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Zinc sensing and regulation in yeast model systems

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ABSTRACT

The Zap1 transcription factor of *Saccharomyces cerevisiae* and the Loz1 transcription factor of *Schizosaccharomyces pombe* both play a central role in zinc homeostasis by controlling the expression of genes necessary for zinc metabolism. Zap1 activates gene expression when cells are limited for zinc, while Loz1 is required for gene repression when zinc is in excess. In this review we highlight what is known about the underlying mechanisms by which these factors are regulated by zinc, and how transcriptional activation and repression in eukaryotic cells can be finely tuned according to intracellular zinc availability.

1. Introduction

Zinc is an essential cofactor in a large number of enzymes and transcription factors, but is toxic when present in excess. The tight regulation of intracellular zinc levels is therefore a basic cellular process that must occur in all eukaryotic cells. Much of what is currently known about the basic mechanisms by which eukaryotic cells regulate zinc levels is derived from studies using yeast model systems. Yeast are easy to use, genetically tractable models, which can survive as haploids or diploids [1]. As yeast can survive as haploids, they can be easily used in genetic screens to identify recessive mutant alleles that lead to a desired phenotype. Many genes involved in zinc transport and zinc homeostasis are also robustly regulated at a transcriptional level in yeast [2,3]. These large changes in gene expression have made yeast a powerful system to identify genes that are important for zinc homeostasis, and to study how zinc-dependent changes in transcription occur. Here we review what is known about the regulatory factors that facilitate zinc-dependent changes in gene expression in the budding yeast *Saccharomyces cerevisiae*, and in its distant cousin, the fission yeast *Schizosaccharomyces pombe*. Specifically, we focus on what is known about the underlying mechanisms by which two factors, Zap1 and Loz1, 'sense' changes in intracellular zinc levels and alter gene expression in response to zinc. Information regarding zinc sensing in other organisms can be found in the following recent reviews [4–6].

2. The *Saccharomyces cerevisiae* zinc sensor Zap1

The first fungal zinc-responsive transcription factor to be identified was Zap1 from *S. cerevisiae* [7]. Since its discovery in the late 1990s, functional homologs of Zap1 have been characterized from *Aspergillus fumigatus*, *Cryptococcus gattii*, *Candida albicans*, and *Candida dubliniensis* [8–11]. Homologs of Zap1 are also widely distributed throughout all of the major fungal phyla suggesting that the majority of the fungal kingdom may use a Zap1-like protein to maintain zinc homeostasis.

Zap1 was discovered in a genetic screen to identify genes required for the zinc-dependent regulation of *ZRT1* - a gene required for high affinity zinc uptake. As further deletion analysis revealed that strains lacking *ZAP1* grew poorly in zinc-limited medium, and had low levels of *ZRT1* expression in zinc-limiting and zinc-replete medium, it was hypothesized that Zap1 was necessary for inducing *ZRT1* expression during zinc starvation [7]. A large body of work has now demonstrated that Zap1 activates the

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expression of ~80 genes in response to zinc deficiency, and that many of these genes are required for zinc homeostasis or surviving/adapting to longer periods of zinc starvation [2,12–14]. Zap1 induces gene expression by binding to one or more Zinc Responsive Elements (ZREs) that are located in the promoter regions of its targets [2,15]. The consensus DNA sequence for a ZRE is 5′-ACCTTNAAGGT-3′.

2.1. Regulation of Zap1 by zinc

Zap1 plays a central role in zinc homeostasis by inducing target gene expression under zinc-limiting conditions. Both in vivo and in vitro studies addressing how Zap1 'senses' zinc have shown that Zap1 is regulated at both a transcriptional level and post-translational level by zinc, and that the combination of these mechanisms allow Zap1 activity to be tightly regulated over a broad range of zinc levels.

2.2. Auto-regulation of ZAP1

At a transcriptional level, Zap1 binds to a ZRE within its own promoter and auto-regulates its own expression [15]. As Zap1 functions as an activator, auto-regulation results in the rapid amplification of *ZAP1* transcripts and Zap1 protein, allowing the rapid induction of target gene expression. Although most Zap1 target genes are induced in response to zinc limitation, global transcript profiling has shown differences in expression patterns in response to zinc. Genes required for zinc homeostasis contain high affinity ZREs within their promoters and are typically induced under mildly zinc-limiting conditions. While genes that help cells to survive the oxidative stress of zinc deficiency, or survive longer periods of zinc starvation are only expressed in severely zinc-limited cells. As these latter genes usually contain low affinity ZREs within their promoters, and auto-regulation of *ZAP1* leads to higher levels of Zap1 protein, this increase may facilitate binding to low affinity ZREs under severely zinc-limiting conditions [2]. Although this hypothesis has not been directly tested, *ZAP1* transcript levels are regulated by zinc in other fungal species [8–10], suggesting that auto-regulation is an important component of zinc homeostasis.

2.3. Post-translational regulation of Zap1

Zap1 is an 880 amino acid protein that contains two transactivation domains, designated AD1 and AD2. Zap1 also contains seven C_2H_2-type zinc finger domains (Fig. 1). Two of the zinc finger domains (ZF1 and ZF2) overlap with AD2. The remaining five zinc fingers (ZF3-7) are located at the C-terminus and are all required for site-specific DNA binding. Combinations of mutagenesis, truncation, and deletion analyses have demonstrated that the activities of AD1, AD2, and the Zap1 DNA binding domain are all independently regulated by zinc, and that the full zinc responsiveness of Zap1 is a combination of these three independent mechanisms [16–19]. Fig. 2 summarizes the contribution of each of the individual regulatory domains to Zap1 zinc-responsiveness.

2.3.1. Post-translational regulation of Zap1 – AD1

AD1 is located between amino acid residues 332–402 and is embedded within a larger region required for zinc-dependent changes in AD1 activity, designated Zinc-Responsive Domain AD1 (ZRD^{AD1}). The zinc-dependent inactivation of AD1 is also strictly dependent upon the Zap1 DNA binding domain [17]. As discussed later, the activity of the DNA binding domain is also independently regulated by zinc. However, the inhibition of DNA binding activity occurs when cells are exposed to high zinc levels, while the inhibition of ZRD^{AD1} occurs over a much lower range of zinc levels [17,18]. These differences indicate that that ZRD^{AD1} and the DNA binding domain form their own unique sensing domains, and their combined actions allow dynamic alterations in Zap1 activity over a much broader range of zinc levels.

Although the underlying mechanism by which AD1 function is regulated by zinc is not known, a number of observations suggest that ZRD^{AD1} directly binds zinc and that this may be the key to the zinc-responsiveness of this domain. ZRD^{AD1} does not contain any characterized zinc-binding motif, but it is enriched for the amino acids cysteine and histidine. Many of these cysteine and histidine residues are conserved in Zap1 homologs from species closely related to *S. cerevisiae* and are necessary for the zinc-dependent inactivation of AD1 [17]. The known amino acid substitutions that significantly alter AD1 activity are highlighted in Fig. 1. As these residues surround AD1, and combinations of cysteine and histidine residues commonly coordinate zinc ions in proteins, it is thought that alterations in zinc ion levels may directly affect AD1 function. In support of this model, recombinant ZRD^{AD1} binds 3 mol equivalents of zinc [17]. However, facets of this model that remain untested include experiments to determine if zinc binding to ZRD^{AD1} is reversible and dependent upon intracellular zinc ion levels. It is also unknown if mutations that disrupt ZRD^{AD1} function in vivo, alter zinc binding in vitro.

Another aspect of the ZRD^{AD1} regulation that remains unsolved is the role of the DNA binding domain. Studies examining the activity of the Zap1 ZRD^{AD1} domain from *S. cerevisiae* and *Ashbya gossypii* indicate that the robust zinc-responsiveness of ZRD^{AD1} is dependent upon the DNA binding domain [17]. Potential models to explain the requirement of DNA binding domain for ZRD^{AD1} regulation include that zinc binding to ZRD^{AD1} triggers an intramolecular interaction with the DNA binding domain masking AD1, or that the DNA binding domain recruits a repressor necessary for the zinc-dependent inactivation of AD1. Future studies examining the precise mechanism by ZRD^{AD1} are therefore still necessary to unravel its dependency on the DNA binding domain.

One remaining observation that may have broader biological significance is that the Zap1 ZRD^{AD1} domain from *A. gossypii* is modestly regulated by zinc in a manner that is independent of the DNA binding domain [17]. This result suggests that the activity of ZRD^{AD1} may be directly regulated by zinc in some Zap1 homologs. In more distantly related fungi, Zap1-like proteins typically contain a region rich in acidic residues at their N-terminus, which is adjacent to, or is embedded within, a region rich in cysteine and histidine residues (Fig. 1). While these features resemble those of ZRD^{AD1} from *S. cerevisiae* and *A. gossypii*, the total number and ratios of cysteine to histidine residues, the clustering of these amino acids, and their positions relative to the acidic residues of AD1, are dependent upon the species. As the role of ZRD^{AD1} in overall Zap1 zinc-responsiveness has not been studied in any of these more distantly related homologs, it is unknown if the sequence differences in this domain affect zinc binding and the zinc responsiveness of Zap1. However, many fungal species have evolved unique mechanisms to acquire zinc from their own environmental niche [20]. Since Zap1 is central to the induction of these genes in response to zinc, it is possible that the differences in ZRD^{AD1} may also be significant.

2.3.2. Post-translational regulation of Zap1 – AD2

The most widely studied regulatory domain from Zap1 controls the activity of AD2, an acidic activation domain that directly overlaps with ZF2. In contrast to AD1, the zinc responsive domain of AD2 (ZRD^{AD2}) is well defined and maps to ZF1 and ZF2 [16]. Another critical aspect of the regulation of ZRD^{AD2} by zinc is that ZF1 and ZF2 both belong to the tandem $CWCH_2$ (tCWCH2) zinc finger family.

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