td>
<td></td>
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<tr>
<td></td>
<td>Isomerase; phosphomannose isomerase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ligase; the major function of zinc is not catalytic in this class (only 39% are catalytic)*</td>
<td>5</td>
</tr>
<tr>
<td>Signaling mediator</td>
<td>Extracellular zinc signaling</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Neuromodulating functions in the central nervous system</td>
<td>104, 367, 394</td>
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<tr>
<td></td>
<td>Activating zinc receptor (ZnR/GPR39)</td>
<td>28, 372</td>
</tr>
<tr>
<td></td>
<td>Reducing insulin secretion and suppressing hepatic insulin clearance</td>
<td>124, 396</td>
</tr>
<tr>
<td>Intracellular zinc signaling</td>
<td>Second messenger functions</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Inhibition of enzyme activity (caspases, protein tyrosine phosphatases, phosphodiesterases)</td>
<td>140, 161, 175, 266, 427</td>
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<tr>
<td></td>
<td>Modulation of signaling pathways (PKC, ERK, JAK/STAT, BMP/TGF-β, NF-κB, cAMP-CREB, PI3K/Akt, B-cell receptor)</td>
<td>111, 112, 144, 146, 156</td>
</tr>
<tr>
<td></td>
<td>Zinc wave; zinc release in cytosol from perinuclear region</td>
<td>401, 451</td>
</tr>
<tr>
<td></td>
<td>Zinc spark; zinc ejection from oocyte, which is necessary for the egg-to-embryo transition</td>
<td>211, 342</td>
</tr>
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*Percentages refer to EC numbers associated with zinc enzymes of known structure (5).

B. Zinc Biochemistry

Unlike iron and copper, zinc is redox neutral and is reactive as a Lewis acid in biological reactions. Because of these features, zinc plays key roles as a structural, catalytic, and signaling component.
As a structural component in proteins, the association of zinc with proteins/peptides was first verified by crystallization of insulin with zinc (364). The “zinc finger” motif was first found in the transcription factor TFIllIA of Xenopus (284). Zinc fingers are now grouped into more than 20 classes of structurally distinct modules and are known to be a functional motif that interacts with a variety of proteins, lipids, and nucleic acids (6, 26, 119, 222, 227) (TABLE 1), clearly indicating the importance of zinc in cellular biochemistry. In catalytic functions, zinc can activate substrates in enzymes by stabilizing negative charges, because of its strong Lewis acid property (264). After the first identification of zinc in erythrocyte carbonic anhydrase as a zinc-dependent enzyme (205), the presence of zinc has been recognized in many enzymes in all six classes defined in the Enzyme Commission (EC) system (5, 420, 421) (TABLE 1).

Within proteins, zinc can be coordinated by nitrogen, oxygen, and sulfur atoms and can have different coordination numbers (216, 263). The zinc proteome estimates that ~9% of proteins are zinc proteins in eukaryotes with the number substantially greater in higher organisms. The number of zinc proteins encoded by humans is ~10% (4). In zinc proteomes, the number of zinc-binding motifs is counted primarily based on mining for intramolecular zinc-binding sites. Intermolecular zinc-binding sites consisting of an interface between two or more proteins are generally difficult to identify (7, 265), although a number of important examples have been found (165, 361). Additionally, the prediction of transient zinc binding sites cannot be identified using sequence homology. Thus the size of zinc proteomes may grow in the future (45).

C. Zinc Signaling

In addition to the roles of zinc described above, zinc is functional as a signaling mediator (TABLE 1), leading to the concept that zinc is the “calcium of the 21st century” (104). The signaling functions of zinc occur by increases in zinc (Zn\(^{2+}\)) concentrations triggered by stimuli. Zinc-activated signaling is associated with pathophysiological functions (111, 156) and therefore has therapeutic potential. Extracellular release of zinc acts as a signaling mediator in endocrine, paracrine, and autocrine systems. In the central nervous system, zinc, which is released from presynaptic neurons upon excitation into synaptic clefts, modulates synaptic transmission by binding to various transporters and receptor channels on postsynaptic neurons (104, 367, 394). Zinc, coreleased from pancreatic \(\beta\)-cells along with insulin by glucose stimuli, can suppress hepatic insulin clearance (396) and reduce insulin secretion from the \(\beta\)-cells (124, 396). Extracellular release of zinc binds to various cell surface proteins, and the most intriguing protein is the zinc receptor, the G protein-coupled receptor 39 (GPR39) (28, 372).

Zinc plays crucial signaling roles as a second messenger in the cytosol (111, 156), where zinc signaling is caused by zinc influx, which originates from extracellular sites and from intracellular organelles. Zinc released from the perinuclear area, including the endoplasmic reticulum (ER), is referred to as a “zinc wave,” which has been shown to be important for cell signaling functions (401, 451).Moreover, zinc, liberated from cytosolic proteins with oxidation-sensitive zinc-binding sites such as metallothionein (MT) via oxidative stimuli, is also involved in intracellular zinc signaling (11, 141). Intracellular zinc signaling is divided into several classes according to the timescale in which it acts (reviewed in Refs. 111, 144, 146, 156). “Fast” or “early” zinc signaling occurs within seconds to minutes after stimulation and does not require transcription of proteins (143, 451). Conversely, “late” zinc signaling requires biosynthesis of proteins to control cytosolic zinc concentrations and occurs over a period of hours after stimulation (215). The word “late” zinc signaling is generally used, to contrast with the word “fast” or “early” zinc signaling. This term describes downstream effects mediated through changes in gene expression, not signaling events. Importantly, intracellular zinc signaling targets a number of enzymes involved in cellular signaling, including protein tyrosine phosphatases (PTPs) (41, 448), phosphodiesterases (PDEs) (161, 427), calcineurin (14), caspases (285, 423), and various kinases such as mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) (72, 149). Physiological levels of intracellular free Zn\(^{2+}\) concentrations regulate these enzymes, e.g., inhibition of caspase-3, T-cell PTP, and PTP-1B is achieved with IC\(_{50}\) values below 10, 200, and 17 nM, respectively (140, 266). Inhibition of the activity of PTPs by zinc is generally operative in intracellular zinc signaling. The enzymatic activity of caspase-9 is reversibly inhibited by zinc binding to cysteine and histidine residues in the active site (175), and similar reversible inhibition mechanisms operate in other enzymes such as PTPs (141). Specifically, inhibition of PTP-1B activity by zinc is probably mediated via a cysteiny1-phosphate intermediate (23). Zinc signaling has been extensively reviewed previously (112).

D. A History of the Discovery of ZIP and ZnT Zinc Transporters

The first zinc transporter protein, Zrc1, was identified in Saccharomyces cerevisiae (199) and confers resistance against high concentrations of cations, including zinc. Subsequently, COT1 of S. cerevisiae and CzCD of Cupriavidus (formerly Ralstonia) metallidurans strain CH34 were identified (64, 298) and also confer resistance against zinc and other metals such as cobalt, manganese, and cadmium. The three proteins show high sequence homology in the transmembrane (TM) regions [the cation efflux domain (pfam01545)] (18). Thus their homologous proteins are named cation diffusion facilitator (CDF) proteins (313), which are found at all phylogenetic levels (115, 196, 286). CDF proteins from mammals were identified a few years later, with the first transporter ZnT1 identified by an elegant...
strategy involving the conferment of zinc resistance to zinc-sensitive mutant BHK cells (309). After this discovery, other ZnT transporters were identified using mutant BHK cells and yeast, as well as EST and genome databases (189, 196, 239, 250). ZnT transporters are assigned to the Solute Carrier family 30A (SLC30A) (310) and designated ZnT1-ZnT10 (FIGURE 1A). However, ZnT9 is probably a misnomer, because ZnT9 acts as nuclear receptor coactivator with sequence homology to a cation efflux domain (51) (see sect. IV).

Compared with ZnT transporters, the identification of ZIP transporters is more complicated. ZIP6, which was referred to as LIV-1 initially, was identified as an estrogen-inducible gene associated with estrogen-regulated growth of human breast cancer cells (259). However, its contribution to zinc homeostasis was confirmed later (399, 405). The first characterized member of ZIP transporters was the iron-regulated transporter 1 (Irt1) of Arabidopsis thaliana (92). Then, zinc-regulated transporter 1 (Zrt1) and Zrt2 were discovered in S. cerevisiae using the sequence of Irt1 (460, 461). The “ZIP (Zrt-, Irt-related protein)” was named after the original members Irt and Zrt (96, 131). ZIP transporters are found in all kingdoms of life (115, 196, 363). Soon afterwards, mammalian ZIP members, ZIP2 and ZIP1, were characterized as zinc transporters that facilitate the

**FIGURE 1.** Structural features and functional domains and motifs of ZnT and ZIP transporters. A: The generalized domain structure of ZnT, including the TM domains, positions of histidine residues (fitting to HX1–6H), and those of serine, arginine, and lysine residues are shown. B: the generalized domain structure of ZIP, including the TM domains, metalloprotease-like motif, the CPALLY motif, and the positions of histidine residues (fitting to HX1–6H) are shown. The length of amino acid residues in both transporters is shown on the right. [Modified from Fukada and Kambe (110) with permission from The Royal Society of Chemistry.]
uptake of zinc into the cells (116, 117). The completion of the human genome sequence led to the identification of all ZIP members in mammals (196, 239). ZIP transporters constitute 14 members designated ZIP1-ZIP14 (FIGURE 1B) and are assigned to the SLC39A family (93, 184). ZIP members mainly transport zinc, but can also mobilize iron (120, 247), manganese, and cadmium (107, 127, 183).

Currently, a number of mutations responsible for inherited disorders have been identified in ZnT and ZIP transporter genes and are therefore of particular clinical interest (194) (see sect. VI).

II. SYSTEMIC AND CELLULAR ZINC HOMEOSTASIS

A. Zinc Homeostasis in the Body

The adult human body contains ~2–3 g of zinc. Approximately 60% of zinc is stored in skeletal muscle, ~30% in bone, ~5% in the liver and skin, and the remaining 2–3% in other tissues (181) (FIGURE 2). Serum zinc accounts for only ~0.1% of the body’s zinc, ~80% of which is loosely bound to albumin and ~20% of that is tightly bound to a2-macroglobulin (17, 346). In the body, ~0.1% of the body zinc is replenished daily through diet. The absorption of zinc in the duodenum and jejunum is strictly regulated; it increases up to 90% when dietary zinc is limited (398), whereas zinc release, when in excess, is facilitated by the gastrointestinal secretion, sloughing mucosal cells and integument, and renal excretion (148, 219). Humans appear to have the capacity to regulate whole body zinc content over a 10-fold change in intake (213).

In general, more than 30 proteins, including ZnT and ZIP transporters, operate under strict coordinated regulation for the maintenance of systemic and cellular zinc homeostasis in mammals; however, humoral mediators, which indicate the zinc status in a cell or tissue to other organs that import and store zinc, have not been identified in the body. Dietary zinc is absorbed in the small intestine (duodenum and jejunum) and then distributed to peripheral tissues. Approximately 60% of zinc is stored in skeletal muscle, ~30% in bones, and ~5% is stored in the liver and skin. The remaining percentage is distributed to other tissues such as the brain, kidney, and pancreas. Excess zinc is excreted through gastrointestinal secretion, sloughing mucosal cells, and integument. Zinc distribution in the body is elaborately controlled through coordinated regulation by ZnT and ZIP transporters.
zinc metabolism. This is in contrast to iron metabolism, where a short peptide hormone hepcidin plays a critical role in systemic iron homeostasis (296). The recent finding that a low-molecular-weight (2 kDa) zinc-regulated humoral factor, which is likely induced in zinc deficiency, controls gene expression particularly associated with immune functions and development in smooth muscle cells is interesting (304) and suggests that a short peptide operates as a humoral factor in systemic zinc homeostasis.

B. Cellular Zinc Distribution

In cells, zinc is distributed in the cytoplasm (50%), nucleus (30–40%), and membrane (10%) (146, 408). The total cellular zinc concentration is thought to range between tens and hundreds of micromolar (61, 221, 309). Zinc is, however, bound with a myriad of proteins and sequestered into organelles and vesicles, and thus the cytosolic labile (“free”) zinc ion concentration is very low and is considered to range between the picomolar and low nanomolar (305, 337, 366, 425) (FIGURE 3). Several lines of evidence indicate that the zinc concentration fluctuates in response to various stimuli, as illustrated by the “zinc wave” (see above) (451) and “zinc spark” (see below) (211) phenomena, and temporal fluctuations of zinc play crucial functions in zinc signaling (265, 305) (see sect. IC). The labile zinc concentrations in intracellular organelles have been measured as 0.14 pM in the mitochondria (311), 0.2 pM in the mitochondrial matrix (279), 0.9 pM in the ER, and ~0.2 pM in the Golgi (337), while much higher (~300 pM and 5 nM) concentrations in the mitochondria and the ER are also described (48). This huge discrepancy in zinc concentrations in the ER and Golgi has not been explained in convincing detail, although the differences may be due to changes of ligand binding or improper folding caused by variables such as pH or the oxidizing environment in their lumen, because the values were measured by different FRET systems. The precise determination of labile zinc concentrations in the cytosol and lumen in subcellular compartments is of current interest and further importance.

The cellular and subcellular zinc homeostasis is achieved through sophisticated regulation of uptake, distribution, storage, and efflux, in which ZnT and ZIP transporters play fundamental roles. Zinc mobilization between intracellular vesicles and organelles by these transporters also contributes to buffer perturbations of cytosolic zinc homeostasis, which is termed “buffering” and “muffling” (62, 262). Buffering and muffling are important in situations of excess zinc conditions and also play a role in zinc ion fluctuations (265).

![FIGURE 3](http://physrev.physiology.org)
C. Zinc Containing Vesicles/Granules in Cells

Zinc has the unique feature that high amounts of chelatable and labile zinc are accumulated in a number of cells and tissues (FIGURE 3). The most well-known is in telencephalic neurons (151, 316); ~20% of zinc is found within the synaptic vesicles of a subset of glutamatergic neurons in the hippocampus and cerebral cortex (60) (see sect. IV). Zinc released from synaptic vesicles is estimated to reach ~100 μM (426), and even up to 300 μM (13). The prostate (138, 276) is another tissue where the accumulation of zinc is 3- to 15-fold higher than levels found in other soft tissues (200 nmol zinc/g wet wt on average) (103). However, high zinc levels are drastically reduced in prostate cancer and carcinomas (125), indicating the importance of zinc in proper prostate metabolism (65). High levels of zinc accumulate in pancreatic β-cells (457), which is required for insulin crystallization. Similarly, a high concentration of zinc is detected in the growth hormone-containing, dense-core secretory granules of anterior pituitary cells (319, 410), as well as submandibular salivary gland epithelial and myoepithelial cells (105), sperm cells (77), pancreatic exocrine cells (223), pigment epithelial cells in the retina (3), Paneth cells in the intestine (289), and mast cells (136). Importantly, the biological and physiological roles of zinc in these cells and tissues remain poorly understood.

Zinc accumulation in subcellular compartments is found in specific processes. For example, large amounts of zinc are imported and localized in the cortical granules of oocytes during meiotic maturation from the prophase I to the metaphase (211, 218, 342), which is required for growth arrest after the first meiotic division (211). The accumulated zinc is ejected from the oocyte to decrease bioavailable zinc during egg activation immediately following intracellular calcium oscillations, which is termed the “zinc spark” (211). In addition to zinc-containing granules and vesicles detected under normal physiological conditions, zinc also accumulates in subcellular compartments under particular pathological conditions. When cells are cultured in high zinc conditions, cytosolic vesicles containing high amounts of zinc appear and are often referred to as zincosomes (31, 441). However, the intracellular vesicles and organelles corresponding to zincosomes are not clearly defined. High zinc accumulation has been reported in lysosomal compartments of cells treated with drugs (58), and in neurons for some neurodegenerative diseases and their model cells (91, 224, 415).

D. Metallothioneins and MTF-1

A portion of the cytosolic zinc pool (5–15%) is bound by MTs. There are ~12 in humans and 4 in mice (70). MTs are composed of 61–68 amino acids, including 20–21 cysteines, and can incorporate up to 7 atoms of zinc and other divalent metals with different affinities. The zinc-binding domain is divided into two clusters: the more stable α-domain and the more reactive β-domain. The number of zinc atoms bound to MT is heterogeneous in vivo, e.g., ~10% of MT is present as the apo-protein in the liver (220). MTs are not simply important for cytosolic zinc storage but are functional as zinc acceptors and donors. Because their sulfur donors coordinating zinc are redox reactive, oxidation of the sulfur donors leads to the release of zinc, which contributes to intracellular zinc signaling (see sect. IC).

In mice, expression of Mt-I and Mt-II is increased significantly by excess zinc through binding of metal-response element-binding transcription factor-1 (MTF-1) to the metal response element (MRE, 5’-TGCRCnCGGCC-3’) in the promoter (387). MTF-1, the only zinc-sensing transcription factor in vertebrates (444), has six C2H2 zinc-finger domains, which contribute to the zinc-sensing and metal-dependent transcriptional activation functions, and function as DNA-binding domains (226). When cellular zinc levels increase, MTF-1 plays a central role in zinc homeostasis by increasing transcription of a host of genes involved in reducing the toxicity of high zinc concentrations, such as MTs, ZnT1, and ZnT2 (134, 229, 387), and in addition, repressing expression of a set of genes involved in zinc uptake such as ZIP10 (241, 449). In the latter case, MTF-1 physically impairs Pol II movement (241). MTF-1 is essential for embryonic liver development (132); however, the relationship of zinc to this process remains unclear. Intriguingly, various zinc-sensing transcription factors have been identified in all kingdoms of life, but their homologs have not been identified in vertebrates (55).

III. STRUCTURAL, BIOCHEMICAL, AND MOLECULAR ASPECTS OF ZnT AND ZIP ZINC TRANSPORTERS

Nine ZnT and 14 ZIP transporters have been identified and characterized over the past two decades (110, 197, 239) (FIGURE 4). ATP hydrolysis is not required for their zinc mobilization across the biological membrane and thus these proteins are assigned to the SLC family members (see sect. ID). The expression levels of “active” zinc transporters at cellular sites, where they normally operate, is critical for defining net zinc transport (191), because zinc (Zn2+) does not require a redox reaction when crossing the cellular membrane, unlike iron and copper. Thus both ZnT and ZIP transporters are indispensable for tightly controlled integrated processes of zinc uptake and efflux, sequestration and release across biological membranes, and a wide variety of zinc functions (see TABLE 1).

A. ZnT Transporters

ZnT transporters mobilize zinc from the cytosol into the extracellular space and the lumens of intracellular compart-
ments, and most of the ZnT transporters operate by transporting zinc into the luminal sides (172, 189, 239, 310) (FIGURES 4 AND 5A). There is no structural information of ZnT transporters; however, there is a body of information describing the structure of a bacterial homolog, YiiP (67, 135, 254, 255) (FIGURE 5B), which greatly contributes to our understanding of ZnT transporters. YiiP has six TM helices with cytosolic NH2 and COOH termini and functions as a homodimer. TM helices I, II, IV, and V form the compact four-helix bundle where four conserved hydrophilic residues of TM helices II and V (three aspartic acids and one histidine, DD-HD) form an intramembranous zinc-binding site (FIGURE 5B). The tetrahedral coordination of zinc by DD-HD is essential, because mutation of aspartic acid residues in the DD-HD motif to nonliganding alanine abolishes zinc transport activity (439). In the zinc transport process, the TM helices of the monomers move closer together, and the cytoplasmic part of the four-helix bundle moves away from the TM helix III–VI pair (67, 135, 254, 255).

YiiP effluxes zinc, which is coupled with proton influx in a 1:1 zinc for proton exchange stoichiometry (49). The zinc/proton (Zn2+/H+) exchange mechanism is conserved in ZnT transporters (303, 375) and involves an alternating access mechanism (67, 135). In this mechanism, YiiP forms inward- and outward-facing conformations, both of which are able to bind Zn2+ or H+, and the extracellular proton provides a driving force for exporting Zn2+ from the cytosol (67). A leucine residue in TM helix V, which is next to the conserved histidine (H) forming the intramembranous zinc-binding site and is located at the interface between the TM helix III–VI pair and the four-helix bundle, is suggested to be important as a principal hydrophobic barrier to control the opening of the intercavity water portal (135). The cytosolic COOH-terminal portion of YiiP forms a binuclear zinc-binding site, which may function to stabilize YiiP homodimers. Alternatively, this region may sense zinc ion concentrations and regulate zinc transport activity of YiiP through triggering a scissor-like conformational change and thereby modulating the coordination of Zn2+ at the intramembranous zinc-binding site in the four-helix bundle (254, 255). In this autoregulation mechanism, YiiP operates allosterically in a zinc-regulated manner. Compared with the TM helices domain, the cytosolic COOH-terminal portion forming the binuclear zinc-binding site has a high degree of sequence variability, but has been shown to be a highly conserved metallochaperone-like structure with an αβα fold in bacterial ZnT homologs (52, 155, 255). Thus the operation mechanism of zinc transport should be conserved among CDF members, including ZnT transporters.

Based on the structural properties of YiiP, ZnT transporters have been biochemically characterized. ZnT transporters are predicted to have six TM helices with the exception that ZnT5 has a long NH2-terminal portion with nine putative TM helices. ZnT transporters form homodimers (114, 180, 230, 231, 291, 353, 391), again with the exception that ZnT5 forms heterodimers with ZnT6 (114, 195, 230). The cytosolic COOH-terminal portion, which is important for
their dimerization (114, 353), contributes to other protein-protein interactions and enables ZnT transporters to control the activity of counterpart proteins or to be controlled by counterpart proteins (114, 186, 189, 355).

ZnT transporters are functional in a Zn\(^{2+}\)/H\(^+\) exchange manner (303, 375), in which two aspartic acids and two histidines of TM helices II and V (HD-HD) in the four-helix bundle are thought to form an intramembranous zinc-binding site (FIGURE 5C). The introduction of mutations in the HD-HD motif abolishes zinc transport activity (106, 113, 303, 375). The position of the histidine in TM helix II probably plays an important role in determining metal substrate specificity, because substitution of HD-HD to

FIGURE 5. Putative topologies of ZnT transporters. A: the schematic topology of ZnT transporter, proposed based on the X-ray structure of their E. coli homolog, YiiP (254, 255) shown in B. ZnT transporters are thought to operate as Y-shaped dimers with six TM helices (except for ZnT5). TM helices I, II, IV, and V probably form a compact four-helix bundle where four conserved hydrophilic residues (HD-HD) of TM helices II and V form the intramembranous zinc-binding site. ZnT transporters are functional as Zn\(^{2+}\)/H\(^+\) exchangers. The cytosolic histidine-rich loop is thought to be implicated in sensing and translocating zinc to the HD-HD site. The actual protein length of each transporter is shown in FIGURE 1A. B: the ribbon representation of the homodimeric YiiP structure (PDB 3H90). The side view (left) and top view (right) from the extracellular side are shown. Yellow spheres represent bound zinc ions in zinc-binding sites. TM helices II and V are colored by blue and purple, respectively. C: the sequences of TM helices II and V of YiiP and human ZnT transporters are aligned. The highlighted conserved hydrophilic residues in multiple alignments are predicted to form the intramembranous zinc-binding site. The indicated TM helices of ZnT5 correspond to TM helices XI and XIV.
DD-HD changes zinc-specific transport to zinc and cadmium transport in ZnT transporters (158). An asparagine is present in ZnT10 (that is ND-HD), which can function as a manganese transporter (see sect. IV) (341, 417) (FIGURE 5C). The DD-HD motif is found in YiiP, which transports both zinc and cadmium, but not manganese (158), while the DD-DD is found in plant ZnT homologs that transport manganese (137, 286). In yeast and plant ZnT homologs, many amino acids other than HD-HD have been shown to affect metal specificity (202, 242), and some of them are conserved in ZnT transporters. Therefore, further clarifying the relationship between the motif and metal specificity is required.

The cytosolic histidine-rich loop between TM helices IV and V is another characteristic of ZnT transporters (FIGURE 1). Deletion and mutational analyses suggest that the histidine-rich loop may be functional as a sensor of cytosolic zinc levels (202, 242), and be involved in controlling the mission of entry of zinc to the HD-HD in the four-helix bundle (202, 203, 391). In several ZnT transporters, another conserved short histidine-rich sequence found in the cytosolic NH2-terminal portion may also contribute to sensing and translocating of zinc to the HD-HD site (12).

Generally, CDF transporters are classified into three groups (with 13 clusters), namely, Zn-CDF, Zn/Fe-CDF, and Mn-CDF. Unlike bacteria and plants, all ZnT transporters belong to the Zn-CDF group (286).

**B. ZIP Transporters**

ZIP transporters function by replenishing cytosolic zinc from the extracellular space and the lumens of intracellular compartments (184, 189, 239) (FIGURES 4 AND 6A). Most ZIP transporters are localized to the plasma membrane, and their cell-surface localization, except for ZIP3, increases...
under zinc-deficient conditions (56, 85, 116, 161, 207, 241, 246, 249, 402, 431). Rapid internalization and degradation occurs to these transporters in response to excess zinc (168, 261, 437). There are no three-dimensional structures of ZIP transporters and their homologs. Hydrophobicity plots suggest that these transporters have eight TM helices with extracellular/luminal NH₂ and COOH termini (184, 189, 239). ZIP transporters and their homologs form homodimers for zinc transport activity (32, 243), and their apparent \( K_m \) values range from hundreds of nanomolar to \( \sim 20 \mu M \) when overexpressed in the cells (10, 78, 84, 116, 117, 246, 323, 431), although ZIP12 has a much lower \( K_m \) (56). Currently, the zinc transport mechanism of ZIP transporters has not been well defined. A bicarbonate/zinc symport mechanism is suggested in several ZIP transporters (116, 127, 152). However, a nonsaturable and electrogenic zinc transport fashion was revealed in a purified bacterial ZIP homolog when reconstituted into proteoliposomes (116, 127, 152). However, a nonsaturable and electrogenic zinc transport fashion was revealed in a purified bacterial ZIP homolog when reconstituted into proteoliposomes (243). The channel-like zinc transport fashion is also supported by evidence that phosphorylation can trigger zinc transport by a ZIP transporter (401).

The zinc substrate specificity for ZIP transporters is not strict, and they are involved in iron, manganese, copper, and cadmium transport. The determinants of metal specificity of ZIP transporters have not been completely defined, but a conserved histidine residue in TM helix V may play a crucial role, which is speculated to be involved in an intramembranous zinc-binding site (FIGURE 6B), if it operates as a “transporter.” The histidine is replaced by glutamic acid in ZIP8 and ZIP14 (FIGURE 6B), both of which are known to have broad metal specificity (107, 127, 183). How ZIP transporters determine their selectivity to zinc or other metals has been of interest.

ZIP transporters are classified into subfamilies I, II, LIV-1, and gufA, according to their sequence similarities (115, 399, 405) (see sect. V). ZIP 1 and guf-A include a single member, respectively. ZIPII consists of three ZIP transporters, and thus the LIV-1 subfamily is the most populous subfamily (405). The sequence diversity of LIV-1 subfamily transporters mirrors their wide range of biological functions. Nonetheless, some features are common, in particular, a potential metalloprotease-like motif (CHEXPHEXGD) in TM helix V and an extracellular CPALLY motif located before the first TM helix (FIGURES 1 AND 8). The functional significance of the CHEXPHEXGD motif has not been elucidated, but may be involved in regulating zinc transport by closing the pore through the formation of a disulfide bond between its first cysteine, which is highly conserved among LIV-1 members localized to the plasma membrane, and that of the CPALLY motif (404). The NH₂-terminal portion containing the CPALLY motif is cleaved in some LIV-1 subfamily transporters (ZIP4, ZIP6, and ZIP10) (referred to as “processing”) (90, 159, 192), and thus processing may operate to open the pore to facilitate uptake of zinc into cells. Moreover, processing is likely to be important for regulating their cellular trafficking (159). Intriguingly, an evolutionary link has been proposed in the extracellular portion between some LIV-1 subfamily transporters (ZIP5, ZIP6, and ZIP10) and the prion proteins, in which the PrP-like amino acid sequence is found (89, 90, 324, 363). These results suggest that particular ZIP transporters may be involved in the etiology of prion diseases.

### IV. CELLULAR AND PHYSIOLOGICAL FUNCTIONS OF EACH \( \text{ZnT} \) TRANSPORTER

#### A. ZnT1

ZnT1 is the only ZnT transporter that is localized mainly to the plasma membrane and functions by exporting cytosolic zinc into the extracellular space (309). Thus ZnT1 protects cells from excess zinc influx under pathological conditions, such as transient forebrain ischemia (414). Upregulated ZnT1 mRNA due to increases in cellular zinc levels, which is mediated by MREs in its promoter in a fashion similar to MT (229), probably contributes to the protection function of ZnT1 (see sect. IID).

In polarized epithelial cells, ZnT1 is localized to the basolateral membrane. Thus ZnT1 facilitates the absorption of zinc by exporting it into the portal blood in enterocytes (280) and similarly the reabsorption of zinc in kidney epithelial cells (239). In contrast, ZnT1 is localized to the apical membrane in pancreatic acinar cells and is implicated in pancreatic secretion of zinc (69). ZnT1 also localizes to intracellular vesicles in some cell types (87, 106) and to the ER in keratinocytes by forming hetero-complexes with EVER proteins, which are responsible for Epidermodysplasia verruciformis (EV), a rare autosomal recessive skin disease (OMIM 226400) (232). The molecular mechanism underlying the specific localization of ZnT1 is not well understood, except for ER localization.

ZnT1 interacts with various intracellular proteins, which contributes to its roles in cellular signaling. ZnT1 binds to the \( \text{NH}_2 \)-terminal regulatory region of Raf-1, a signal transducer of the Ras-ERK pathway, which promotes Raf-1 activation. This activation is likely to occur through lowering the cytosolic zinc level, which inhibits their interaction, possibly releasing zinc from the cysteine-rich domain of Raf-1 (186). The regulation of Raf-1 activity by ZnT1 contributes to the protection of cells from ischemia reperfusion (21). ZnT1, which forms a hetero-complex with EVER proteins, downregulates the transcription factors stimulated by MTF-1, c-Jun, and Elk. ZnT1 also interacts with the regulatory \( \beta \)-subunit of the L-type calcium channel (LTCC), which reduces the capacity of the \( \beta \)-subunit to chaperone the pore-forming \( \alpha_1 \)-subunit of LTCC to the cell surface (236). ZnT1 enhances the activity and surface expression of...
the T-type calcium channel, which is regulated via activation of Ras-ERK signaling (287). Upregulation of ZnT1 in response to lipopolysaccharide (LPS) stimulation is involved in TLR signaling by decreasing intracellular zinc levels in dendritic cells (215). ZnT1 is essential during embryonic development, because deletion of the *Znt1* gene in mice results in embryonic lethal (9) (TABLE 2).

B. ZnT2

ZnT2 is a zinc transporter that confers cells with zinc resistance by transporting surplus cytosolic zinc into the lumen of vesicular compartments, including endosomes/lysosomes, to which it is localized (100, 180, 307). ZnT2 is also localized to the ER in breast cancer cells (38), the zymogen granules in pancreatic acinar cells (134, 249), and the inner mitochondrial membrane of mammary cells (370). The alternative splicing variant of ZnT2 locates to the plasma membrane (253). Abundant ZnT2 mRNA expression is observed in mammary glands, prostate, pancreas, small intestine, kidney, and retina (235, 248, 308), which is upregulated by high zinc levels via the MRE located downstream from the ZnT2 transcription start site (134). Upregulation of ZnT2 transcription also occurs via a glucocorticoid receptor and STAT5 interaction in pancreatic acinar cells (134), or via the prolactin-induced JAK2/STAT5 signaling

<table>
<thead>
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<td>Slc30a10</td>
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Phenotypes of each Znt8 KO mice are shown, because general consensus is lacking among mouse colonies. KO, knockout; –, no report.
pathway in mammary epithelial cells (334). ZnT2 mRNA expression is ~20-fold higher in the ventral prostate of aged rats when compared with levels in young rats (176).

ZnT2 is indispensable for zinc mobilization to breast milk in humans. Mothers with mutations in the ZnT2 gene produce zinc-deficient milk (75–90% reduction), and thus infants exclusively breast-fed by mothers carrying this mutation experience transient neonatal zinc deficiency (TNZD; OMIM 608118) (57, 180, 231, 283) (see sect. VI.B). ZnT2-mediated zinc secretion is posttranslationally regulated by prolactin; prolactin stimulates ZnT2 ubiquitination, which targets ZnT2 to exocytic vesicles to transiently enhance zinc secretion, after which ZnT2 is degraded by the ubiquitin proteasome pathway (369). Overexpression of ZnT2 decreases invasive phenotypes of breast cancer cells (38).

C. ZnT3

ZnT3 is a critical transporter of zinc into synaptic vesicles of a subset of glutamatergic neurons in the hippocampus and neocortex (60). The synaptically released zinc from presynaptic vesicles plays pivotal roles in modulating neuronal transmission and plasticity. This is achieved through zinc binding to multiple neuronal ligand- and voltage-gated ion channels, transporters, and receptors of neurotransmitters on postsynaptic neurons (104, 157, 300, 367, 368, 394, 424). ZnT3 probably contributes to the prevention of aging-related cognitive loss, because ZnT3 expression levels fall with age (1) and in patients with Alzheimer’s disease (1, 34) or Parkinson’s disease dementia (446). Consistent with these results, aged Znt3-KO mice exhibit deficits in learning and memory (1, 269, 377). However, young Znt3-KO mice were first reported to show almost no obvious phenotypes without slightly higher thresholds to seizures than wild-type mice, despite the observation that synaptic zinc is essentially absent (59). Currently, it is reported that Zn3-3-KO mice show a reduction in hypoglycemia-induced progenitor cell proliferation and neuroblast production (389). The fundamental changes in expression of proteins and genes important in neurotransmission are also reported in Znt3-KO mice (1, 294). Zinc accumulated in synaptic vesicles by ZnT3 has been shown to play an essential role in presynaptic Erk1/2 signaling during hippocampus-dependent learning (377). In addition, the importance of the synaptic zinc mediated by ZnT3 has also been revealed in several knock-in mice studies, where mutations are introduced in zinc-binding sites of receptors of neurotransmitters (157, 300). Synthetic zinc may contribute to amyloid deposition in an age-dependent manner (233). Thus proper handling of zinc into synaptic vesicles by ZnT3 should be critical for preventing the onset of Alzheimer’s disease.

A brain-specific adaptor protein, AP-3, conducts the trafficking of ZnT3 to the synaptic vesicles through interacting with the COOH-terminal portion of ZnT3 (355). Therefore, mocha mice expressing a mutant form of AP-3 show disturbed localization or loss of ZnT3 and synaptic zinc (201). Zinc transport activity by ZnT3 in neuronal cells is potentiated by a chloride channel (354) and a vesicular glutamate transporter (352).

In addition to the brain, ZnT3 expression is detected in pancreatic β cells where it is involved in the regulation of insulin production (379). ZnT3 mRNA is also abundantly expressed in the testis; however, the ZnT3 protein is not expressed (308).

D. ZnT4

Znt4 is known as a responsible gene for a spontaneous mutant lethal milk (lm) mouse (167), which is characterized by reduced milk zinc levels. Pups die before weaning because of a deficiency in zinc when nursed by affected lm/- dams (321). There is no evidence of mutations in the ZnT4 gene of women who produce low milk zinc levels (194, 281), suggesting that zinc supply into breast milk is controlled by species-specific mechanisms. The lm mice can grow if foster nursed by normal dams, but show congenital defect of otolith (calcium carbonate crystals in the inner ear) with delayed righting, “tail-spinning,” and abnormal swimming (98). The lm mice also exhibit extensive hair loss, dermatitis, and skin lesions over 8 mo of age (98), which are typical features of zinc deficiency. Thus ZnT4 may be involved in a zinc absorption process in adults.

ZnT4 is found in the endosomes/lysosomes, cytoplasmic vesicles, Golgi apparatus, and trans-Golgi network (TGN), which changes during differentiation and high zinc conditions (169, 224, 248, 282, 343). In polarized cells, vesicles where ZnT4 locates are concentrated to both the apical and the basal sides, and this localization is dependent on the cell type (207, 248, 343). ZnT4 plays a role in the maintenance of cytosolic zinc homeostasis by controlling zinc translocation to the lysosomes, cooperatively with a lysosomal zinc leak channel, the transient receptor potential mucolipin 1 (TRPML1) (224). Zinc translocated by ZnT4 into TGN is likely to be supplied to zinc-requiring enzymes, such as carbonic anhydrase V1 (278).

ZnT4 mRNA is ubiquitously expressed, but expression is relatively higher in the brain and digestive tract (167). In the villus of the small intestine, ZnT4 expression is dramatically increased during differentiation (16). Upregulation of ZnT4 mRNA expression, along with ZnT7 mRNA by GM-CSF in macrophages, may contribute to the phagocyte antimicrobial effector function by shunting zinc away from phagosomes through the mobilization of zinc into the Golgi apparatus (388). ZnT4 expression decreases in the progression from benign to invasive prostate cancer (153).
**E. ZnT5**

ZnT5 is localized to the early secretory pathway including COPII-coated vesicles and the Golgi apparatus (195, 390). ZnT5 uniquely forms heterodimer complexes with ZnT6 (114, 230, 391), which is conserved in ZnT5 orthologs (94, 101). ZnT5-ZnT6 heterodimers and ZnT7 (see below) have important biosynthetic functions by delivering zinc into the early secretory pathway, which is required for the activation of zinc-requiring enzymes such as alkaline phosphatases (ALPs) (113, 390). The activation of ALPs occurs in a two-step mechanism through stabilizing the enzyme, and then the conversion of the enzyme from the apo- to the holo-form with zinc loading (113, 390). ZnT5 contributes to the homeostatic maintenance of the secretory pathway functions by supplying zinc into the lumen during zinc deficiency, which induces the unfolded protein response (UPR) (95, 163, 179). ZnT5 is implicated in the regulation of cellular signaling, e.g., translocation of PKC to the plasma membrane (299). The splicing variant of ZnT5 (referred to as hZTL1 or ZnT5B) mediates zinc transport in the opposite or bidirectional directions at the plasma membrane, which may be because this variant has an extra TM helix next to the cation efflux domain (71, 419).

ZnT5 mRNA expression is significantly increased by the UPR inducer through the conserved UPR element (179), which is a probable feedback regulation mechanism that maintains homeostasis of the secretory pathway. ZnT5 mRNA is relatively highly expressed in the pancreas, and the ZnT5 protein is associated with insulin granules in pancreatic β-cells (195, 373). However, its contribution to insulin crystallization is minor (190). Meanwhile, ZnT5 may play an important role in regulating GH secretion (318). Zinc-induced transcriptional repression of ZnT5 is shown to be under the control of the Zn transcriptional regulatory element (ZTRE) (63). The disruption of Znt5 gene in mice causes poor growth, osteopenia, and male-specific sudden cardiac death because of the bradyarrhythmia (177), and also impairs the mast cell-mediated, delayed-type allergic reaction (299).

**F. ZnT6**

ZnT6 is a zinc transporter localized to the Golgi apparatus and TGN (169). However, ZnT6 itself lacks zinc transport activity, because two histidine residues of the intramembranous zinc-binding site in the four-helix bundle are substituted with leucine and phenylalanine (114, 189) [FIGURE 5C]. A serine residue of the cytosolic COOH-terminal portion of ZnT6 is identified as a potential phosphorylation site (160), which may indicate that ZnT6 is a modulator subunit of zinc transport activity of ZnT5-ZnT6 heterodimers (114). ZnT6 mRNA expression is less ubiquitous than ZnT5 mRNA expression (169, 195, 371), and the subcellular localization of ZnT6 is somewhat distinct from that of ZnT5 (169, 390), e.g., its subcellular localization is altered under high zinc conditions (169). Therefore, ZnT6 may have specific biological functions in some cell types that do not involve the formation of the ZnT5-ZnT6 complex.

**G. ZnT7**

ZnT7 is localized mainly to the early secretory pathway, including the Golgi apparatus (214). ZnT7 is homologous to ZnT5 in the cation efflux domain, but ZnT7 cannot interact with ZnT6, which is attributed to the lower homology in the cytosolic COOH-terminal region between ZnT7 and ZnT5 (114, 189). ZnT7 is involved in the activation of ALPs (113, 188, 391) and contributes to homeostatic control in the early secretory pathway, cooperatively with ZnT5-ZnT6 heterodimers (179). ZnT7 is also involved in cellular signaling, e.g., overexpression of ZnT7 increases Irs2 and Akt phosphorylation in skeletal muscle cells (170).

The disruption of the Znt7 gene in mice causes poor growth, decreased adiposity, and reduced absorption of dietary zinc, indicating the involvement of ZnT7 in dietary zinc absorption and in the regulation of body adiposity. However, dermatitis and hair abnormality, typical symptoms of zinc deficiency, are not observed (173). Male Znt7-deficient mice fed with a high-fat diet are more susceptible to diet-induced glucose intolerance and insulin resistance (170). The absence of ZnT7 may promote the early onset of prostate tumors (407).

**H. ZnT8**

ZnT8 was identified as a pancreatic β-cell-specific zinc transporter, which enhances glucose-stimulated insulin secretion in a high glucose challenge (53, 54), and afterward was shown to be expressed in α-cells (139). ZnT8 transcription in β-cells is regulated by the β-cell-enriched transcription factor Pdx-1 through an intronic enhancer (326). ZnT8 plays an indispensable role in supplying zinc to insulin granules in β-cells to form insulin-zinc crystals. In a line of Znt8-KO mice, the dense core of zinc-insulin crystals is lost because of a reduction in zinc content in β-cells (234, 297, 327, 396, 447).

Nonsynonymous single nucleotide polymorphism [SNP; rs13266634 (R325W)] of the ZnT8 gene is associated with a high risk of type 2 diabetes mellitus (OMIM 125853) (359, 365, 378, 458) (see sect. VIC). The ZnT8 variant of the elevated risk reduces the zinc transport activity of ZnT8 (297). In the evaluation of quantitative traits, the risk allele (R325) carriers have abnormalities in both glucose-stimulated insulin secretion (GSIS) and insulin processing (35, 385).

In addition to loss of zinc-insulin crystals, Znt8-KO mice show the effect of ZnT8 abnormalities on glucose-stimu-
lated insulin secretion, glucose tolerance, and body weight, often when fed a high-fat diet (150, 234, 297, 327, 328, 396, 447), although the observed effects have been highly variable among mouse colonies (302) (Table 2). Some effects are influenced by sex and genetic background (328), and probably age. Zinc, which is accumulated by ZnT8 in insulin granules, can reduce insulin secretion from β-cells and suppress hepatic insulin clearance, when being cosecreted with insulin following glucose stimuli (396). Thus ZnT8 is an essential component of β-cell functions, and dysfunctional ZnT8 in β-cells leads to an increase in the risk of type 2 diabetes. Additionally, non-β-cell-specific effects of ZnT8 may contribute to the risk of developing type 2 diabetes (150). However, contrary to the above evidence, a rare variant association study has shown that haploinsufficiency of ZnT8 is protective against type 2 diabetes (102). This controversial relationship between pathogenesis of type 2 diabetes and ZnT8 function requires further investigation.

ZnT8 is also targeted by autoantibodies in 60–80% of new-onset type 1 diabetes (442). The R risk allele is shown to be to a key determinant in humoral autoreactivity to ZnT8 (443) (see sect. VI).

I. ZnT10

ZnT10 is a zinc transporter localized to early/recycling endosomes or the Golgi apparatus (37, 312), which is altered to the plasma membrane at higher extracellular zinc concentrations (37). Expression of ZnT10 is downregulated by IL-6 (108) and is suggested to be under the control of ZTRE, as in ZnT5 (63). The downregulation of ZnT10 along with ZnT3 by angiotensin II may be associated with cellular senescence in vascular smooth muscle cells (312). Homozygous mutations of the ZnT10 gene cause Parkinsonism and dystonia with hypermanganesemia, polycthemia, and hepatic cirrhosis (OMIM 613280) (341, 417). In these patients, not zinc, but systemic and cellular manganese homeostasis was disturbed (341, 417), suggesting that the primary function of ZnT10 is manganese transport (see sect. IIIA). The ability of ZnT10 to transport manganese has been shown recently at the molecular level (327). ZnT10 mRNA expression is high in the liver and brain, which is consistent with high manganese accumulation in these tissues for patients with ZnT10 mutations (341, 417).

ZnT9 was classified to ZnT transporter members, based on its low sequence similarity to cation efflux domains of ZnT transporters. However, ZnT9 is thought to have no zinc transport functions, because it lacks an essential histidine constituting the intramembranous zinc-binding site, and has not been shown to interact with other ZnT transporters, unlike ZnT6. Moreover, ZnT9 is predominantly localized in the cytoplasm, and its cell membrane localization was ruled out (376). ZnT9 acts as nuclear receptor coactivator and is renamed as GAC63 (GRIP1-associated coactivator 63) (51).

V. CELLULAR AND PHYSIOLOGICAL FUNCTIONS OF EACH ZIP TRANSPORTER

A. ZIP1 (ZIPII Subfamily)

ZIP1 functions as an importer of zinc at the plasma membrane (84, 117). Zinc uptake by ZIP1 contributes to the activation of microglia by inducing ATP release and autocrine/paracrine activation of P2X7 receptors (154). The overexpressed ZIP1 on the cell surface is endocytosed, thereby decreasing in response to excess zinc (429), which is mediated through the dileucine motif in the cytosolic loop between TM helices III and IV (168). ZIP1 expression, however, is not zinc responsive in vivo (437). Expression of ZIP1 is significantly reduced in prostate cancer and carcinoma cells, and parallel decreases in zinc levels are observed (66, 465), suggesting that ZIP1 expression is indispensable for normal prostate metabolism. Zip1-KO mice are more likely to develop abnormally during pregnancy than wild-type mice, when dietary zinc was limited (82) (Table 3).

B. ZIP2 (ZIPII Subfamily)

ZIP2 imports zinc from the extracellular space (116). Zinc uptake by ZIP2 is necessary for the differentiation of keratinocytes and for the turnover of the epidermis (178). ZIP2 mRNA expression is relatively low in limited tissues (84, 116), but is significantly induced by zinc deprivation in monocytes (68) and by GM-CSF in macrophages (388), as well as during keratinocyte differentiation (178). In the prostate, ZIP2 expression decreases in carcinomas (79). Similarly to Zip1-KO mice, Zip2-KO mice are sensitive to dietary zinc deficiency during pregnancy (317).

C. ZIP3 (ZIPII Subfamily)

ZIP3 imports zinc from the extracellular space. The plasma membrane localization of ZIP3 changes to intracellular compartments following zinc treatment (207). The apical membrane localization of ZIP3 in lactating mammary glands suggests its role in zinc reuptake from the alveolar lumen (208). ZIP3 expression increases during differentiation to a secretory phenotype in mammary epithelial cells in response to prolactin (206).

Zip3-KO mice are more likely to develop abnormally in zinc-deficient pregnancy (81). Moreover, Zip1- and Zip3-double or Zip1-, Zip2-, and Zip3-triple KO mice show zinc-sensitive phenotypes during pregnancy, which is similar to the corresponding single KO mice (82, 193), indicating that each has unique cell-specific functions for adaptation to a zinc deficiency during development. The involvement of ZIP1 and ZIP3 in zinc entry-induced neural
degeneration has been shown using Zip1- and Zip3-double KO mice (333).

ZIP1, ZIP2, and ZIP3 expression is often regulated in a synchronized manner, e.g., their mRNA expression is decreased in lymphocytes in older people when compared with their expression in young people (126). Moreover, protein expression of these three transporters decreases in prostate carcinomas (79), suggesting their potential roles as tumor suppressors in the prostate. ZIP1 and ZIP3 mRNA expression significantly increases in allergic airway inflammation (228).

D. ZIP4 (LIV-1 Subfamily)

The ZIP4 gene is responsible for inherited zinc deficiency, acrodermatitis enteropathica (AE; OMIM 201100) (225, 432). ZIP4 is essential in dietary zinc absorption in mammals, which was confirmed by enterocyte-specific deletion of ZIP4 (123). ZIP4 also plays a pivotal role in intestinal integrity (123). ZIP4 mRNA is highly expressed in the small intestine, including the duodenum and jejunum (85). ZIP4 expression is strictly regulated by zinc levels and multiple posttranscriptional mechanisms; ZIP4 mRNA is stabilized and ZIP4 accumulates on the apical membrane of the small intestine during zinc deficiency (83, 85, 249, 261, 430, 437). ZIP4 undergoes processing (the proteolytic cleavage of the extracellular NH2-terminal portion, see sect. III) during prolonged zinc deficiency (192). AE-causing mutations in ZIP4 result in reduced expression, trafficking defects to the plasma membrane, decreased zinc uptake activity (97, 430), and inhibition of processing (192). Complete Zip4-KO mice are embryonic lethal by E8-E9. The heterozygosity of the Zip4

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<td>KO: abnormal embryonic development and thymic pre-T cells depletion in zinc-limited condition</td>
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</tr>
<tr>
<td>Slc39a11</td>
<td>Zip11</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Slc39a12</td>
<td>Zip12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Slc39a13</td>
<td>Zip13</td>
<td>KO: growth retardation, abnormal development of skeletal and connective tissue</td>
<td>109</td>
</tr>
<tr>
<td>Slc39a14</td>
<td>Zip14</td>
<td>KO: growth retardation, dwarfism, impaired skeletogenesis, impaired gluconeogenesis, increased body fat, insulin levels and liver glucose, hypoglycemia, and impairment of hepatocyte proliferation</td>
<td>15, 22, 161</td>
</tr>
</tbody>
</table>

KO, knockout; –, no report. Phenotypes of Zip1 and Zip3-double KO and Zip1, Zip2 and Zip3-triple KO are summarized in the row of Zip3-KO.
ROLE OF ZINC TRANSPORTERS IN ZINC HOMEOSTASIS AND METABOLISM

gene in mice is teratogenic and embryotoxic and reveals pleiotropic phenotypes (86), indicating its essential role during development.

In other tissues, ZIP4 expression may be involved in diseases. Aberrant expression of ZIP4 is associated with pathogenesis and progression in cancer and carcinomas (238, 244, 438). In the case of pancreatic cancer, ZIP4 enhances CREB activity by the uptake of zinc, which transcriptionally increases miR-373 expression, leading to silencing of molecules with tumor suppressor activity (459). High ZIP4 expression was associated with higher grade of gliomas and shorter overall survival (244). Conversely, in prostate carcinoma, ZIP4 expression is downregulated and thus ZIP4 may act as a tumor suppressor (50).

E. ZIP5 (LIV-1 Subfamily)

ZIP5 is functional as a zinc importer. ZIP5 has moderate sequence similarity to ZIP4 (36% similarity), particularly in the COOH-terminal half (49% similarity). However, their expression in response to zinc and subcellular localization are reciprocally regulated (83, 437). ZIP5 expression is not increased but decreased in zinc-depleted environments. In polarized cells, ZIP5 is localized to the basolateral membrane (83, 431, 437). ZIP5 expression is not regulated at the transcriptional level, but at the translational level (437). ZIP5 translation is enhanced, when zinc is available, which is mediated by a conserved stem-loop and two overlapping miRNA seed sites in the 3'-untranslated region (435). ZIP5 is abundantly expressed in the small intestine and pancreas (83). ZIP5 is also expressed during the whole process of eye development, mainly in the sclera and retina (133). Zinc mediated by ZIP5 is likely to be involved in the regulation of Smad protein expression (133). Complete disruption of the Zip5 gene in mice, and intestine- or pancreas-specific disruption of the Zip5 gene in mice, clearly indicates that ZIP5 participates in the control of zinc excretion. Pancreas-specific Zip5-KO mice revealed that ZIP5 in pancreatic acinar cells likely functions in zinc accumulation/retention and protects cells from zinc-induced acute pancreatitis (122).

ZIP5 has the PrP-like ectodomain (see sect. IIIB). Intriguingly, ZIP5 can be colocalized with the cellular prion protein (PrPc) at the plasma membrane and in Rab5 immuno-reactive endosomes in neuroblastoma cells (324).

F. ZIP6 (LIV-1 Subfamily)

ZIP6 is localized to the plasma membrane and functional as zinc transporter (218, 402). ZIP6 can be a growth factor-elicted signaling molecule through its zinc influx functions from the extracellular space, and thereby enhanced zinc influx mediated by altered ZIP6 expression has been shown to be closely associated with invasive and metastatic ability and cancer progression (374, 462). Elevated expression of ZIP6 is involved in epithelial-mesenchymal transition (EMT) of breast cancer cells (159), in pancreatic carcinoma (418), and in prostate cancer cells (256). In particular, the intimate association of ZIP6 with breast cancer is supported by a number of studies. Zinc influx mediated by ZIP6 activates glycogen synthase kinase 3β (GSK-3β), resulting in activation of Snail and leading to anoikis resistance (159). Expression of ZIP6 is associated with estrogen receptor α status (259, 413) and positively correlated with lymph node involvement (260). Importantly, the ZIP6 gene is a downstream target of STAT3 (159, 452) that is constitutively activated in many human malignancies (358). These results indicate that ZIP6 is a new target for the prediction of clinical cancer spread and also an attractive therapeutic target for improving metastatic tumors in breast and other tissues (159, 400). ZIP6 also plays a role in immune functions, e.g., ZIP6 contributes to dendritic cell maturation by negatively regulating LPS-induced cell surface MHC class II expression (215), or to tuning the TCR activation threshold in T cells (455) through increasing zinc influx.

ZIP6 often plays a role in conjunction with its close homolog ZIP10. ZIP6 and ZIP10 promote cell motility of breast cancer cells by high glucose stimulation (393). Maternally derived ZIP6 and ZIP10 import zinc into the mammalian oocyte for proper meiotic progression, in which ZIP6 and ZIP10 transcripts are upregulated in a synchronized manner (218).

The trafficking of ZIP6 to the plasma membrane is regulated by processing on the ER (159). Thus the extracellular NH2-terminal portion containing the PrP-like domain is missing on the cell surface ZIP6.

G. ZIP7 (LIV-1 Subfamily)

ZIP7 is localized to the early secretory pathway including the ER and Golgi apparatus (171, 403). Zinc transport activity of ZIP7 is regulated by phosphorylation by casein kinase 2 (CK2) protein kinase (401), and its regulated zinc release from the intracellular stores promotes the activation of tyrosine kinases, AKT and ERKs, both of which are involved in regulating cell proliferation and migration (401). ZIP7 expression levels inversely correlate with phosphorylated GSK3 levels and cellular zinc levels (130), although the molecular mechanism underlying their regulatory relationships is unknown. ZIP7 is implicated in glycolytic control in skeletal muscle (292). Glucose-induced ZIP7 expression may facilitate the processing and storage of insulin in pancreatic β-cells (24).

Disturbed zinc homeostasis caused by aberrant ZIP7 expression is thought to contribute to growth and invasion, leading to a more aggressive phenotype in breast cancers (406). ZIP7 is among 10% of genes consistently overex-
pressed in many breast cancers with poor prognosis (160). Moreover, ZIP7 expression has been shown to be progressively lost in the occipital lobe of neuronal ceroid lipofuscinosis (CLN6 disease) affected sheep, which suggests that ZIP7 contributes to deregulation of subcellular zinc homeostasis in the disease (130).

H. ZIP8 (LIV-1 Subfamily)

ZIP8 is localized to the plasma membrane and to the apical surface in polarized cells (152, 245), and is also localized to the lysosome (14, 20). In both cases, ZIP8 activity increases the cytosolic zinc status. ZIP8 mRNA expression is induced significantly by the stimulus of BCG in monocytes (20). Increased mRNA expression of ZIP8, with accompanying lower plasma zinc levels, is reported in sepsis patients (27). ZIP8 mRNA expression is a transcriptional target of NF-κB, and ZIP8 negatively regulates proinflammatory responses through zinc-mediated downmodulation of IkB kinase (IKK) activity, thereby inhibiting NF-κB activity (245). Thus ZIP8 plays pivotal roles in a negative-feedback loop that directly regulates the innate immune function (245). The lysosome-located ZIP8 has functions in TCR-mediated T-cell activation by releasing zinc from the lysosomal lumen (14).

Higher ZIP8 expression is found in the cartilage of patients with osteoarthritis (212), whose serum zinc levels have been shown to be higher in clinical studies (306). The higher expression of ZIP8 in chondrocytes mediates greater zinc influx into the cells, which activates MTF1, thereby enhancing the expression of matrix-degrading enzymes such as MMPs that induce cartilage breakdown, and thus is implicated in the pathogenesis of osteoarthritis (212). Ectopic expression of ZIP8 in cartilage tissue causes cartilage destruction, while surgically induced osteoarthritis pathogenesis is suppressed in chondrocyte-specific conditional Zip8-KO mice (212). MiR-488 has been shown to regulate ZIP8 expression during pathogenesis of osteoarthritis (381). ZIP8 plays indispensable roles in both multiple-organ organogenesis and hematopoiesis during early embryogenesis, because hypomorphic Zip8 mice show hypoplasia of multiple organs, such as the spleen, liver, kidney, and lung, and defects in hematopoiesis (118).

The ZIP8 gene was also identified as being responsible for conferring cadmium-induced testicular toxicity in some inbred mouse strains (76). Multiple biochemical studies have revealed that ZIP8 can transport cadmium, manganese, and iron (107, 127, 183, 246). The involvement of ZIP8 in enhancing the risk of cadmium-mediated toxicity in lung tissue by cigarette smoke is suggested, because ZIP8 mRNA expression is enhanced by cadmium in an NF-κB-dependent manner and is higher in the lungs of chronic smokers (295).

I. ZIP9 (ZIPI Subfamily)

ZIP9 is the only member belonging to the ZIPI subfamily in mammals (115, 196). ZIP9 is localized to the Golgi apparatus and the cell surface (274, 409). The Golgi-located ZIP9 plays a crucial role in B-cell receptor (BCR) signaling through regulation of Akt and ERK activity by translocating zinc from the Golgi lumen (397). The cell surface ZIP9 can function as a membrane androgen receptor and contributes to testosterone-induced prostate and breast cancer apoptosis through both zinc transporter functions and androgen signaling (25, 409). The increased ZIP9 expression is reported in malignant breast and prostate biopsies (409).

J. ZIP10 (LIV-1 Subfamily)

ZIP10 functions as a cell surface zinc importer (241). ZIP10 is indispensable for B-cell development in early and mature stages (162, 285). The genetic ablation of ZIP10 in the early B-cell stage causes splenoatrophy with diminished numbers of peripheral B cells and immunoglobulin levels (285). Zinc uptake through ZIP10 is important for anti-apoptotic signaling by inhibiting caspase activity and thus is essential for early B-cell survival (285). ZIP10 is also essential in mature B-cell functions for proper humoral immune responses following BCR activation (162). The mature B cells in B-cell-specific Zip10-KO mice grow poorly because of dysregulated BCR signaling. The Zip10 deficiency in mature B cells attenuates both T-cell-dependent and -independent immune responses (162). ZIP10 mRNA is highly expressed in follicular B lymphoma (285), and higher ZIP10 mRNA expression is correlated with lymph node metastasis in clinical breast cancers (187). Thus ZIP10 is involved in invasive behavior of cancer cells (187), as observed for ZIP6 (see sect. VF).

ZIP10 transcription is regulated by cytokine signaling via the JAK/STAT pathway, which is under the control of two STAT-binding sites in the promoter (285). Moreover, ZIP10 transcription is upregulated in zinc-depleted cells (351) and attenuated in zinc excess conditions. The zinc-regulated transcription is mediated by pausing Pol II transcription by MTF-1 (241) (see sect. ID).

ZIP10 is processed by zinc starvation as observed for Z7P4 (192) (see sect. IIIB), and a similar cleavage of the extracellular NH2-terminal portion results when starved of manganese (90). The portion of ZIP10, which has a PrP-like domain, is also cleaved in prion-infected mouse brain. Intriguingly, the shedding of the NH2-terminal domain occurs in PrP in zinc-deficient conditions (90). The processing regulation of ZIP10 by the status of zinc may represent significant meaning in the spread of the prion disease.
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K. ZIP11 (gufA Subfamily)

ZIP11 is the sole member assigned to the gufA subfamily in mammals (115, 196). ZIP11 is localized to the nucleus or Golgi apparatus (210, 270), and its mRNA is abundantly expressed in testes and the digestive system (270, 456). The presence of multiple MREs upstream of the first exon of the ZIP11 gene suggests its zinc-dependent expression, but the expression is not significantly upregulated by excess zinc, compared with that of MT (270, 456). The physiological and cellular functions of ZIP11 have not been well defined.

L. ZIP12 (LIV-1 Subfamily)

ZIP12 is localized to the plasma membrane and imports extracellular zinc into the cytosol (56). ZIP12 probably has a higher affinity to zinc than other ZIP members because the apparent $K_m$ of ZIP12 is $<10$ nM (56) (see sect. IIIB). ZIP12 mRNA is specifically expressed in the central nervous system (56, 198, 293). ZIP12 has critical functions in neuronal differentiation, such as tubulin polymerization and neurite extension, by facilitating zinc uptake into the cytosol, which mediates CREB activation via phosphorylation (56).

M. ZIP13 (LIV-1 Subfamily)

ZIP13 is localized to the Golgi apparatus and cytoplasmic vesicles (32, 109, 185). ZIP13 mobilizes zinc from the lumen of these compartments and plays pivotal roles in cellular signaling, such as the BMP/TGF-β signaling pathway through regulating the nuclear translocation of Smad proteins (109) and in ER homeostatic maintenance (185).

The ZIP13 gene is responsible for spondylodysplastic Ehlers-Danlos syndrome (SCD-EDS; OMIM 612350), which is characterized by a variety of symptoms, including skeletal and connective tissue abnormalities (109, 128) (see sect. VIB). ZIP13-deficient mice show phenotypes reminiscent of those of SCD-EDS patients (109). ZIP13 mRNA expression is relatively high in these affected tissues (109). Mutant ZIP13 proteins, which cause SCD-EDS, are subject to more rapid degradation via the Valosin-containing protein-linked ubiquitin proteasome pathway (33).

N. ZIP14 (LIV-1 Subfamily)

ZIP14 has two alternative splicing isoforms, ZIP14A and ZIP14B. Both isoforms are localized to the plasma membrane and import zinc (127, 161, 251). ZIP14A and ZIP14B show exclusive apical surface localization in polarized cells (127). Cell surface ZIP14 expression is upregulated significantly by cytokines and LPS (251), which is mediated by NO production by inducible nitric oxide synthase (iNOS) (240). ZIP14 expression is also induced by the UPR stimulated by zinc deficiency via ATF6 binding to the ER stress response element (ERSE) upstream of the translational start site (163). The extreme upregulation of ZIP14 by IL-6 in the liver contributes to hypozincemia of the acute-phase response through facilitating zinc uptake (251).

ZIP14 is important for regulating systemic growth; Zip14-KO mice exhibit dwarf body sizes and impaired skeletogenesis, because ZIP14 modulates G protein-coupled receptor (GPCR)-mediated cAMP-CREB signaling through suppression of the basal PDE activity (161). The ZIP14-deficient mice also have more body fat and are hypoglycemic and hyperinsulinemic, showing enhanced insulin receptor phosphorylation and higher levels of liver glucose (22). In Zip14-KO mice, hepatocyte proliferation, following partial hepatectomy, is impaired, which indicates the importance of ZIP14 in liver regeneration (15).

Similarly to ZIP8, ZIP14 can mobilize various divalent cations, including iron, manganese, and cadmium (107, 323). The ability of ZIP14 to transport transferrin unbound iron transport (NTBI) is thought to physiologically contribute to iron homeostasis (22), because hereditary hemochromatosis protein HFE inhibits iron uptake via downregulation of ZIP14 (120). Iron deficiency promotes ZIP14 degradation through proteasomes (463).

VI. ZnT and ZIP Transporters in Integrative Physiology and Disease Pathogenesis

Growing evidence has illustrated the involvement of ZnT and ZIP transporters in the pathophysiology and pathogenesis of multifarious diseases. The elected aspects of these diseases are herein overviewed and discussed concisely. Moreover, mutations of ZnT and ZIP transporter genes, which are implicated in inherited diseases (194, 436), are also summarized. In addition, a discussion about the associations between SNPs of ZnT and ZIP transporter genes and disease traits, which have been revealed by genome-wide association studies, are presented.

A. Involvement of ZnT and ZIP Transporters in Pathophysiology

Both ZnT and ZIP transporters play a variety of pivotal roles in immune cell functions by controlling zinc signaling, and thus dysfunction of both transporters and dysregulation of their expression results in abnormal immune functions (144, 146, 156, 290). Moreover, recent literature has revealed that ZnT and ZIP transporters are involved in novel functions of zinc in immunity, “nutritional immunity” (384, 388), as in the case of transporters of other
nutritional metals such as iron (360). Here, the microbial pathogen is starved of micronutrients through spatial isolation (164). Intoxication of zinc has also been suggested to be involved in another operation of the immune system by inhibiting the growth of phagocytosed microorganisms (39, 428), where ZnT and ZIP transporters may play a role, as observed for copper transporters (445). The involvement of zinc at the host-pathogen interface is complex (271) and represents a topic that requires greater focus in the future (145).

Chronic perturbations of brain zinc homeostasis have been suggested to be closely related to the development of neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis (ALS) (73, 104, 392). The zinc levels (and other divalent cations) in the cerebrospinal fluid or in the brain of the patients are significantly altered (73, 166, 345, 392). Free zinc ($Zn^{2+}$) at a high concentration is a potent killer of neurons (217, 440). Conversely, zinc deficiency has been shown to induce the SOD1 protein to be an ALS-linked pathogenic mutant conformation (163). A number of ZnT and ZIP transporters show altered expressions at both the mRNA and protein levels in the brains of patients with neurodegenerative diseases (1, 29, 30, 36, 200, 380, 392). Thus how the alteration of their expression is involved in these diseases should be extensively investigated.

Abnormal zinc metabolism is associated with the risk of cancer pathogenesis in breast and prostate cancers (65, 74). The altered or ectopic expression of ZnT and ZIP transporters is thought to be associated with cancer progression and invasive and metastatic phenotypes in many carcinomas and cancers, and thus both transporters have received significant attention in terms of new targets for cancer therapy. Such information is briefly described in each transporter section.

**B. Genetic Diseases of Zinc Transporters and Therapeutic Approaches**

ZIP4/SLC39A4 gene mutations cause AE (8, 362) (Table 4), characterized by eczematous dermatitis, alopecia, and diarrhea (288) at an estimated frequency of $\sim$1 in 500,000 (275). The molecular basis underlying the severe dermatitis in AE patients was recently shown (204). In this study, irritant contact dermatitis through loss of Langerhans cells is revealed to cause dysregulation of ATP-
mediated inflammatory signals (204). Zinc-deficient phenotypes in AE patients can be ameliorated with a simple, daily oral zinc supplementation (1–3 mg·kg⁻¹·day⁻¹) (362). ZIP4 is now the only zinc transporter indispensable for the uptake of zinc from the intestinal lumen, but some AE mutations, which are not linked to the chromosomal region of the ZnT4/SLC39A4 gene by homozygous mapping, are present (362, 432). Thus another ZIP transporter may operate as a second zinc uptake route in the digestive tract.

TNZD is caused by low zinc levels in breast milk. The symptoms, similar to AE, only develop during breast-feeding, and do not reoccur after weaning, so termed TNZD. TNZD can be alleviated by a zinc supplement to the infant, but not to the mother, who secretes low levels of zinc in breast milk because of ZnT2/SLC30A2 gene mutations (57, 180, 231, 283). Because zinc levels in breast milk are considerably high, particularly in colostrum (47, 252), ZnT2 function is required for the secretion of large amounts of zinc (1–3 mg zinc/day) into breast milk during lactation (209).

SCD-EDS is attributed to homozygous loss-of-function mutations of the ZIP13/SLC39A13 gene (109, 128). The patient displays a variety of symptoms, including postnatal growth retardation, skeletal and connective tissue abnormalities, and hyperelastic and easily bruised skin, but these zinc deficiency-related symptoms are not alleviated by zinc supplementation, unlike the two inherited diseases described above. The accelerated degradation of SCD-EDS-causing ZIP13 mutants is associated with the disease pathogenesis, and therefore, rescue of ZIP13 protein expression by inhibiting its degradation may potentially improve the symptoms of SCD-EDS (33).

Nonsense and missense mutations of ZIP5/SLC39A5 have recently been shown to be associated with nonsyndromic high myopia (133), which may be caused in an autosomal dominant or haploinsufficient manner.

Homozygous loss-of-function mutations of the ZnT10/SLC30A10 gene cause Parkinsonism and dystonia with hypermanganeseemia, which are ameliorated by metal chelation therapy (341, 417).

**C. Single Nucleotide Polymorphism and Gene Dosage Effects of ZnT and ZIP Transporters Related to Human Disease Pathogenesis**

The SNP rs13266634 of the ZnT8/SLC30A8 gene results in a R325W substitution in ZnT8 (TABLE 5). The R allele increases the risk of type 2 diabetes (359, 365, 378, 458) because of lower zinc transport activity (297), whereas single-nucleotide variants, which cause truncation of ZnT8, decrease the risk in heterozygous individuals (102). This discrepancy requires further investigation. The SNP rs13266634 is implicated as a key determinant in humoral autoreactivity to ZnT8 (443) and is also related to a favorable cardiometabolic lipid profile in human immunodeficiency virus (HIV)/hepatitis C virus-coinfected patients (322) and skeletal muscle strength and size (383).

The SNP rs13107325 of the ZIP8/SLC39A8 gene causes an A391T substitution in ZIP8, and the T allele (8% frequency) is revealed to be associated with relatively lower circulation of high-density lipoprotein cholesterol (434). The same SNP (rs13107325) is associated with body mass index/obesity (382) and blood pressure (88) as well as and the risk of schizophrenia (46).

The SNP rs4806874 of the ZIP3/SLC39A3 gene has been shown to be associated with bipolar disorder (19). The SNP rs1871534 in the ZIP4/SLC39A4 gene results in a L372V substitution in ZIP4, and the V variant may be advantageous in Sub-Saharan Africa, because certain pathogens would be starved of zinc (97).

Much attention and interest has been paid to the SNPs of ZnT3/SLC30A3 in terms of brain functions and neurological disorders. The SNPs rs6547521, rs11126936, rs2083363, and rs11126931 of the ZnT3/SLC30A3 are shown to be associated with a gender-specific association with schizophrenia (315). The SNP rs11126936 is suggested to be associated with memory deficits (75). A rare copy number variant (duplication) of the ZnT3/SLC30A3 gene may be involved in monogenic determination of autosomal dominant, early-onset Alzheimer’s disease (350). However, this is in contrast to reports describing decreased ZnT3 expression in patients with Alzheimer’s disease (1, 34). Moreover, a rare copy number variation (deletion) of the ZIP12/SLC39A12 gene is a plausible candidate gene of the autism spectrum disorder (121). Possible genetic links to the autism spectrum are described in the SNPs of several ZnT and ZIP transporter genes (268).

**VII. ZnT and ZIP TRANSPORTER FUNCTIONS FOUND IN NONMAMMALIAN MODEL ORGANISMS**

Investigations using nonmammalian model organisms aid our efforts to understand the functions of ZnT and ZIP transporters. In particular, important evidence has been reported in Drosophila, zebrafish, and nematode, which are briefly described here. Evolutionary trees among ZnT or ZIP transporters of human and mouse, and their homologs in Drosophila, zebrafish, and nematode, are shown to clarify their relationships (FIGURE 7).

In Drosophila, many ZnT and ZIP homologs have been extensively characterized. Both dZip1 (dZip42C.1) and dZip2 on the cell surface are responsible for importing zinc...
from the lumen into the enterocyte (257, 336), and the imported zinc is released into circulation by dZnt1 (dZnT63C) and ZnT77C (CG5130, no vertebrate ZnT homologs) on the basolateral membrane (257, 336). Gut-specific silencing of dZnt1 increases lethality under zinc-deficient conditions, which indicates importance of dZnt1 function on zinc absorption (433). Zinc repletion suppresses dZip1 and dZip2 mRNA expression and also suppresses dZnt1 protein expression (336). Expression of dZnt1 (dZnT63C) and dZnT35C (possibly a ZnT2 homolog) is regulated by dMTF1 (454). FOI (273), a dZip6 and dZip10 ortholog, is essential for normal cell migration (320) and gonad morphogenesis through posttranscriptional control of DE-cadherin expression (272, 422). A Catsup mutant, in which dZip7 is defective, shows abnormally high levels of catecholamines and is probably semi-dominant lethal (386). The mutant also exhibits defects in membrane protein trafficking and elevated ER stress (129). In contrast to mammalian ZIP13, dZip13 is identified as an iron exporter to supply iron into the secretory pathway (450). Phenotypes of knockdowns or overexpression of Drosophila ZnT and ZIP transporters, and genetic interactions between them have been extensively investigated and summarized (257, 258).

In zebrafish, zZip1 is a cell surface, zinc uptake transporter (339, 340). Zebrafish Zip6 (zLiv1) is essential for gastrulation by controlling EMT under STAT3 activation (452), indicating the conserved importance of ZIP6 in organ and tissue remodeling and cancer metastasis. Zebrafish Zip7 is required for eye, brain, and skeleton formation during early embryonic development (453). Expression of zZnt1 is upregulated in the presence of excess zinc and downregulated by zinc deprivation, and zZip10 is negatively regulated through MREs (464), both of which are conserved responses in mammals. In puffer fish, pZip1 and pZip2 facilitate zinc uptake from the extracellular site as high- and low-affinity zinc transporters, respectively (339, 340). In Xenopus, xZip12 plays an important role in nervous system development, including neurulation and neuronal differentiation (56).

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP No. or Gene Dosage Effects</th>
<th>Chr. No. Region</th>
<th>Nucleotide Change</th>
<th>Effect on Protein</th>
<th>Comments</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnT3/SLC30A3</td>
<td>rs6547521 rs2083363 rs11126931 rs11126936</td>
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<td>NA</td>
<td>Gender-specific association with schizophrenia</td>
<td>315</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Memory deficits, gender-specific association with schizophrenia</td>
<td>75, 315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Early-onset of Alzheimer’s disease</td>
<td>350</td>
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<tr>
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<td>8 Exon 2</td>
<td>c.101_107del</td>
<td>K34SfsX50</td>
<td>Reduction of risk of type 2 diabetes in heterozygous individuals</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.412C&gt;T</td>
<td>R138X</td>
<td>Reduction of the risk of type 2 diabetes in heterozygous individuals</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c.973C&gt;T</td>
<td>R325W</td>
<td>Increase in the risk of type 2 diabetes, cardiometabolic lipid profile, skeletal muscle strength and size, humoral autoreactivity to ZnT8</td>
<td>322, 359, 365, 378, 383, 443, 458</td>
</tr>
<tr>
<td>ZIP3/SLC39A3</td>
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<td>NA</td>
<td>Bipolar disorder</td>
<td>19</td>
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<tr>
<td>ZIP4/SLC39A4</td>
<td>rs1871534</td>
<td>8 Exon 6</td>
<td>c.1114C&gt;G</td>
<td>L372V</td>
<td>Advantageous in Sub-Saharan Africa</td>
<td>97</td>
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<td>rs13107325</td>
<td>4 Exon 8</td>
<td>c.1171G&gt;A</td>
<td>A391T</td>
<td>Risk of schizophrenia, blood pressure, body mass index/obesity, lower circulation of high-density lipoprotein cholesterol</td>
<td>46, 88, 382, 434</td>
</tr>
<tr>
<td>ZIP12/SLC39A12</td>
<td>Copy number variant</td>
<td>10 Deletion of all exons except for exon 12</td>
<td>NA</td>
<td>NA</td>
<td>Autism spectrum disorders</td>
<td>121</td>
</tr>
</tbody>
</table>

Chr. No., chromosomal number; NA, not available.
In the nematode, the ZnT1 ortholog CDF1 is essential for conferring zinc resistance in conditions with high levels of zinc (44, 182). CDF1 positively regulates Ras-ERK signal transduction by reducing the concentration of cytosolic zinc through its zinc efflux functions (44). This evidence confirms the conserved function of ZnT1 in the regulation of the Ras-ERK pathway. The regulatory function similar to CDF1 is conducted by SUR-7 (no vertebrate ZnT homologs), which is probably localized to the ER. In homeostatic control, CDF2 (a ZnT2 homolog) or TTM-1 (no vertebrate ZnT homologs) also plays a crucial role by transporting zinc into gut granules (lysosome-related organelles) or excreting zinc from the apical plasma membrane in intestinal cells, respectively (348, 349).

**VIII. CONCLUSIONS AND FUTURE PERSPECTIVES**

The progressive understanding of ZnT and ZIP transporters has facilitated our understanding of the physiological, biochemical, and molecular roles of zinc. Concurrently, this knowledge base clearly illustrates that ZnT and ZIP transporters are relevant in many clinical fields, and understanding the function of these transporters should help in the prevention and treatment of zinc-related pathological conditions. Nonetheless, questions remain in our effort to understand the molecular mechanisms and specific activities of zinc transporters. Detailed three-dimensional structures, mechanisms of zinc transport, and regulation of zinc transport activity following specific stimuli require further investigation. Furthermore, physiological and pathological interactions with other membrane proteins require further analysis, because increasing evidence indicates that these interactions contribute to zinc metabolism and diseases relating to the dysfunction of zinc homeostasis (40, 91, 104, 194, 415). The answers to the above questions will lead to a comprehensive understanding of the temporal-spatial regulation of zinc dynamics in physiology, and the indispensable roles of zinc as structural, catalytic, and signaling components in specific proteins and reactions. Additionally, the involvement of ZnT and ZIP transporters in disease pathogenesis, including a variety of neurodegenerative diseases, clearly suggests that future discoveries will pave the way for the design of specific small compounds that can modulate zinc transport activity by ZnT and ZIP transporters, which should be of significant therapeutic benefit.

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