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Zinc homeostasis in the secretory pathway in yeast

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Abstract

It is estimated that up to 10% of proteins in eukaryotes require zinc for their function. While the majority of these proteins are located in the nucleus and cytosol, a small subset is secreted from cells or is located within an intracellular compartment. As many of these compartmentalized metalloproteins fold to their native state and bind their zinc cofactor inside an organelle, cells require mechanisms to maintain a supply of zinc to these compartments even under conditions of zinc deficiency. At the same time intracellular compartments can also be the site for storing zinc ions, which then can be mobilized when needed. In this review, we highlight insight that has been obtained from yeast models about how zinc homeostasis is maintained in the secretory pathway and vacuole.

Graphical Abstract

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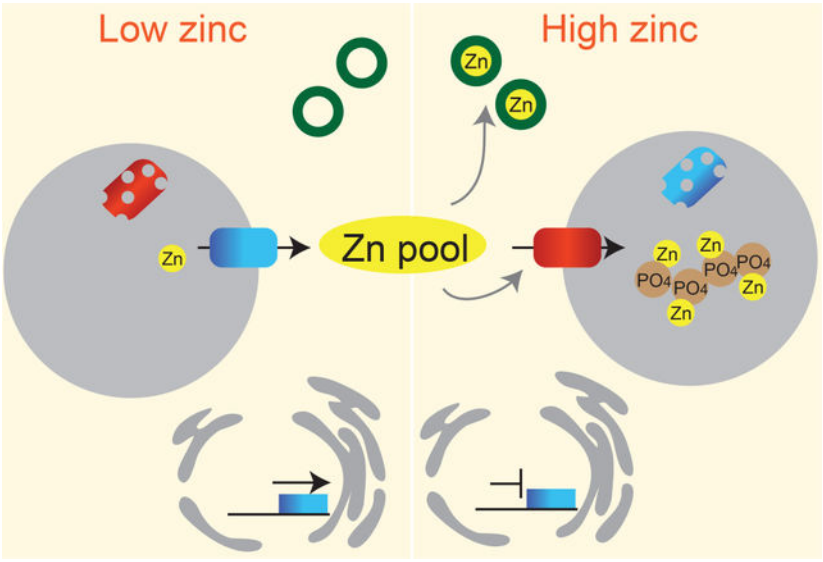
Conflict of interest

None



Introduction

In a zinc-replete environment up to 14% of zinc proteins are secreted from cells or are localized to organelles [1]. As some of these proteins fold to their native state and bind zinc within the lumen of an organelle, cells need to maintain an adequate supply of zinc ions within these organelles for the activation of these proteins. Organelles are also sites for the storage of zinc ions and where labile zinc ions accumulate for more specialized functions (Table 1). In some eukaryotes, zinc ions function as intracellular secondary messengers [2,3]. In these organisms, zinc ions are held in reserve in the endoplasmic reticulum or other intracellular vesicles until the appropriate signal triggers their release into the cytosol [4–6]. New studies in *Arabidopsis* have also revealed that zinc ions travel between cells to the xylem via a network of interconnected endoplasmic reticuli under conditions of zinc deficiency [7]. Thus, in some organisms, organelles may provide routes for the long distance transport of zinc ions.

Zinc homeostasis in the secretory pathway: insight from yeast

While zinc ions have important functions inside of organelles, genetic or chemical approaches that lead to the buildup of zinc ions within the endoplasmic reticulum cause increased ER stress and reduced cell viability [8–10]. Multiple genetic diseases are also caused by mutations that affect the function of zinc transport proteins that supply or release zinc ions from compartments [11,12], whereas single nucleotide polymorphisms and gene dosage effects that alter the expression or function of intracellular zinc transporters frequently correlate with increased risks for multiple complex diseases [11,13,14]. Together these observations suggest that cells rely on mechanisms to tightly regulate zinc concentrations inside of organelles.

Yeast are genetically tractable systems that have been extensively used to study zinc homeostasis in the secretory pathway. In the budding yeast *Saccharomyces cerevisiae*, 4

proteins belonging to the Cation Diffusion Facilitator (CDF) family and 2 proteins belonging to the Zrt1- and Irt1-like protein (ZIP) family, transport zinc ions into and out of the secretory pathway and vacuole (Figure 1). Studies of these proteins have revealed that their activity can be regulated at a transcriptional and post-translational level in response to cellular zinc status, which in turn allows cells to balance the levels of zinc inside of the cytoplasm and organelles.

The importance of transcriptional control

At a transcriptional level, genes encoding Zrt3, Zrg17, and Zrc1 are all expressed at higher levels under low zinc conditions in a manner that is dependent upon the zinc-responsive transcription factor Zap1 [15]. As discussed in past reviews, the Zap1-dependent regulation of *ZRT3* creates a negative feedback circuit to help cells release zinc from stores when zinc ions in the cytosol are low (Figure 2A), whereas the Zap1-dependent regulation of *ZRG17* creates a positive feedback circuit, enabling yeast to maintain a supply of zinc to organelles when zinc is limiting (Figure 2B) [15,16]. More recent studies suggest that related feedback mechanisms operate in other fungi with Zap1 homologs, as well as in other eukaryotes that use different zinc-responsive factors to control zinc homeostasis [17–20].

In most cases increased transcription of a zinc transporter gene correlates with a need for increased fluxes of zinc ions into or out of an organelle or the cytosol under a given growth condition. *ZRC1* is an unusual Zap1 target gene as the Zap1-dependent increase in *ZRC1* expression does not increase the transport of zinc ions into the vacuolar stores in low zinc, instead it is critical for protecting cells from a zinc shock - a growth condition where zinc-deficient cells are suddenly supplied with zinc ions [21].

The studies with Zrg17 and Zrc1 raises the question of why increased expression of some CDF proteins facilitates the transport of zinc ions out of a zinc-deficient cytosol, yet increased expression of others do not. One explanation is that there are other differences in the regulation or properties of the zinc transporters that affect their function. New studies in *Schizosaccharomyces pombe* provide additional evidence to support this hypothesis. In *S. pombe*, three members of the CDF family are localized to the secretory pathway. Cis4 and Zrg17 (the homologs of Msc2 and Zrg17) transport zinc ions into the cis-Golgi, and Zhf1 (the homolog of Zrc1 and Cot1) transports excess zinc ions into the endoplasmic reticulum [22,23]. By using genetically encoded high and low affinity zinc-responsive FRET reporters to examine changes in the labile pools of zinc in the cytosol, Choi et al. found that deletion of *zhf1* had no effect on the concentrations of labile zinc in the cytosol under low zinc conditions, whereas deletion of *zrg17* or *cis4* resulted in higher levels of zinc accumulating in the cytosol [24]. On the other hand, when zinc-limited cells were exposed to zinc, Zhf1 is critical for the rapid removal of zinc ions from the cytosol, whereas Zrg17 and Cis4 have a more minor role in this process. As the expression of *cis4*, *zrg17*, and *zhf1* is not affected by intracellular zinc status in fission yeast, these results suggest that there may be other regulatory mechanisms or differences in the intrinsic properties of the transporters that affect zinc transporter function.

How else could the activity of zinc transporters be affected by cellular zinc status? While investigating mechanisms of membrane protein degradation in *S. cerevisiae*, other studies have revealed one possible answer. By testing whether the stability of the zinc transporters Zrt3 and Cot1 was dependent upon the levels of zinc, the Emr lab found that Zrt3 was ubiquitinated and targeted for degradation in high zinc, whereas Cot1 was ubiquitinated and targeted for degradation in low zinc [25]. In both cases polyubiquitination of each protein led to its degradation via the vacuole membrane recycling and degradation pathway (vReD) (Figure 3). Although the effects of these changes on zinc homeostasis remain to be tested, these analyses reveal a different mechanism by which cells can control the flux of zinc into and out of organelles in response to intracellular zinc status. Given that many zinc transporter genes are constitutively expressed, it is possible that similar mechanisms may be widespread in other eukaryotes, and that other mechanisms are present to control the activity of zinc transporters in response to cellular zinc status [26,27].

Coping with extreme zinc deficiency

While much is known about how cells alter gene expression to maintain zinc homeostasis, until now it has been unclear what happens to the entire zinc proteome under conditions of zinc deficiency. New studies from the Eide lab have used a combination of proteomic, in silico, and ionomic analyses to gain insight into what happens to the *S. cerevisiae* zinc proteome when intracellular zinc levels are low. Key findings from these analyses show that during conditions of zinc deficiency there are ~3-fold more zinc binding sites than there are zinc ions [1]. These results suggest that when zinc is limiting, the majority of zinc-binding sites in proteins are not occupied by zinc or are potentially mis-metalated with the wrong metal.

If there are insufficient zinc ions to metalate all proteins under conditions of zinc deficiency, how do cells prioritize the delivery of zinc ions to essential compartmentalized metalloproteins under this condition? As discussed above, one strategy is to control the levels or activity of zinc transporters that facilitate the flux of zinc into or out of organelles. Another strategy is to reduce the expression of non-essential zinc binding proteins to conserve zinc for more important functions. Such ‘zinc-sparing’ mechanisms typically result in reduced expression of abundant cytosolic zinc binding proteins [28,29]. However, in *S. cerevisiae* the vacuolar zinc-binding Pho8 alkaline phosphatase is degraded when cells are starved of zinc [30]. The targeted removal of non-essential compartmentalized metalloenzymes could therefore be a mechanism to facilitate the reallocation of zinc to more essential metalloproteins under conditions of extreme zinc deficiency.

In fission yeast Pho8 activity is also dependent upon zinc. In this yeast, *pho8* is expressed at a higher level under conditions of zinc deficiency, which leads to the accumulation of the inactive apo-Pho8 protein that can be rapidly activated as soon as zinc is available [31]. As a number of the enzymes that potentially bind zinc in the endoplasmic reticulum are essential for life, the proactive accumulation of apo-Pho8 under conditions of zinc deficiency suggests that there are mechanisms present to prioritize the delivery of zinc to the other essential zinc-requiring proteins when zinc levels are low. While this hypothesis remains to be tested, in humans the activation of some secreted zinc proteins requires specific CDF family

members [32–34]. New studies of the chaperone ERp44 have shown that it only forms zinc-bridged homodimers in the slightly acidic environment of the cis-Golgi, resulting in the exposure of amino acid residues critical for the retrieval of its client proteins back to the ER [35]. Thus, the environment of a subcellular compartment as well as other factors may affect metalation and protein activity of zinc proteins within the secretory pathway.

Coping with too much zinc

In the cytoplasm excessive concentrations of zinc ions are potentially toxic because they can displace other redox-active metals from their cognate sites. An important unanswered question is therefore how can high concentrations of metal ions be stored inside intracellular compartments without causing toxicity? In some organisms, specific molecules have been identified that help to buffer zinc ions inside of cells [36]. However, for the most part, detailed knowledge is lacking about the ligands that buffer zinc ions inside of the cytosol and organelles. Gaining knowledge of the intracellular buffering environment for zinc ions in vivo is complicated by the fact that there are many low molecular weight (LMW) small molecules that could potentially buffer zinc ions [37–42]. These ligands also include the N, O, and S donors from the side chains of the amino acids histidine, glutamate/aspartate and cysteine, raising the possibility that these side chains on exposed surfaces of proteins and peptides also contribute to the zinc buffer. The extent to which each of these molecules contribute to the zinc buffering environment is also likely to be influenced by the pH of the compartment, the concentration of ligand within the compartment, and competition effects from other metal ions. The zinc buffering environment could therefore be specific to individual compartments, as well as the number and types of buffering molecules produced under a different growth condition.

Despite this complexity, in yeast the labile zinc pools within the vacuole are affected by the concentrations of glutathione and its derivatives [43]. New studies in *S. cerevisiae* have also revealed that multiple zinc-containing LMW complexes accumulate in the vacuole, most of which were removed by phosphatase treatment [44]. While these studies provide some insight into the speciation of zinc within compartments, to date, enzymes involved in polyphosphate synthesis and degradation have only been found to be important for resistance to high zinc in some fungal species [45]. As multiple other zinc buffering molecules accumulate within compartments, future studies are needed to determine the extent to which these molecules contribute to zinc buffering inside organelles.

Conclusions

A growing amount of evidence suggests that the zinc ion concentrations within intracellular compartments are dynamic in nature and are highly regulated. Studies in yeast have also revealed that transcriptional and post-transcriptional mechanisms contribute to organelle zinc homeostasis and that these mechanisms include altering the zinc proteome and expression of zinc transporters that supply and release zinc ions from intracellular compartments.

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• of special interest

•• of outstanding interest

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Highlights

- Labile pools of zinc ions are present in many different organelles
- Cells tightly regulate the levels of zinc ions inside of organelles
- Cells have multiple mechanisms to control zinc transport into and out of organelles
- When zinc is limiting, most zinc-binding sites in proteins are not occupied by zinc.

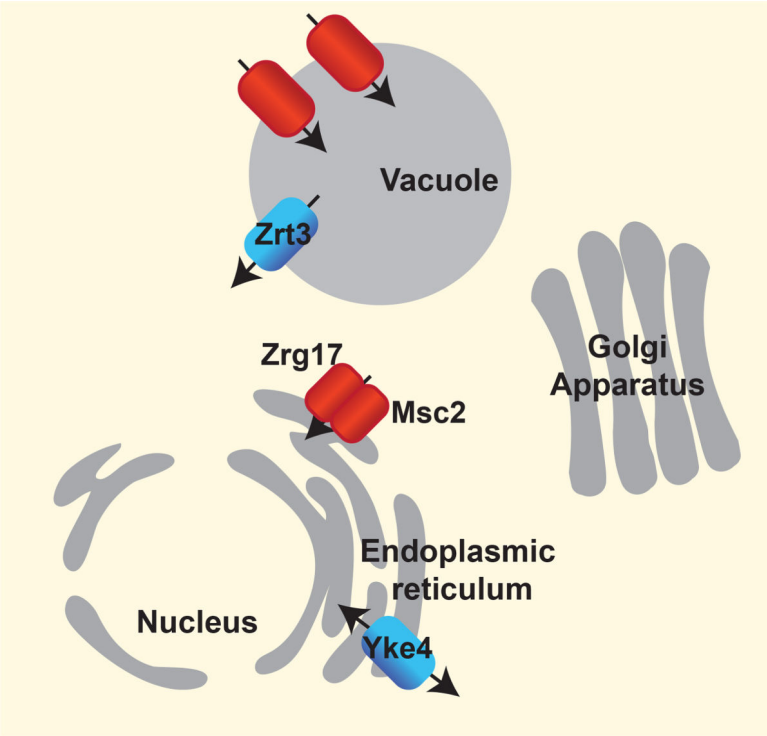

Figure 1.

Subcellular localization of zinc transporters that are localized to the secretory pathway in *S. cerevisiae*. Proteins belonging to the CDF and ZIP families are highlighted in red and blue respectively. Arrows indicate the direction of zinc transport.

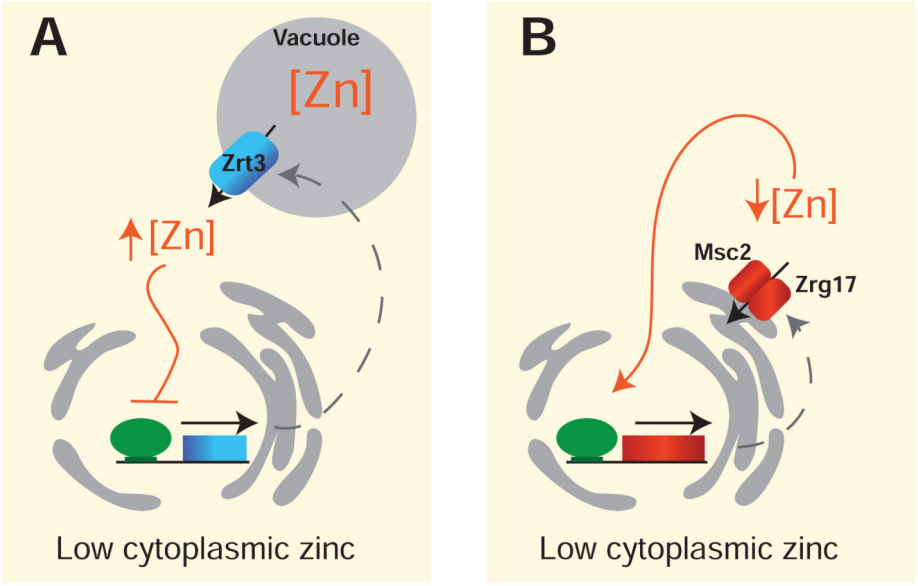

Figure 2.

The effects of Zap1-dependent regulation of *ZRT3* and *ZRG17* expression during zinc deficiency in *S. cerevisiae*. (A) Low levels of zinc in the cytoplasm increase Zap1 activity and the transcriptional activation of *ZRT3*. Increased expression of *ZRT3* leads to increased levels of the Zrt3 protein and increased mobilization of zinc ions from the vacuolar stores. When cytosolic zinc ion concentrations are returned to an optimal level, Zap1 activity is inhibited creating a negative feedback circuit to prevent the accumulation of toxic levels of zinc in the cytosol. (B) Low cytoplasmic zinc levels also increase *ZRG17* expression and Zrg17 protein levels. As Zrg17 works together with Msc2 to transport zinc ions out of the cytosol into the ER, a positive feedback circuit is generated, which maintains the supply of zinc to proteins in the ER, despite low zinc availability in the cytoplasm.

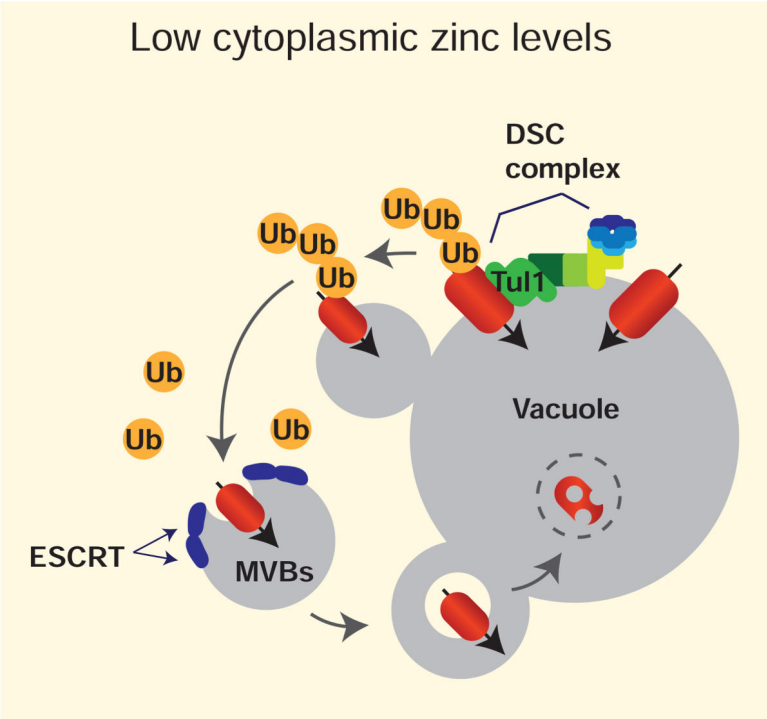

Figure 3.

Degradation of Cot1 in *S. cerevisiae* under low zinc conditions. When zinc levels are low, Cot1 is targeted for degradation in a manner that is dependent upon the Tul1 RING domain-containing E3 ligase and other members of the defective for SREBP cleavage (DSC) complex. While the signal that leads to the ubiquitination of Cot1 is unknown, polyubiquitination leads to its degradation via the vacuole membrane recycling and degradation pathway (vReD).

Table 1.

Intracellular compartments that contain high concentrations of zinc ions

Zinc storage		
Intracellular compartment with high zinc accumulation	**Organisms, cell, or tissue type**	**Reference**
Vacuole	*Saccharomyces cerevisiae* *Cryptococcus neoformans* *Toxoplasma gondii* *Suillus luteus* *Arabidopsis halleri* *Thlaspi caerulescens Frey* *Thlaspi goesingense* *Cucumis sativus* (cucumber) *Oryza sativa* (rice)	[26,27,45–51]
Endoplasmic reticulum	*Schizosaccharomyces pombe*	[23]
Zincosomes*	*Candida albicans*	[52]
Acidocalcisome	*Leishmania amazonensis* *Phytomonas serpens*	[53]
Gut granules	*Caenorhabditis elegans*	[54]
Storage granules	*Drosophila melanogaster* *Drosophila hydei*	[55,56]
Granules	*Crassostrea gigas* (Pacific oyster)	[57]
Endosome/lysosome	Kidney cells	[58]
Other Functions		
Synaptic vesicles	Neuronal cells	[59]
Secretory vesicles	Pancreatic beta cells Mast cells	[60,61]

*As outlined in more detail in the following review [62], zincosomes are membrane bound vesicles of unknown origin that have been observed in a variety of cells types and organisms.