

Sequence Similarity and Functional Relationship Among Eukaryotic ZIP and CDF Transporters

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ZIP (ZRT/IRT-like Protein) and CDF (Cation Diffusion Facilitator) are two large metal transporter families mainly transporting zinc into and out of the cytosol. Several ZIP and CDF transporters have been characterized in mammals and various model organisms, such as yeast, nematode, fruit fly, and zebrafish, and many candidate genes have been identified by genome projects. Unexpected functions of ZIP and CDF transporters have been recently reported in some model organisms, leading to major advances in our understanding of the functions of mammalian counterparts. Here, we review the recent information on the sequence similarity and functional relationship among eukaryotic ZIP and CDF transporters obtained from the representative model organisms.

Key words: zinc transporter, ZIP, CDF, ZnT

Introduction

Zinc is an essential trace element for living organisms, because it is required for the catalytic activity of numerous metalloenzymes (1) and can also serve as a key structural component of a large number of zinc-dependent proteins (2, 3). Zinc homeostasis in the cells, therefore, is achieved through the coordinate regulation of zinc influx, efflux, and distribution to intracellular organelles (4). Zinc transporters have essential functions in such processes and a number of zinc transporters have been identified in many organisms (4–7).

Zinc transporters are largely classified into two metal transporter families, the ZIP (ZRT/IRT-like Protein) and CDF (Cation Diffusion Facilitator) families (4, 5, 7). In bacteria, the ABC transporters and P-type ATPases have been shown to function as zinc transporters (8), but neither of them plays a physiological role in zinc transport in eukaryotes (5). The ZIP and CDF families are also assigned as solute carrier 39 (SLC39A) and SLC30A families (9, 10), and both seem to have a very ancient origin because they are identified in diverse organisms from archaea and eubacteria to eukaryotes (5). ZIP family transporters function in zinc influx into the cytosol, while CDF family transporters mobilize zinc in the opposite direction. All members of both families are thought to

transport zinc across the biological membranes, but certain proteins are speculated to transport other metals such as iron, nickel, and manganese as a major substrate. In fact, ZIP and CDF transporters have been shown to transport these metals as physiologically important substrates in certain plants (11–13), and manganese not zinc is described as a more selective substrate of ZIP8/BIGM103 in the competitive assay of cadmium uptake (14).

Recently, interesting functions of ZIP and CDF transporters have been found in various organisms. A comprehensive deliberation on these functions together with integrative comparison of the sequence similarity within each ZIP and CDF transporter family would provide a clue to speculate functions of the uncharacterized ZIP and CDF proteins and to elicit further functions of the characterized ones. Here we review the physiological and cellular functions of ZIP and CDF transporters with emphasis on these matters. The plant ZIP and CDF proteins are referred to other reviews (15–17).

ZIP Transporters

Arrangement of ZIP proteins found in the genome sequences of the representative model organisms

To date, fourteen ZIP proteins have been molecularly characterized or identified in human and mouse (4, 9).

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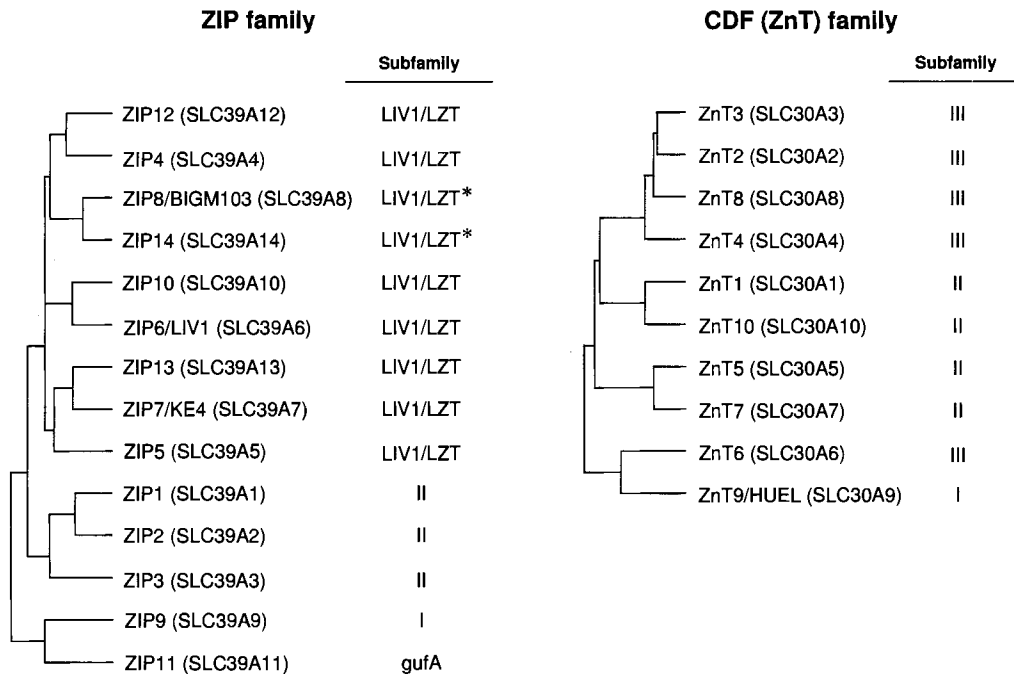


Fig. 1 A dendrogram showing the sequence similarity and the class of subfamily of human ZIP and CDF (ZnT) family members. The dendrogram was generated by using the GENETYX-MAC software, and the class of subfamily follows the assignment by Gaither and Eide (5). The class of members of the LIV1/LZT subfamily lacking the initial histidine in the HEXPHEXGD motif is indicated as LIV1/LZT* subfamily. [This figure has been modified from Kambe *et al* (4) with permission from Birkhäuser Publishing, Basel, Switzerland.]

The ZIP family is divided into subfamilies I, II, LIV1/LZT, and gufA, based on their degrees of sequence conservation (5, 18) (Figure 1). The LIV1/LZT subfamily is characterized by having a metalloprotease motif (HEXPHEXGD) around the membrane-spanning domain V. Although the initial histidine in the HEXPHEXGD motif is thought to be requisite for the zinc transport activity of LIV1/LZT transporters, ZIP8/BIGM103 and ZIP14 lacking it have zinc transport activity (19–22). ZIP proteins are predicted to have eight membrane-spanning domains with a membrane topology in which the N- and C-terminal ends are located outside the plasma membrane, and have a cytoplasmic His-rich loop between membrane-spanning domains III and IV, which is thought to function as a zinc-binding site. However, ZIP11, ZIP12, and ZIP13 lack the His-rich loop.

Table 1 shows the ZIP proteins found in the genome sequences of human, mouse, chicken, zebrafish, fruit fly, nematode, and yeast according to the similarity to the human ZIP proteins. As shown in Table 1, most LIV1/LZT proteins in the indicated organisms are arranged to each human LIV1/LZT member except in yeast, where the LIV1/LZT pro-

tein is only found as the homologous protein to ZIP7/KE4. In LIV1/LZT subfamily, ZIP12 and ZIP4, ZIP8/BIGM103 and ZIP14, ZIP10 and ZIP6/LIV1, or ZIP13 and ZIP7/KE4 are similar (Figure 1), but not completely homologous. For example, ZIP12 lacks the His-rich loop between membrane-spanning domains III and IV while ZIP4 has; ZIP13 lacks histidine residues in N-terminal portion and between membrane-spanning domains II and III, or III and IV, but ZIP7/KE4 has many histidine residues in these portions. ZIP8/BIGM103 and ZIP14, or ZIP10 and ZIP6/LIV1 have a high identity (48% or 38% identity, respectively) and are homologous in the length of amino acid sequence, the property of the His-rich loop, and the distribution of histidine residues in their sequences (19, 23). The expression of only one or the other of ZIP8/BIGM103 and ZIP14 in nematode, or ZIP10 and ZIP6/LIV1 in fruit fly and nematode, may be sufficient for the biological function, judging from the genome sequences (Table 1). Compared with LIV1/LZT subfamily, ZIPII subfamily has similar amino acid length, topology, and subcellular localization (at the plasma membrane) (4, 5, 24). Interestingly, the numbers of homologous proteins of ZIP1,

Table 1 Arrangement of ZIP Proteins Found in the Genome Sequences of the Representative Model Organisms*

	<i>H. sapiens</i>	<i>M. musculus</i>	<i>C. gallus</i>	<i>D. rerio</i>	<i>D. melanogaster</i>	<i>C. elegans</i>	<i>S. cerevisiae</i>
ZIP12 (SLC39A12) BAB70848	73 AAH89362	60 XP_418616	-	-	-	-	-
ZIP4 (SLC39A4) NP_570901	71 NP_082340	-	(66) ^a XP_691232	42 NP_648732	40 NP_503096	-	-
ZIP8/BIGM103 (SLC39A8) NP_071437	88 NP_080504	59 XP_426331	54 XP_686745	-	30 NP_499995	-	-
ZIP14 (SLC39A14) BAA06685	86 NP_659057	73 XP_427108	62 XP_696513	-	-	-	-
ZIP10 (SLC39A10) BAA86579	87 BAC65765	65 XP_426562	51 NP_956965	34 Q9VSL7 (FOI)	22 NP_872140 ^c	-	-
ZIP6/LIV1 (SLC39A6) NP_036451	94 NP_631882	64 XP_419071	44 BAD18961	-	-	-	-
ZIP13 (SLC39A13) AAH08853	87 NP_080997	67 NP_001008471	58 CAD58734	46 Q9VAF0	39 NP_509719	-	-
ZIP7/KE4 (SLC39A7) CAA20238	87 AAC69903	-	50 AAP83181	45 Q9V3A4 (Catsup)	49 (NP_510563) ^d 37 (NP_503070) ^d	31 NP_012241	-
ZIP5 (SLC39A5) NP_775867	82 NP_082368	-	37 XP_690258	-	-	-	-
ZIP1 (SLC39A1) NP_055252	94 NP_038929	-	46 AAP83180 (DrZIP1) ^b	28 CAC14873	30~20 ^e	28 NP_011259 (Zrt1) ^f	-
ZIP2 (SLC39A2) AAF35832	78 XP_139051	-	-	29 NP_650440	30~20 ^e	25 NP_013231 (Zrt2) ^f	-
ZIP3 (SLC39A3) NP_653165	83 NP_598896	75 XP_426607	-	29 CAC14874	30~20 ^e	-	-
ZIP9 (SLC39A9) BAA92100	92 XP_484158	89 NP_001007934	81 NP_001013558	48 NP_651919	49 NP_506393	27 NP_014722 (Atx2)	-
ZIP11 (SLC39A11) BAC04504	89 BAC33713	65 XP_415697	52 NP_294756	54 NP_610712	53 NP_491614	23 NP_012746 (Zrt3)	-

*The representative model organisms include: human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), zebrafish (*Danio rerio*), fruit fly (*Drosophila melanogaster*), nematode (*Caenorhabditis elegans*), and yeast (*Saccharomyces cerevisiae*). ZIP proteins in the indicated organisms are arranged according to the similarity to each human member from the homology search using NCBI BLAST server (<http://www.ncbi.nlm.nih.gov/BLAST/>) and available information. The upper number indicates the identity (%) between the sequence and the corresponding human counterpart. The lower number indicates the accession number with the name in the parentheses if known. ^aPartial sequence but shows significant similarity to ZIP4; ^bCharacterized as a high-affinity zinc transporter (58, 59); ^cHas a short N-terminal portion and does not conserve the HEXPEHXGD sequence; ^dTwo highly homologous proteins are found; ^eThe sequences include NP_493626, NP_491660, NP_496876, NP_495126, NP_500517, and NP_001026796. They are not arranged because of almost equal similarity to ZIP1, ZIP2, and ZIP3; ^fAffinity of ZIP1 and ZIP2 to zinc is unknown although Zrt1 (high affinity) and Zrt2 (low affinity) are arranged.

Table 2 Arrangement of CDF Proteins Found in the Genome Sequences of the Representative Model Organisms*

	<i>H. sapiens</i>	<i>M. musculus</i>	<i>G. gallus</i>	<i>D. rerio</i>	<i>D. melanogaster</i>	<i>C. elegans</i>	<i>S. cerevisiae</i>
ZnT3 (SLC30A3) Q99726	88 NP_035903	-	-	-	-	-	-
ZnT2 (SLC30A2) CAI17131	81 XP_131731	71 XP_423325	59 XP_691997	53 NP_609741	48 NP_510091 (T18D3.3) ^d	28 NP_014961 (Cot1) ^f	
ZnT8 (SLC30A8) AAM80562	80 NP_766404	48 XP_418398	47 XP_689129	39 NP_723732 ^c	-	-	
ZnT4 (SLC30A4) O14863	91 O35149	68 XP_413826	58 NP_956937	37 NP_610185	40 NP_499691 (Y39E4A.2) ^{d,e}	29 NP_013970 (Zrc1) ^g	
ZnT1 (SLC30A1) Q9Y6M5	83 Q60738	58 XP_419437	57 AAH63939	43 NP_647801	30 NP_509096 (CDF1)	-	
ZnT10 (SLC30A10) NP_061183	77 XP_136506	53 XP_419410	(65) ^b XP_683409	26 NP_649233	-	-	
ZnT5 (SLC30A5) AAM09099	92 AAL96438	86 AAV98201	76 AAH96996	-	39 NP_740931 (Y105E8A.3) ^d	36 NP_010491 (Msc2)	
ZnT7 (SLC30A7) AAM21969	94 NP_075703	82 AAV98202	74 AAH44151	58 NP_650049	-	-	
ZnT6 (SLC30A6) NP_060434	92 NP_659047	90 AAV53770	69 NP_991214	-	41 NP_498127 (toc-1, ZC395.3) ^d	- - - h NP_014437 (Zrg17)	
ZnT9/HUEL (SLC30A9) NP_006336	87 AAV85854	75 XP_420731	76 NP_001008575	53 NP_610806	39 NP_497603	-	
Not classified ^a							NP_013902 (Mft1) NP_015100 (Mft2)

*CDF proteins in the indicated organisms are arranged according to the similarity to each human member as described in Table 1. ^aIndicates the absence of the corresponding proteins in human ZnTs; ^bPartial sequence but shows significant similarity to ZnT10; ^cHas long N- and C-terminal portions and shows higher similarity to ZnT2 (44%); ^dSequence name indicated in parentheses is taken from Yoder *et al* (51); ^eShows almost equal similarity to ZnT2 and ZnT8; ^fMay functionally be arranged to ZnT4 orthologue; ^gShows the highest similarity to ZnT1; ^hNot found by homology search using BLASTP but functionally homologous between ZnT6 and Zrg17; ⁱShows weak similarity to yeast Mft1 and Mft2.

ZIP2, or ZIP3 found are: one in chicken and zebrafish, two in yeast, and six in nematode (Table 1), which may be linked to the fact that nematode has extra CDF proteins (see Table 2 and below). All eukaryotes have proteins with similarity to ZIP9 of ZIP1 subfamily or ZIP11 of *gufA* subfamily, which suggests that they may have retained important functions during evolution.

Interesting characteristics of ZIP transporters obtained from the model organisms

An interesting function of ZIP6/LIV1 was found in zebrafish (25). Zebrafish ZIP6/LIV1 is essential for epithelial-mesenchymal transition (EMT), which is one of the central events of embryonic development, organ and tissue remodeling, and cancer metastasis, by regulating the nuclear localization of the zinc-finger transcription factor Snail, a master regulator of EMT, because it represses the transcription of E-cadherin (25). The expression of zebrafish ZIP6/LIV1 is dependent on STAT3, which is required for the cell migration, and this characteristic is conserved in human and mouse ZIP6/LIV1 (25). As ZIP6/LIV1 was identified as an estrogen-regulated gene in breast cancer cells (26) and was shown to be significantly associated with the spread of breast cancer to the lymph nodes (27), the presented function of ZIP6/LIV1 is very interesting in that it may be a novel therapeutic target for improving tumor therapy (28).

FOI, a homologous protein of ZIP10 in fruit fly, can act as a zinc transporter (29), and is required for both germ cell ensheathment and gonad morphogenesis in order to control germ cell migration without affecting gonad cell identity (30). It controls the level of E-cadherin in the gonad that is essential for the cell-cell adhesion (31). FOI was reported as the closely related protein to ZIP6/LIV1 (30), but its sequence is the most homologous to ZIP10 (Table 1). ZIP10 and ZIP6/LIV1 are homologous in amino acid sequence (38% identity) and the property of many histidine residues in the His-rich loop or N-terminal portion, therefore they are likely to have very similar functions. Either of them may function as a backup system if expressed simultaneously.

Another LIV1/LZT protein, Catsup, a fruit fly ZIP7/KE4 orthologue, down-regulates tyrosine hydroxylase activity (32). Interestingly, IAR1, an *Arabidopsis* ZIP7/KE4 homologue, is supposed to regulate auxin conjugate hydroxylase activity by export-

ing inhibitory metal (zinc) out of the secretory pathway (33). Actually, ZIP7/KE4 is localized to the endoplasmic reticulum (ER) and the Golgi apparatus (34, 35) and transport zinc out of the Golgi apparatus (35). Since the expression of mouse ZIP7/KE4 cDNA complements the defects of *iar1* mutant (33), ZIP7/KE4 and all of its orthologues may export zinc out of the secretory compartments to fine-tune the activity of zinc-requiring enzymes and other metal-requiring enzymes like hydroxylases.

In yeast, a high-affinity zinc uptake transporter, Zrt1, is rapidly endocytosed from plasma membrane through a ubiquitin-mediated mechanism and degraded in vacuoles in response to high levels of extracellular zinc (36, 37). This type of posttranslational distribution operates in mammalian ZIP proteins; not only in the Zrt1 homologous proteins ZIP1 and ZIP3 (38, 39) but also in the LIV1/LZT protein ZIP4 more clearly (40, 41). These characteristics of ZIP transporters indicate that the traffic of ZIP proteins in response to extracellular zinc would be essential for physiological and cellular zinc homeostasis.

CDF Transporters

Arrangement of CDF proteins found in the genome sequences of the representative model organisms

To date, ten CDF proteins designated as ZnT (Zn Transporter) proteins of human or murine origin have been molecularly characterized or identified (7, 10, 42). CDF transporters are divided into three subgroups, CDF subfamilies I, II, and III, based on their sequence similarities (5). Most eukaryotic members are assigned to subfamilies II and III (5) but ZnT9/HUEL and its homologous proteins are classified into CDF subfamily I (Figure 1), which contains mostly prokaryotic members from both eubacterial and archaeal sources (5). There are sequence similarities among ZnT2, ZnT3, ZnT4, and ZnT8, between ZnT1 and ZnT10, and between ZnT5 and ZnT7 (Figure 1), which suggests that these closely related proteins have similar functions in the cells. CDF transporters have the same predicted membrane topology of six membrane-spanning domains with both N- and C-terminal ends thought to reside intracellularly and a cytoplasmic His-rich loop between membrane-spanning domains IV and V, although ZnT5 and its homologous proteins have a long N-terminal portion

with extra membrane-spanning domains (43). In ZnT6 and its orthologues, the His-rich loop is not rich in histidine residues but retains serine residues instead (44). In ZnT10 and its orthologues, the loop lacks histidine residues but bears a long loop rich in serine and basic amino acid residues (42). The His-rich loop is thought to function as a metal-binding site and is shown to have essential functions (45). ZnT9/HUEL has a cation efflux domain (pfam01545); therefore, it has been assigned to the CDF family (7, 10). However, ZnT9/HUEL and its homologous proteins have significant homology to the DNA-binding domain and the nuclear receptor interaction motif (46). Furthermore, ZnT9/HUEL is predominantly localized to the cytoplasm and translocates to the nucleus in a cell cycle-dependent manner (46). Thus, whether ZnT9/HUEL belongs to the CDF family remains open to question.

The CDF proteins found in the human, mouse, chicken, zebrafish, fruit fly, nematode, and yeast genome sequences are arranged in Table 2 according to the similarity to human ZnT proteins as in Table 1. Compared with ZIP proteins, CDF proteins in the indicated organisms are arranged to each human ZnT member except for ZnT3. ZnT3 is specifically expressed in the brain, which suggests that ZnT3 has important neural functions in mammals (47). Since the zinc transported by ZnT3 into the synaptic vesicles is implicated in β -amyloid plaque formation (48), the expression level of ZnT3 may be an important factor in the incidence of Alzheimer's disease.

The sequences of ZnT5 and ZnT6 are found simultaneously in all organisms except for fruit fly (Table 2), which is consistent with their characteristic to form hetero-oligomeric complexes (49). In fruit fly, the ZnT7 homologous protein is found (Table 2). As ZnT5 and ZnT7 have similar functions in the secretory pathway (see below and ref. 45), the expression of either ZnT5 (with ZnT6) or ZnT7 would be sufficient in fruit fly, nematode, and yeast.

Mft1 and Mft2, which were identified as mitochondrial iron transporters in yeast (50), and SUR7, which is the nematode CDF protein involved in Ras signaling (51), are not classified into human members because of low homology and different subcellular localization and functions (Table 2). Nematode has five more CDF proteins that fail to show similarity to human members (Table 2). Further investigation is needed to identify their functions and the relationship between these unclassified CDF proteins and mammalian members.

Interesting characteristics of CDF transporters obtained from the model organisms

Like the ZIP proteins, interesting CDF functions have been found in the model organisms. In yeast, Msc2 and Zrg17 form hetero-oligomeric complexes and have essential functions to maintain homeostasis in the ER by transporting zinc into the ER (52). They are counterpart proteins of ZnT5 and ZnT6, although Msc2 is homologous to ZnT5 only in the C-terminal portion including six membrane-spanning domains (43, 53) and Zrg17 is the distant homologue of ZnT6 (52). The hetero-oligomeric formation of ZnT5 and ZnT6 has been evidenced by using chicken DT40 cells deficient in ZnT5, ZnT6, and ZnT7 (49). ZnT7 is homologous to ZnT5 in cation efflux domains (pfam01545), but it fails to form hetero-oligomeric complexes with ZnT6 (unpublished data), instead, it forms homo-oligomeric complexes (49). In vertebrates, these two different zinc transport complexes, ZnT5/ZnT6 hetero-oligomeric complexes and ZnT7 homo-oligomeric complexes, operate to activate zinc-requiring enzymes like alkaline phosphatases that are synthesized and activated by binding with zinc in the secretory pathway (49). Moreover, since Msc2 and Zrg17 are involved in the unfolded protein response (UPR) because the mutant yeast strains lacking either or both of the genes are defective in the ER-associated degradation (ERAD) and show the increased UPR under low-zinc conditions (52, 54), ZnT5, ZnT6, ZnT7, and their orthologues may have such functions. In fact, zinc deficiency can up-regulate the UPR in mammalian cells (54).

CDF1, a nematode ZnT1 orthologue, positively regulates the Ras-Raf-MEK-ERK signal transduction by promoting zinc efflux and reducing the concentration of cytosolic zinc (55, 56). CDF1 binds to Raf-1 and promotes the biological and enzymatic activity of Raf-1 (57). This interaction occurs between the intracellular C-terminal tail of CDF1 and the N-terminal regulatory portion of Raf-1. ZnT1 complements all of these characteristics of CDF1 (55, 57). As the binding of ZnT1 to Raf-1 is inhibited by zinc (57), it is plausible that ZnT1 lowers the cytosolic zinc, which promotes its binding to Raf-1 and facilitates Raf-1 activation. However, it has not been elucidated whether the mammalian Ras-mediated signaling pathway is fine-tuned by ZnT1 in physiological condition.

The divergent CDF protein in nematode, SUR7, which is probably localized to the ER, also positively

regulates Ras signaling through modulating the activity of kinase suppressor of Ras (KSR; ref. 51), suggesting that other CDF proteins may regulate Ras signaling in nematode. In fact, the *toc-1* protein (ZC395.3) that shows homology to ZnT6 is reported to be involved in Ras signaling (51). The *toc-1* protein seems to have essential functions of supplying zinc to proteins in the secretory pathway by forming hetero-oligomeric complexes with the ZnT5 orthologue protein (Y105E8A.3), because the *ZnT7* gene is not found in the nematode genome sequence (Table 2). The putative hetero-oligomeric complexes may have important functions in Ras signaling in nematode.

Conclusion

Various roles of ZIP or CDF transporters have been clarified, but further studies are needed to fully elucidate their physiological functions. A comprehensive comparison of similarities and differences in the functions and regulations in transcription, translation, trafficking, and turnover of homologous proteins of ZIP and CDF among mammals and other organisms should help elucidate the true role of each transporter in zinc homeostasis. By elucidating which of the redundant transporters is the principal or the backup, and identifying which transporter forms homo-oligomeric complexes or hetero-oligomeric complexes to express zinc transport activity, we should be able to ultimately solve the intriguing question why living organisms including humans need so many zinc transporters to survive.

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