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Review

Cation Diffusion Facilitator family: Structure and function

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ABSTRACT

The Cation Diffusion Facilitators (CDFs) form a family of membrane-bound proteins capable of transporting zinc and other heavy metal ions. Involved in metal tolerance/resistance by efflux of ions, CDF proteins share a two-modular architecture consisting of a transmembrane domain (TMD) and C-terminal domain (CTD) that protrudes into the cytoplasm. Discovery of a Zn^{2+} and Cd^{2+} CDF transporter from a marine bacterium *Maricaulis maris* that does not possess the CTD questions current perceptions regarding this family of proteins. This article describes a new, CTD-lacking subfamily of CDFs and our current knowledge about this family of proteins in the view of these findings.

1. Divalent metal cations and the importance of their transport

Many heavy metal ions constitute essential trace elements (known as micronutrients) in many biological systems. Such elements perform vital biological functions at low levels but can cause toxic effects at higher cellular concentrations. Consequently, living organisms have evolved transport mechanisms for the active uptake and/or extrusion of these ions in order to control their intracellular levels [94]. Essential trace elements commonly found in cells include zinc, cobalt, iron, manganese and copper and although other heavy metal ions are found in cells they have yet to have clear physiological functions defined. Focusing on the known essential trace elements, cobalt is required in vitamin B12 and other cobalamins [112] while manganese is essential in many enzymes and is necessary for glucose metabolism [91]. Essential in the electron transport chain, iron and copper are found at the center of cytochromes and in the active site of cytochrome *c* oxidases, respectively [48,109]. Many enzymes contain zinc in the active center or in other structurally important sites. In addition to enzyme catalysis, zinc has also been shown to be crucial for cell growth, development and differentiation by contributing to processes such as gene expression, DNA synthesis, hormone storage and release, neurotransmission, memory and apoptosis [4]. In humans, zinc has been found to be required for the function of various bone growth hormones such as testosterone and thyroid hormones, and indeed other vital hormones, including insulin [8].

Four major families of zinc transporting proteins have been identified: (i) RND (Resistance, Nodulation and Division) multidrug efflux transporters, (ii) P-type ATPases (iii) ZIP (ZRT, IRT-like Protein) transporters and (iv) CDF (Cation Diffusion Facilitator) transporters. While the RND type transporters are only found in a few Gram-negative bacteria [37], the zinc transporting ATPases are found widely in bacteria and plants [108,104]. On the other hand ZIPs are mammalian transporters [47] and the CDFs build an ubiquitous family of proteins found in all major phyla of living organisms [74]. The latter have evolved a strong preference for the trafficking of zinc ions in many biological systems. In humans, zinc homeostasis is mediated mainly by two zinc transporter families, the zinc import proteins (ZIP/Slc39) and CDF proteins for zinc export (ZnT/Slc30). To date, 14 ZIP and 10 ZnT proteins have been identified [24]. The disturbed zinc homeostasis often caused by a mutation in one or more zinc transporters has been associated

Abbreviations: CDF, Cation Diffusion Facilitator; TMD, transmembrane domain; CTD, C-terminal domain; ITC, isothermal titration calorimetry; SEC-MALS, size exclusion chromatography multi-angle light scattering; IL2, intracellular loop 2; ZnT, zinc transporter; RND, resistance nodulation and division; SlC, solute carrier; Zrc, zinc resistance conferring; EL, extracellular loop; SAXS, small angle X-ray scattering; MD, Molecular Dynamics

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with a number of diseases such as neonatal zinc deficiency, Alzheimers Disease, diabetes and prostate cancer. The pathophysiological roles of ZnT (Zinc Transporter) proteins have been extensively reviewed recently in [42].

This review will summarize the current knowledge of the CDF transporters in the view of the recently described subfamily of CDFs that lack the C-terminal cytoplasmic domain (CTD).

2. CDFs – more than heavy metal efflux proteins

The CDF family of transporters was first described in the late 1990s and its first characterized member, CzcD, shown to play a role in heavy metal resistance in *Cupriavidus metallidurans* [73,80] (formerly *Alcaligenes eutrophus*, *Ralstonia metallidurans*, *Wautersia metallidurans* strain CH34 [31]). Members of this ubiquitous family of proteins can be found in bacteria, archaea, and eukaryotes [74]. Although initially believed to just transport Zn^{2+}, Cd^{2+} and Co^{2+}, CDFs have been shown to mediate the transport of other divalent metal ions including Mn^{2+}, Ni^{2+} and Fe^{2+} [72,87,18,83], though the human members of this family, the ZnT proteins, mainly transport Zn^{2+}. Plant CDFs are commonly called MTPs (Metal Tolerance Proteins) and have primarily been characterized as Mn^{2+} transport proteins. There are a number of exceptions in terms of metal specificity within the CDF family. CzcD from *Bacillus subtilis* is reported to play a role in Cu^{2+} tolerance and PbtF from *Achromobacter xylosoxidans* A8 reported to play a role in Pb^{2+} efflux, however more characterization is needed in both cases [71,39].

Based on early phylogenetic analysis, the CDF family was divided into three major groups according to metal ion specificity: (1) Mn^{2+}-transporting CDFs, (2) Fe^{2+}/Zn^{2+}-transporting CDFs and (3) CDFs transporting Zn^{2+} and other metal ions but not Fe^{2+} or Mn^{2+} [70]. More recent phylogenomic analysis of the CDF family has seen substrate defined clades provided for Ni^{2+}, Cd^{2+} and Co^{2+}. While 7 out of 18 identified clades (6 of Zn^{2+} and 1 of Mn^{2+} specificity) agree with the previous system, the Fe^{2+}/Zn^{2+} group was separated into 5 independent clades with Zn^{2+}/Cd^{2+}, Co^{2+}/Ni^{2+}, Fe^{2+} and $Zn^{2+}/Cd^{2+}/Fe^{2+}/Mn^{2+}$ specificities defined. The new phylogeny for CDFs contains 18 clades including 13 clades with at least one characterized CDF and 5 clades containing only uncharacterized CDFs. Zn^{2+} transporting CDFs are present in 8 of these clades making Zn^{2+} transport polyphyletic. This new grouping of CDFs into defined clades also suggests that Mn^{2+} transport, via CDFs, in eukaryotes and prokaryotes is polyphyletic [17].

Bacterial CDFs are primarily involved in metal tolerance/resistance and homeostasis by efflux of divalent metal cations from the cell. This has been shown for bacteria such as *B. subtilis* [97,103], *Staphylococcus aureus* [111,53], *Escherichia coli* [29,30], *Thermus thermophilus* [96], *Corynebacterium glutamicum* [100], *Deinococcus radiodurans* [98] and *C. metallidurans* [1]. In addition, it has been suggested that bacterial CDFs may be capable of other activity such as mediating antibiotic resistance as is the case of CepA of *Klebsiella pneumoniae* that has been linked to chlorhexidine resistance [23], while two CDFs, MamB and MamM from *Magnetospirillum gryphiswaldense* were recently shown to be involved in magnetosome formation [101]. Interestingly, MamM has been used as a platform for studying CDF-related type II diabetes because of the ease of measurement of its magnetism-related phenotypes [114].

Eukaryotic CDFs are known to have an additional function as intracellular transporters of their substrates. A CDF, termed MSC2, from *Saccharomyces cerevisiae* mediates specific export of ions from the nucleus to the cytoplasm [58], while another two CDFs from *S. cerevisiae*, MMT1 and MMT2, have been suggested to function as mitochondrial Fe^{2+} exporters [57] with evidence of this export being recently reported [59]. Two further CDFs from the yeast, Zrc1 and Cot1, are responsible for the transport of Zn^{2+} into the vacuole for storage, detoxification and re-use [65,66,69]. A similar function was reported for CDFs from the plants *Arabidopsis thaliana* and *Oryzasativa* L. [52,19,54,68].

Among human CDFs, only ZnT1 is found uniquely in the cell membrane and functions as a Zn^{2+} ions exporter to the extracellular space [113,82]. Other ZnT transporters are localized in the membrane of intracellular organelles to sequester cytoplasmic zinc into various cell compartments such as lysosomes (Znt2; [22]) endosomal/secretory vesicles (ZnT2 and Znt4; [61,78]), synaptic vesicles (Znt3; [14,79]), Golgi apparatus and cytoplasmic vesicles (Znt5, Znt6, Znt7; [51,46,43]) or secretory granules (Znt8; [13]). CDF-2 found in *Caenorhabditis elegans* was shown to transport zinc ions into gut granules whilst TTM-1B, also from *C. elegans*, promoted excretion of zinc ions from intestinal cells into the intestinal lumen [85,86]. Certain CDFs have also been shown to function when expressed in other plant or yeast model hosts. The cucumber CDF, CsMTP8, can increase Mn^{2+} tolerance in both *S. cerevisiae* and *A. thaliana*. In the yeast it localizes to the vacuolar membrane whilst in *A. thaliana* it is localized to the protoplast. Similarly, OsMTP8.1, a rice plant CDF, and MTP10 and MTP11 from *Beta vulgaris* ssp. *maritima* increases Mn^{2+} tolerance when expressed in *S. cerevisiae* [11,21]. CDF proteins may also function as divalent metal importers when the metal concentration in the cytoplasm is too low. Isoforms of ZnT2 [61] and ZnT5 [102] localized in the plasma membrane were shown to function in both the import and efflux of zinc across the membrane. Similarly, ZAT1p from *A. thaliana* was found to function as an uptake system in certain situations [7]. More recently, Sll1263, a CDF from the cyanobacterium *Synechocystis* sp. strain PCC 6803, has been reported to be involved with the import of Fe^{2+} rather than its efflux, possibly due to the low-iron habitats it is associated with the high iron requirements inherent to cyanobacteria [45].

3. Current understanding of signature motifs and metal specificities

Although relatively little is known about the metal specificity of the CDF transporters, there is some evidence for varying sites which can control and influence metal specificity in CDFs. These studies are primarily based on whole cell functional assays and/or bioinformatics studies. Whilst much of this data has not been validated with more robust in vitro assays, such as reconstitution of purified CDFs into proteoliposomes, the current literature proposes several CDF associated sites of interest. The majority of reports in this regard are focused on plant CDFs, mainly due to the possible role of tailored transporters in biofortification. Bioinformatics studies indicate that the metal specificity of CDFs may reside in the cytoplasmic domain [6] while other studies report that a His-rich loop, known as IL2 (intracellular loop 2), is responsible for metal selectivity [49,81,92]. In AtMTP1 from *A. thaliana* the sequence within IL2 that restricts the protein to solely Zn^{2+} transport was reduced to the five N-terminal residues of said IL2 [81]. However, residues outside of the cytoplasmic domain and His-rich loop regions have also been shown to influence metal specificity of CDFs which hampers efforts to define a common motif for metal selectivity (Fig. 1). Mutation of two residues (L87H and E97G) within transmembrane helix 3 (TM3) of the *S. cerevisiae* Zrc1 transporter were found to change metal selectivity completely from Zn^{2+} to Fe^{2+} and Mn^{2+} [60]. Interestingly, homologous residues within TM3 of AtMTP1 (I135F and E145G) were also found to influence metal selectivity, possibly through conformational changes induced at the active site or at the CTD [81]. Homologous residues in YiiP have been shown to be part of an