Membrane transport proteins in metal toxicity and defense

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1 Abstract

Membrane transport proteins are essential components in cellular metal homeostasis, playing a significant role in both metal uptake and detoxification. Although metals like Copper, Zinc and Iron are crucial for various cellular functions, their excess can lead to toxicity via oxidative stress and disruption of specific biochemical pathways. This review discusses how membrane transport proteins mediate metal toxicity and resistance, focusing on key proteins like HMT1, ABC-type vacuolar transporters and P1B-type ATPases. These proteins facilitate the detoxification of heavy metals by sequestering in intracellular compartments or expelling them from the cell, thus maintaining metal balance. We highlight their structural characteristics, the mechanisms that underly their transport activity, and their importance in defending against metal-induced cellular damage.

2 Introduction

Metal homeostasis plays a crucial part in most of the known organisms. Whether a metal is detrimental or beneficial can be determined by their effect on the cell, but the consensus is that cells require metals in specific concentrations to function. Eukaryotes use metals in multiple configurations such as cofactors in plants [1] and hemoglobin in humans. Zinc as a redox neutral trace nutrient is indispensable for life as a structural, catalytic and signaling compound further proving the importance of metals [2]. Some heavy metals however have been shown to negatively affect cellular organelles and components such as the cell membrane, lysosomes, enzymes and the DNA among many [3]. Their toxicity resides in the ability to generate oxidative stress, to promote conformational changes that lead to the disruption of cell respiration, degradation of cellular structures and in acute cases carcinogenesis and apoptosis. Moreover, essential metals can also induce toxic effects in the cell as essential heavy metals in high or extreme concentrations adversely affect water homeostasis and tip the balance of Calcium Magnesium and Kalium uptake [4].

As physiologist Walter B. Cannon articulated, "Homeostasis does not occur by chance, but is the result of organized self-government." [5] This principle highlights the criticality of maintaining (metal) homeostasis to ensure proper cellular function. To abide by this statement, cells use different methods while monitoring both intra- and extracellular circumstances. Although phytochelatins, metallothionines, and other components aid the sequestering and binding of

metals, protein transporters play one of the most significant roles in metal uptake and toxicity [6]. Transport membrane proteins provide the highest level of tolerance against metal toxicity as metal or metalloid removal from the cytosol is the most effective detoxification method. Transporters on the cell membrane expel excess and import essential metals through the cell membrane while transport proteins on the vacuolar membrane facilitates compartmentalization [7]. Depending on whether the transport membrane protein is importing or exporting metals from the cytosol, they are called influx or efflux transporters respectively.

2.1 Influx Transporters

[8] Metal import mainly appears in influx transporters which are a large group of complexes that play a prominent role in metal uptake. A smaller group of them are the transport membrane proteins with influx affecting capabilities of which the ZRT-, IRT-Like Proteins (ZIP) and the Natural Resistance-Associated Macrophage Proteins (NRAMP) have the highest effect in higher eukaryotes. The ZIP family consists of proteins that are about 400 amino acids in length and consists of 8 domains. They have representatives in all eukaryotic kingdoms and have an important role in the passive transportation of different metal ions like Zinc, Iron, Manganese and cadmium [9]. The NRAMP metal ion transporters are consisting of 12 trans membranal domains and can be found in multiple organisms ranging from bacteria to humans. They are general metal ion transporter in the sense that they show little specificity towards substrates therefore the NRAMPs are able to transport Manganese, Zinc, Copper, Iron, Cadmium, Nickel and Cobalt cations [10].

2.2 Efflux Transporters

Efflux transporters like ATP-binding cassettes (ABC), Cation Diffusion Facilitators (CDF) and P-type ATPases are the first and major line of defense against metal toxicity. Efflux transport membrane proteins are located on the vacuoles' and on the cell's membrane, promoting sequestration and expelling excess metal ions. ABC transporters have a dual role as members of the family are located on both the vacuolar membrane where they act as sequesters and on the cell membrane where they take part in metal uptake [11]. The CDF family has many different members although most of them are found in the vacuolar and cell membrane. They have a strict role in different metal expulsion however they show an increased efficiency therefore play a key role in

Zinc transport [12]. Finally, the P-type ATPases most importantly the P1B-type ATPases function as an efflux transporter that effects transition metals like Copper, Zinc and Manganese with 8 trans membranal domains at play [13].

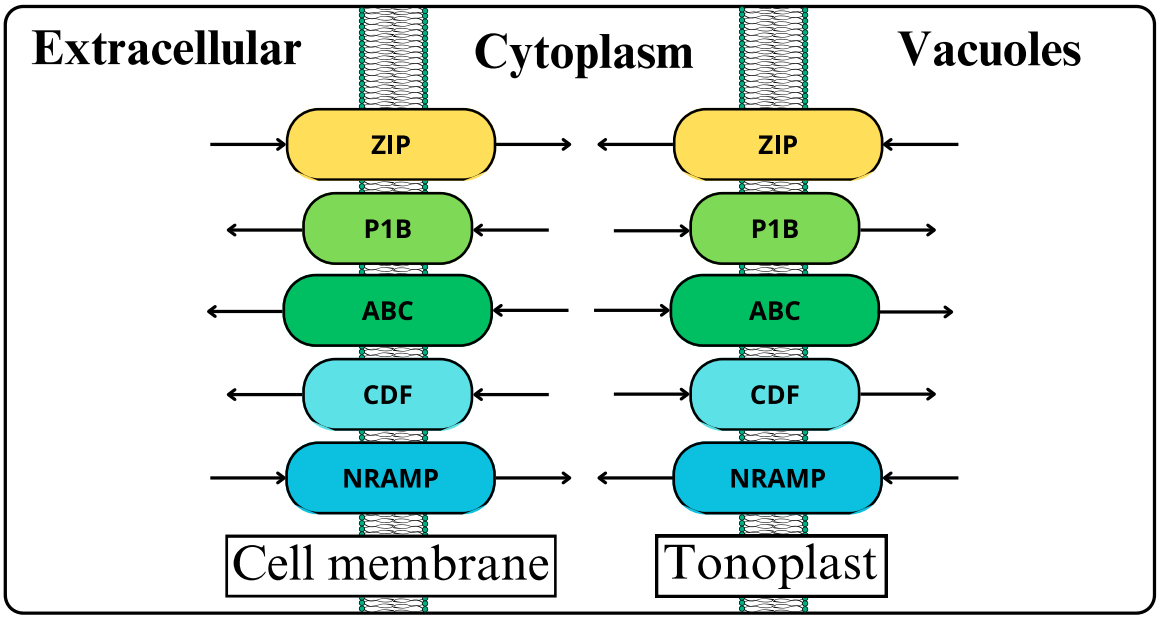

Figure 1. Transport membrane proteins in the cell membrane and tonoplast of vacuoles. The ZIP and NRAMP families act as influx transporters to the cytosol while the P1B-type ATPases, the ABC and the CDF family exports metals out of the cytosol. The mechanism of transport differs in each transport protein, but the main principles can overlap. Some membrane transport proteins form passive channels (Yellow), others function as primary transporters using ATP (Green), while secondary transport systems are also present with their proton gradient fueled transport (Blue).

3 Results

The three reviewed articles' findings reveal the distinct mechanisms behind the metallic defense of membrane transport proteins. The first study focuses mainly on the HMT1 transporter in Schizosaccharomyces pombe, an ABC-type protein involved in the vacuolar sequestration of cadmium through the formation of phytochelatin-metal complexes. This study demonstrated that overexpression of HMT1 enhances cadmium tolerance by increasing intracellular sequestration

[14]. The second article presents the P1B-type ATPase, particularly a copper-transporting ATPase, revealing its structural components and copper transport pathway using X-ray crystallography. This transporter expels toxic amounts of Copper ions from the cytoplasm, with a mechanism tightly linked to ATP hydrolysis [15]. Finally, the third article investigates metal tolerance in yeast using genomic phenotyping, identifying membrane transporters that differentially affect resistance to cadmium and nickel. This study highlights the roles in a number of vacuolar and plasma membrane transporters in detoxification, pointing out that the vacuole is a central organelle for metal tolerance [16].

4 Discussion

Membrane transporters are vital players in the delicate balance of metal homeostasis. Metals such as copper, zinc, and cadmium are essential for cellular functions, yet toxic in excess. The role of transport proteins in mitigating this toxicity cannot be understated, as they are responsible for maintaining appropriate intracellular metal concentrations. These transporters act as gatekeepers, controlling both the import of necessary metals and the export or sequestration of toxic excess. In this discussion, I focus on two key players: HMT1, an ABC-type transporter, and P1B-type ATPases, a family of efflux transporters. I will also touch on how genomic studies in yeast have further elucidated their importance.

4.1 The discovery of HMT1 and its function

One of the first important membrane transporters involved in heavy metal detoxification to be discovered was HMT1 in *Schizosaccharomyces pombe*. HMT1, found in a cadmium-sensitive mutant of fission yeast, was shown to be localized to the vacuolar membrane and functions by sequestering cadmium in the form of phytochelatin-bound complexes. The discovery of HMT1 was a critical step forward in understanding how cells protect themselves from heavy metal toxicity. This ABC transporter is part of a larger family of proteins that use ATP hydrolysis to translocate substrates across membranes. In this case, HMT1 moves cadmium-phytochelatin complexes into the vacuole, effectively isolating the toxic metal from vital cellular components.

The importance of HMT1 exceeds far beyond simple cadmium detoxification. The study has shown that overexpression of HMT1 in yeast results in increased metal tolerance, suggesting that this transporter plays a significant role in intracellular metal compartmentalization. This ability

to sequester metals is essential for mitigating and dampening the potential damage they can cause, like oxidative stress and disruption of enzymatic activities. In plants and fungi, similar transporters play matching roles, which raise interesting possibilities for bioengineering, especially in crops. Overexpression of HMT1-like transporters in plants could lead to enhanced heavy metal sequestration in non-edible tissues, offering a way to reduce human exposure to metals in contaminated soils which became a crucial yet common challenge in the last century.

4.2 Structure and mechanism of P1B-type ATPases

Another family of transporters crucial for metal detoxification are the P1B-type ATPases, with a focus on copper-transporting ATPases. These transporters are essential in removing toxic levels of metals such as copper, zinc, and cobalt from the cytosol. The study [15] provided a detailed structural analysis, revealing the mechanism by which these ATPases transport copper across the membrane. By comparing the structure of CopA to other P-type ATPases, the researchers demonstrated that copper is translocated through the membrane via a highly conserved transport pathway, driven by ATP hydrolysis.

The structural features of P1B-ATPases, particularly the transmembrane helices that form the metal-binding site, are critical for their function. These proteins alternate between different conformational states, known as E1 and E2, which correspond to high and low affinities for the metal substrate. The P1B-ATPases are extremely efficient, with an equally high affinity for their substrates. This is particularly important for copper, a metal that is both essential for cellular function and is highly toxic at elevated levels.

The clinical implications of P1B-ATPases are significant. In humans, mutations in ATP7A and ATP7B, two P1B-type ATPases, are responsible for Menkes' and Wilson's diseases, respectively. These disorders arise from imbalanced copper homeostasis, highlighting the criticality of these transporters in human health. Understanding the structure and function of bacterial homologs like CopA provides insights that could aid in therapeutic approaches to these and other metal-related disorders.

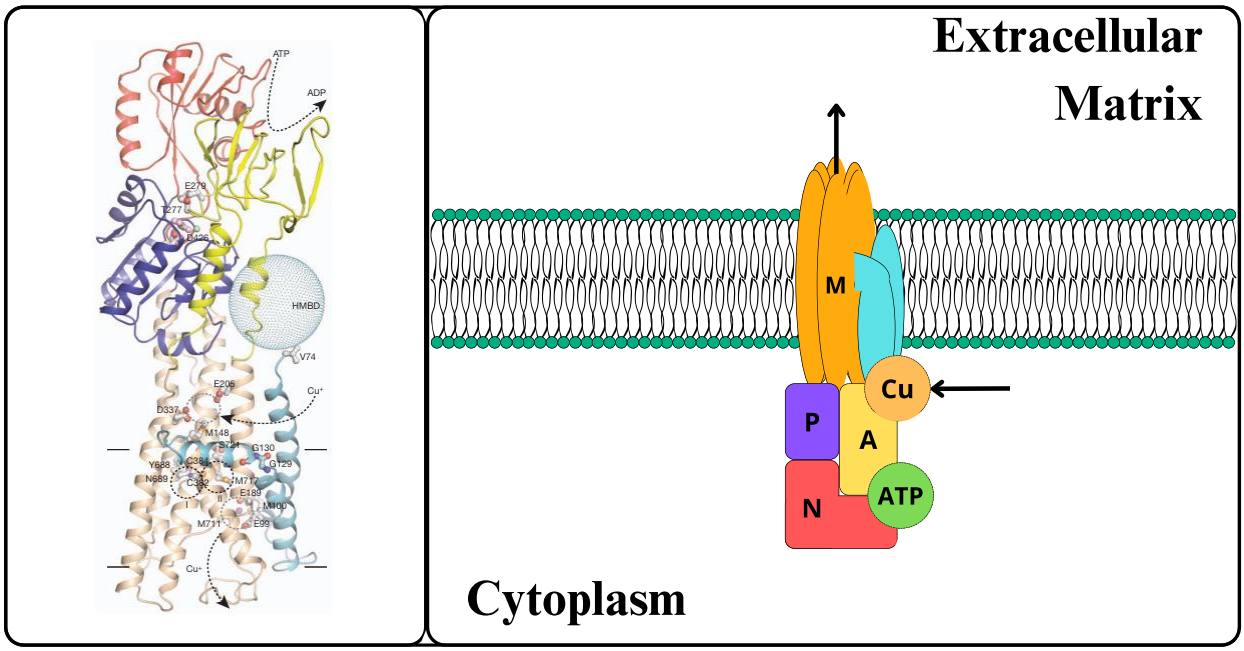

Figure 2. Cartoon representations of the crystal structure of an *L. pneumophilia* CopA after the analysis of the complex's electron density map. On the left-hand side comprehensive domain structures can be seen derived from the electron density map of the complex in the original article [15]. On the right-hand side simplified representation of the domains and their relative position in the membrane is visible. The cyan- and orange-colored domains are transmembrane M helical domains that guide the copper ions through the membrane. The purple, yellow and red domains are P, A and N domains respectively positioned in the cytoplasm that builds up the ATP binding site (green) and the Heavy Metal Binding Site (HMBD, Cu in this example). The HMBD shows low electron density in the region therefore the exact mechanism is obscured.

4.3 Genomic studies: metal tolerance and transport

While structural studies offer valuable insights into the mechanisms of metal transport, genomic studies have broadened our understanding of the physiological roles of these transporters. The study [16] utilized genomic phenotyping in *Saccharomyces cerevisiae* to identify a variety of transporters involved in metal tolerance. This large-scale screen uncovered numerous genes involved in the detoxification of cadmium and nickel, many of which were related to membrane transport and protein trafficking pathways.

One of the key findings from this study was the vacuole's central role in detoxification, which supports the importance of transporters like HMT1. The vacuole acts as a storage compartment, sequestering toxic metals away from the cytoplasm. Interestingly, the study also highlighted how different metals affect distinct cellular pathways. For instance, while vacuolar transport is crucial for cadmium detoxification, different pathways, such as nucleocytoplasmic transport, were more prominently affected by nickel exposure. These results emphasize the specificity of metal toxicity and defense mechanisms, as well as the versatility of membrane transporters in coping with these stresses.

The differential effect of transporters on various metals outlines the complexity of metal homeostasis. The interplay between vacuolar sequestration and plasma membrane export demonstrates that no single transporter is responsible for maintaining metal balance but rather, it is the coordination of multiple transport systems including transport membrane proteins that ensure cellular survival under toxic conditions.

5 Summary

Membrane transporters are central to cellular defenses against metal toxicity. Both HMT1 and P1B-type ATPases play key roles in detoxifying heavy metals, however with different mechanisms. HMT1 sequesters metals within vacuoles, while P1B-type ATPases extrude toxic metals from the cell. The importance of said proteins is further supported by genomic studies in yeast, which have uncovered a wide range of transporters involved in metal homeostasis. Understanding the mechanisms of these transporters not only advances our knowledge of cellular metal detoxification but also provides potential avenues for improving metal tolerance in plants and addressing human metal-related diseases.

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