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*Review Article*

# AN OVERVIEW OF ZINC TRANSPORT IN LIVING ORGANISM

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**ABSTRACT**
This review aims at concentrating the knowledge underlying zinc uptake and transport from bacteria to plants and providing a better understanding of zinc homeostasis at a glance. Zinc (Zn) is an essential micronutrient and has particular physiological functions in all living systems, such as the maintenance of structural and functional integrity of biological membranes and facilitation of protein synthesis and gene expression. On average, one-third of the world's population, ranging from 4 to 73 % in different countries affect from Zinc deficiency. It is documented that plasma membrane built transporters not only aid in Zinc transport from the media but also act as sensor and signal the uptake of even minute quantity of this essential micronutrient thus zinc transporters act as efficient transceptors and are found in vast range of living organism ranging from bacteria to human beings. A profound research related to metal uptake in plants may allow for biotechnological improvement of plant mineral uptake and acquisition, hence meeting health related problems of plant and humans.

**KEY WORDS:** Heavy metals, Transporters, Transceptors, Zinc uptake, Zinc transport regulation

## INTRODUCTION

Heavy Metal ions such as $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Ni^{2+}$and $Mn^{2+}$ represent essential elements for plant metabolism. These ions are constituents of diverse protein molecules and are required in trace amounts, the amount of these trace elements found in soil is sometimes so small that they are barely detectable, but without them, plants fail to thrive but when present in excess, these, and non-essential metals such as $Cd^{2+,}$ $Hg^{2+}$ and $Pb^{2+}$, can become extremely toxic. The availability of mineral nutrients in the soil dramatically fluctuates in both time and space. In order to optimize their nutrition, plants need efficient sensing systems that rapidly signal the local external concentrations of the individual nutrients. The mechanisms existing to satisfy extra cellular nutrient acquisition for cellular metabolism is still not clearly defined. However the involvement of receptors / sensors in nutrient signaling pathway has been described in recent researches and a number of genes have now been identified which encode potential transporters.

### Transmembrane transport of heavy metals

A number of membrane transporters that have been implicated in metal homeostasis have been broadly categorized into following five families. The ABC (ATP Binding Cassette)family known to participate in plant iron transport although the family shows affinity with a wide range of substrates including ions, sugars, antibiotics, sugar, peptides and lipids. CDF (Cation Diffusion Facilitator) family mainly identified in bacteria, archaea and eukaryotes and are involved in transport of $Zn^{2+}$, $Co^{2+}$and $Cd^{2+}$, and a role in $Zn^{2+}$ vacuolar sequestration has been suggested for plant representatives (Hall *et al*., 2003), Nramp (Natural resistance associated macrophage proteins) family. The Nramp proteins have been involved in transport of $Fe^{2+}$, $Cu^{2+}$, $Co^{2+}$, $Mn^{2+}$ and $Cd^{2+}$ ions in prokaryotes and eukaryotes (Portony *et al*., 2000). PIB-ATPases (also referred as heavy metal ATPases) are members of the P-type ATPases superfamily, a large group of ATP-driven pumps involved in the transport of various cations across biomembranes. P-type ATPases have been identified in archaea, prokaryotes and eukaryotes, and some of them have been thoroughly studied (Toyoshima *et al*., 2002). ZIP (Zinc Regulated Transporter/Iron Regulated Transporter related Proteins) family, proteins of the ZIP family are involved in $Fe^{2+}$, $Zn^{2+}$, $Ni^{2+}$, $Mn^{2+}$ and $Cd^{2+}$ transport (Wintz *et al*., 2003)

### Transporters as nutrient sensors: Transceptors

Nutrient uptake efficiency to date has been attributed to genes encoding sensors, transcription factors, transporters, and metabolic enzymes that have been identified as potential candidates to regulate nutrient use efficiency. Transporters involved in an additional regulatory function triggered by sensing of the nutrient have been reported (Gojon *et al*., 2011). Transceptor or nutrients transporter related receptor already been discovered in yeast and other eukaryotes (Holsbeeks *et al*., 2004). Two types of transceptor are known in yeast and eukaryotes which are nontransporting homologoues of nutrients transporters as in the case of the yeast glucose sensors SnF3 and Rgt2 (Özcan *et al*., 1999) and amino acid sensor Ssy1 etc (Iraqui I *et al*., 1999). Moreover several transporter which are actively transporting nutrients have also been found to be transceptor, which are PHO84 phosphate carrier, Arabidopsis nitrate transporter NRT1.1 (Van Nuland A, *et al*., 2006) *etc*.

**Transporting transceptors**

These transceptors originally act as regular nutrient transporters that are induced during starvation for their nutrient and play a key role in rapid adaptation of cells to the presence of essential nutrients in the medium. Several examples have been described reporting to the receptor activities of transporters in *E. coli* (One copy of a duplicated gene encodes a glucose-6-phosphate (Glc6P) transporter, UhpT, whereas the other copy encodes a sensor for external Glc6P, UhpC (Schwöppe *et al*., 2003). UhpC displays low residual Glc6P transport activity and triggers induction of UhpT expression) and yeast A nutrient sensor protein with residual transport activity has been discovered in *Escherichia coli.*

Several examples supporting the sensor activity of transporters have been described to date, in *Arabidopsis*, a nitrate transporter was suggested to have a direct nitrate-sensing role in a nitrate signaling pathway affecting root architecture (Walch-Liu and Forde, 2008). Recently, a cis-acting natural antisense transcript member has been identified in rice which can regulate the PHO1; 2 expression to maintain the phosphate homeostasis. Moreover the regulation of PKA pathway in yeast cells through transporter signaling open gateways for a better understanding of transporting transeptors. Mep2 (Van Nuland *et al*., 2006) and Pho84 (Giots *et al*., 2003) a nitrogen and phosphate transporter protein respectively play a key role in activation of PKA pathway by sensing the presence and absence of nutrient in the media. In these cases, nutrients exert a hormone-like effect similar to the hormone-like stimulation of cAMP synthesis by glucose in glucose-deprived yeast cells which is triggered by the glucose-sensing G protein–coupled receptor (GPCR) Gpr1 (Kraakman *et al*., 1999**).**

Evidence for transporters functioning as transceptors has also been obtained in other systems. The yeast Mep2 ammonium transporter and homologues in other fungi, for example, *Ustilago maydis* and *Candida albicans*, have been implicated in control of filamentation (Pan *et al*., 2000; Smith *et al*., 2003; Biswas and Morschhäuser 2005). A transceptor function has also been suggested for the SNAT2 amino acid transporter in mammalian cells (Hyde *et al*., 2007).Also in animal and plant cells, evidence has been found for the existence of transceptors (*Holsbeeks et al*., 2004; Hundal and Taylor 2009; Taylor 2009). In *Drosophila*, two amino acid transporters were found to affect growth independent of their amino acid transport function (Goberdhan *et al*., 2005).

**Nontransporting transceptors**

These transceptors are nutrient receptors with a transporter-like sequence and structure but without transport capacity, and G protein–coupled receptors (GPCRs) that have nutrients as ligands. Recent results have revealed that transceptor signaling requires a specific ligand-induced conformational change, which indicates that transceptors function in a similar way as regular receptors as revealed from bioinformatic analysis for Rgt2 as the closest homologue of the glucose-sensing GPCR Gpr1 in yeast (Thevelein, 2009). The receptor property of transceptors can be attributed to the nontransported signaling agonists that were able to trigger the signaling function of the transceptor: L-Leu-Gly for Gap1 (Van Zeebroeck *et al*., 2009), glycerol-3-phosphate and other organic phosphate esters for Pho84 (Popova *et al*., 2010) and D-glucosamine 2-sulfate for Sul1, 2. In addition, the compounds turned out to be interesting new tools for studying the signaling function of the transceptors independently of their transport function, but have also turned out to be powerful tools to investigate other outstanding questions with respect to transporter functionality and regulation (Joep Schothorst *et al*., 2013).

**Molecular mechanism underlying zinc uptake through ZIP transporter family**

Zinc transporters typically act as zinc sensors, responding to zinc availability to maintain intracellular zinc homeostasis. The mode in which cellular homeostasis is achieved through zinc and zinc transporters are complex and comprehensive. Zinc transport from extracellular compartment is mediated by members of ZIP family that transport zinc into the cytosol (Pence *et al.*, 2000). Members of ZIP family are upregulated in roots under zinc-deficient conditions, suggesting that they participate in zinc uptake (Palmer and Guerinot, 2009). However, so far it is unknown which member plays the dominant role in zinc transport.

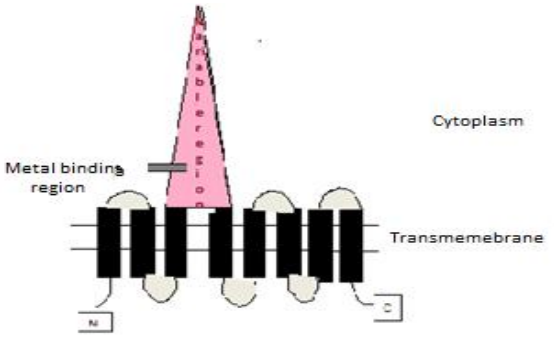


**FIGURE 1:** General topology of ZIP transporters

ZIP transporters were first identified as "Zrt-, Irt-like Proteins" following their identification in yeast *Saccharomyces cerevisiae* (Zrt; zinc regulated transporter) and their similarity to the Fe (II) transporter Irt1 protein from the plant *Arabidopsis thaliana* [44, 45]. High affinity Zn uptqake was seen concomitant to over expression of

Zrt1 in yeast cells and was reduced subsequently in Zrt1 mutant cells resulting in poor growth in zinc-limited media (Zhao and Eide, 1997).

Most ZIP transporters are predicted to have six to eight transmembrane (TMD) domains. Many of the family members have a long loop region between TMD III and IV that frequently contains a histidine-rich region that is suggested to be a putative zinc-binding domain (Fig-1). Usually the sixth TM has a CPC, CPS, SPC, TPC or CPH ("xPx sequence") upstream the phosphorylation site (DKTGT) in prokaryotes.

The majority of ZIP proteins share a similar predicted topology where both the N and C-termini are extra cytoplasmic. Most ZIP proteins range from 309 to 476 amino acids in length, containing a variable region between transmembrane domains III and IV of varying lengths. The most conserved portion of the ZIP family proteins occurs in transmembrane domain IV, which is predicted to form an amphipathic helix with fully conserved histidine residues. A detailed experiment by Brookhaven team suggest an auto-regulatory mechanism for zinc transport owing to conformational changes in the portion of protein traversing cellular memebrane caused by binding of Zinc within the cell triggering hinge-like movements of two electrically repulsive portions of the protein that lie within the cell's interior.. So when zinc levels inside the cell rise too high, this shape shifting somehow pushes zinc ions through the membrane and out of the cell thus regulating zinc uptake. Although mechanism underlying zinc transport is not known exactly but the involvement of transcription factors of the basic-region leucine zipper (bZIP) family: bZIP19 and bZIP23 have been documented in ZIP4 in *Arabidopsis Thaliana*.(Assunção *et al*., 2010a). These transcription factors bind to a ZDRE (zinc deficiency response element), present in the upstream region of *ZIP4* and other members of ZIP family namely *ZIP1*, *ZIP3*, *ZIP9* and *IRT3* giving ample evidence in support of similar regulation for these ZIP transporters. Post transcriptional regulation of transcription factors of the bZIPfamily have been studied in other regulatory networks as well. In general, bZIP transcription factors (in particular bZIP19 and bZIP23) are known to dimerize (Jakoby *et al*., 2002) forming homodimers (Assunçãoet al., 2010a), although formation of heterodimers have also been reported (Fig 2)

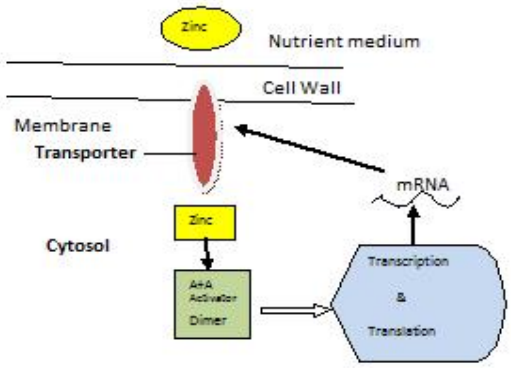


**FIGURE 2:** Diagrammatic model of Zinc uptake and regulation

A key feature of ZIP transporters is that they facilitate the influx of zinc into the cytosol from the extracellular space and from the lumen of intracellular compartments into the cytoplasm. Not only transport but recent discovery also highlights the identification of transporters that modulate the nutrient distribution in the aerial portion of plants. OsHMA2 was first shown to function in the root to shoot translocation of Zn in rice (Satoh-Nagasawa *et al*., 2012), but is also expressed in the phloem of the vascular bundles and functions in the preferential distribution of Zn into sink organs during the reproductive stage of rice (Yamaji *et al*., 2013b).

Many trasporters are involved in regulation of influx and efflux pathway of Zinc uptake. The transporters involved in efflux of Zinc from the cells are responsible for maintenance of zinc concentrations to prevent toxicity and aids in hyper accumulation of the micronutrient into the cells. The efflux pathway transporters HMA2 and HMA4 are known to transport Zinc from root to shoot (Hanikenne *et al*., 2008). In Arabidopsis halleri, a Zinc hyper accumulator species the same ZIP transporters are found as non hyper accumulators, but different HMA4 genes. Moreover, studies in different plant species have shown that hyperaccumulators possess multiple copies of *HMA4* in their genome that results in higher expression levels and more efficient root-to-shoot transport of zinc (Ó Lochlainn *et al*., 2011).

Apart from yeasts, ZIP family of proteins has grown to more than 100 members including those from insects, bacteria, nematodes and mammals. In mammals, members of this family are designated SLC39 (Zip) and SLC30 (ZnT) solute carriers. ZnT and Zip family zinc transporters have opposing roles in regulating cellular zinc homeostasis; ZnT transporters reduce cytosolic zinc bioavailability by promoting zinc efflux and Zip transporters function by increasing cytosolic zinc. (Carmen p wong, 2014). Methylation of the SLC30A5 promoter region contributes to the age-related decline in Zn status in mice (Karweina *et al*., 2015).

**Zinc transport in prokaryotes**

A large number of enzymes and proteins such as alkaline phosphatase, RNA polymerase, aspartate trans carbamylase, FtsH ($Zn^{2+}$-dependent protease) and zinc

finger proteins, playing very diverse functions contain zinc as a structural or catalytic cofactor. In many bacteria zinc export systems have been identified that were found to be essential for competence and supply of micronutrients (Dinthilhac *et al*., 1997 and Lu *et al.,* 1997). Many bacteria use an ABC transporter for high-affinity uptake of zinc with a cluster 9 solute-binding protein however low-affinity ZIP-type zinc transporters in bacteria have also been identified. In *E. coli*, ZnuABC a high-affinity periplasmic-binding protein-dependent transport system has been identified (Dinthilhac *et al*., 1997) for $Zn^{2+}$ in *E. coli.* Manganese and zinc binding protein-dependent transport system were described in *Streptococcus pneumoniae* **(**Dinthilhac *et al*., 1997). The ORFs for the three zinc transporter genes of *S. pneumoniae adcABC* have 22% to 36% identity to the corresponding Znu proteins. From the same transporter family, a $Zn^{2+}$-binding protein, Pzp1, in *Haemophilus influenzae* was described **(**Lu *et al*., 1997). Structural studies of ABC transporters (Fig 3a) showed the presence of two transmembrane domains (TMDs) form the ligand binding sites and provide specificity, and two NBDs bind and hydrolyze ATP to drive the translocation of the bound ligand. These NBDs are homologous throughout the family (Kenneth J linton, 2007). Most high-affinity zinc uptake systems are regulated by Zur proteins, which form at least three unrelated subgroups of the Fur protein family (regulators of iron transport). In *Bacillus subtilis*, peroxide induced zinc transporter ZosA play a key role in zinc uptake. ZosA functions together with two Zur-regulated uptake systems and one known efflux system to maintain Zn (II) homeostasis. Low affinity zinc uptake has been studied in *Escherichia coli wherein zupT* (*ygiE*), encoding a ZIP family member, mediated zinc uptake mechanism is present.

Recent studies also revealed the role of cation diffusion facilitator family, CDF in zinc homeostasis. CzcD from *Ralstonia metallidurans* and ZitB from *Escherichia coli* are prototypes of bacterial members of the cation diffusion facilitator (CDF) protein family (Anton *et al*., 2004). These proteins are secondary carriers which utilize the pmf and function by H antiport (for metal efflux). One member, CzcD of *Bacillus subtilis***,** has been shown to exchange the divalent cation ($Zn^{2+}$ or $Cd^{2+}$) for two monovalent cations ($K^+$ and $H^+$).

**Zinc transport in Eukaryotes**

Zinc transport mechanism is totally different in eukaryotes as compared to prokaryotes where neither ABC type nor P type zinc transporter is present rather two different transport systems namely ZIP and CDF families are implicated in zinc uptake and transport. The members of CDF family in Eukaryotes totally differ in structure and phylogeny but have similar mechanism of function. Members of the Zrt and Irt protein (ZIP) family are a central participant in transition metal homeostasis as they function to increase the cytosolic concentration of zinc and/or iron. The Zip families of transporters are highly conserved in nature and operate in wide range of organism ranging from fungi to humans. In eukaryotes Zinc transport mechanism was first studied in in *S. cerevisiae* and revealed the central role of the ZIP (Zrt-, Irt-like protein) family of zinc transporters (Eide, 2006). The name of this family, also known as SLC39 (solute carrier 39), refers to the first members to be functionally characterized, the *S. cerevisiae* zinc transporters Zrt1 and Zrt2 and the *Arabidopsis thaliana* iron transporter Irt1 (Eide, 2004). The transcriptional responses to zinc deprivation of fungal cells are regulated by the Zap1 transcription factor. The first characterization of the role of Zap1 in regulating zinc homeostasis was performed in *S. cerevisiae* (Zhao and Eide, 1997). In nickel hyper accumulator plant *Thlaspi japonicum* a histidine-rich region present between transmembrane regions three and four of TjZNT1 ZIP transporter was necessary for zinc selectivity (Nishida *et al*., 2008). In plants, we also have evidence that expression of the zinc transporters is metal responsive. ZIP1, ZIP3, and ZIP4 mRNAs are all induced in zinc-limited plants. Furthermore, ZIP1 shows zinc-induced inactivation when expressed in yeast.

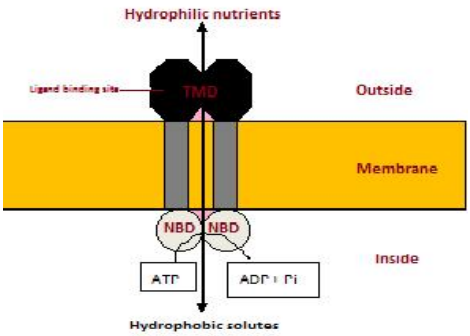


**Figure 3 a**

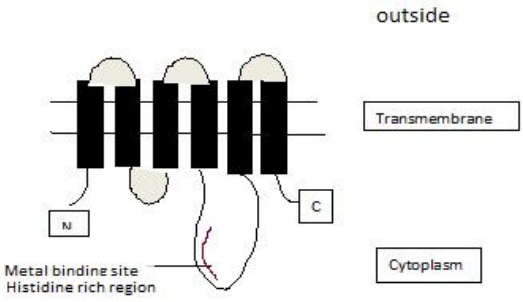


**Figure3 b**

Predicted topology of Zinc transporters: Figure 3a; ABC transporters, Figure 3b; CDF transporter family.

The CDF (Fig3b) family designation stands for "cation diffusion facilitator" Znt/SLC30 (in mammals). Cation diffusion facilitators (CDF) are part of a highly conserved protein family that maintains cellular divalent cation homeostasis in all domains of life The key feature of this family is that they transport zinc and/or other metal ions from the cytoplasm into the lumen of intracellular organelles or to the outside of the cell. Thus, CDF proteins work in opposition to the ZIP transporters. Most members of the CDF family possess six putative transmembrane

spanners with N- and C-termini on the cytoplasmic side of the membrane, but MSC2 of *S. cerevisiae* (TC #2.A.4.4.1) and Znt5 and hZTL1 (2.A.4.4.3) of *H. sapiens* exhibit 15 and 12 putative TMSs, respectively (Cragg *et al*., 2002). These proteins exhibit an unusual degree of sequence divergence and size variation (300-750 residues).

**Summary and Future prospect**

Several strategies are being used to enhance zinc micronutrients in edible plant tissues for overcoming the harmful effects of zinc deficiency. Continuing research in the field has made it increasingly evident that maintaining Zn homeostasis in plants requires tightly controlled mechanisms. This phenomenon is likely to provide great advantages for plants to cope with the fluctuations in the availability of Zn in various environments and stress conditions. Although heavy metal ions are essential components of a variety of enzymes, transcription factors and other proteins, the mechanisms that contribute to metal ion homeostasis are only starting to be elucidated in higher plants. Research findings over a decade have led to the identification of several components from the membrane to the cytoplasm and to the nucleus that are involved in Zn sensing, transport and downstream response. Any particular metal will have two or more relatively specific transport systems categorized into high-affinity systems that are active in metal limiting conditions and low-affinity systems that function when substrates are more abundant. We already know that the metal are sensed and taken up by plant roots from soil and redistributed to various compartments and hence, manipulation of the mechanisms by which plants take up, extrude and compartmentalize heavy metals, and also redistribute them around the plant, can enhance significant biotechnological applications which can be exploited, both in terms of human nutrition and phytoremediation. Heavy metal transport is a very exciting and developing field in plant biology and we are poised at the discovery of a range of new ion transporters that will undoubtedly change our concepts of metal nutrient acquisition and homeostasis in higher plants.

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