Free Radical Biology and Medicine 121 (2018) 136–148

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Histone variant H2A.Z antagonizes the positive effect of the transcriptional activator CPC1 to regulate *catalase-3* expression under normal and oxidative stress conditions

Qing Dong, Yajun Wang, Shaohua Qi, Kexin Gai, Qun He, Ying Wang*

State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing 100193, China

ARTICLE INFO

Keywords:
Neurospora crassa
Oxidative stress
Gene regulation
H2A.Z
Catalase-3

ABSTRACT

In eukaryotes, deposition of the histone variant H2A.Z into nucleosomes through the chromatin remodeling complex, SWR1, is a crucial step in modulating gene transcription. Recently, H2A.Z has been shown to control the expression of responsive genes, but the underlying mechanism of how H2A.Z responds to physiological stimuli is not well understood. Here, we reveal that, in *Neurospora crassa*, H2A.Z is a negative regulator of *catalase-3* gene, which is responsible for resistance to oxidative stress. H2A.Z represses *cat-3* gene expression through direct incorporation at *cat-3* locus in a SWR1 complex dependent pathway. Notably, loss of H2A.Z or SWR1 subunits leads to increased binding of a transcription factor, CPC1, at *cat-3* locus. Additionally, introduction of plasmids containing gene encoding H2A.Z or SWR1 complex subunits into wild-type strains decreased CAT-3 expression, indicating that H2A.Z counteracts the positive effect of CPC1 to achieve low level *cat-3* expression under non-inductive condition. Furthermore, upon oxidative stress, H2A.Z is rapidly evicted from *cat-3* locus for the recruitment of CPC1, resulting in robust and full *cat-3* gene expression in response to external stimuli. Collectively, this study strongly demonstrates that H2A.Z antagonizes the function of transcription factor to regulate responsive gene transcription under normal conditions and to poise for gene full activation under oxidative stress.

1. Introduction

Eukaryotic DNA is highly condensed into chromatins. The basic unit of chromatins is the nucleosome, consisting of DNA wrapped around an octamer of histone proteins (two copies of histone H2A, H2B, H3 and H4). The compacted chromatin alters the accessibility and behavior of the DNA and recognition by the transcription factors and RNA polymerase [1]. Thus, it is generally believed that nucleosomes must be remodeled at certain regions of a given gene to increase DNA accessibility in order to trigger efficient gene expression by transcription factors during development or stress responses. Histone variants are specialized histones which replace their canonical counterparts in specific nucleosomes on the genome. This is catalyzed by specific ATP-dependent chromatin remodelers. The replacement of histone variants on the core histones of nucleosomes and post-translational modifications on the tails of core histones contribute to the stability of the modified nucleosomes at defined regions of the genome [2].

Although many histone variants are specific to higher eukaryotes, H2A.Z is an evolutionarily conserved histone variant [3] which shares higher sequence similarity among different eukaryotic species than to the canonical H2A within the same organism, suggesting a functionally distinct role for H2A.Z on gene expression and genomic stability [4]. H2A.Z is characterized by an extended acidic patch on the surface of nucleosomes and a unique C-terminal tail [5], changing the surface of the H2A.Z-H2B dimer and affecting chromatic compaction [6]. Therefore, H2A.Z is commonly found at the regulatory regions of genes, such as promoters [7], transcription start-sites (TSSs), enhancers, gene bodies [8], and heterochromatin [6,9,10]. The distribution of H2A.Z in the genome is mediated by the H2A.Z-specific nucleosome-remodeling complex. In *Saccharomyces cerevisiae*, the deposition of H2A.Z into nucleosomes is catalyzed by an ATP-dependent chromatin remodeling complex SWR1 [11] consisting of the catalytic Swr-1 subunit and 13 other subunits [11]. This complex is responsible for the histone dimer exchange by removing H2A-H2B dimer and incorporating of H2A.Z-

Abbreviations: CAT, catalase; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide; ChIP, chromatin immunoprecipitation assay; RT-qPCR, reverse transcription qPCR

* Corresponding author.
E-mail address: wangying@cau.edu.cn (Y. Wang).

https://doi.org/10.1016/j.freeradbiomed.2018.05.003
Received 8 March 2018; Received in revised form 1 May 2018; Accepted 3 May 2018
Available online 05 May 2018

H2B dimer [11–13].

Much of what we know regarding the function of H2A.Z stems from studies performed in the *S. cerevisiae*, where it has been implicated principally in regulating gene expression both positively [14–16] and negatively [17]. In *S. cerevisiae*, H2A.Z located at promoter can exert positive effects on gene expression, including recruiting RNA polymerase II and TATA-binding protein to the *GAL1–10* promoters after galactose induction [15]. Moreover, H2A.Z deposited proximal to telomeres serves as boundary to prevent the spreading of heterochromatin into euchromatic regions [18]. Furthermore, early reports [7,19–21] have shown that H2A.Z is preferentially localized within a few nucleosomes of inactive/basal promoter regions in the yeast genome, suggesting that the variant histone poises genes for activation. Indeed, H2A.Z is preferentially evicted from promoters during gene activation in exchange for H2A [14,21]. These findings indicate that H2A.Z nucleosomes are predisposed for disassembly to allow for a rapid transcriptional response.

In mammalian cells, the histone variant H2A.Z is preferentially incorporated at enhancer regions [22]. It was described as an essential determinant of the inducible transcriptional activation mediated by the estrogen receptor alpha (ERα) [23,24]. Current views suggest that H2A.Z present at active enhancers bound by the ERα promotes RNA polymerase II recruitment, enhancer RNAs (eRNAs) production and enhancer-promoters interactions [24]. In line with the observations in yeast, it was found that H2A.Z dissociates from enhancers during inducible gene activation [23,25]. Consistent with this, recent studies show that the histone variant H3.3 antagonizes the chromatin compaction mediated by H2A.Z with retinoid acid (RA) induction, leading to the quick eviction of nucleosomes and activating RAR/RXR-regulated genes transcription [26].

Comparison of RNA-seq data with genome-wide H2A.Z enrichment analyses showed that high levels of H2A.Z in *Arabidopsis thaliana* gene bodies correlates with lower transcription levels and higher capacity of gene responsiveness in wild-type, whereas lack of H2A.Z caused unusual regulation/expression of genes in response to environmental and developmental stimuli [8]. Consistent with this, previous observations in *Arabidopsis* revealed that H2A.Z-containing nucleosomes are depleted from genes that are up-regulated by increasing temperature, suggesting that H2A.Z may act as a thermo-sensor in plants [27]. Similar studies performed recently demonstrated that levels of H2A.Z in the coding region of drought-responsive genes correlate with transcript levels. Meanwhile, H2A.Z is removed from nucleosome upon transcriptional activation [28]. Thus, these results indicate that the histone variant H2A.Z plays a vital role in mediating responsiveness of genes to the external cues.

Aerobic organisms are potentially exposed to internal or external stimuli such as reactive oxygen species (ROS) via respiration, metabolism as well as effective defense against exogenous pathogen [29]. ROS mainly consist of singlet oxygen, superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals [29]. In *Neurospora crassa*, ROS act as cellular signal molecules responsible for mycelial and conidial development [30]. Imbalance between endogenous generation of ROS by oxidants and antioxidants results in oxidative stress or signal molecules. In order to enhance fitness and survival, aerobic organisms generated ROS scavengers such as catalases (CAT) that reduce H_2O_2 produced in cells to water and oxygen [31]. Catalases are prevalent in most organisms, such as CAT-1, CAT-2 and CAT-3 in *N. crassa*. Catalases in *N. crassa* are inducible by oxidative stress and are differentially expressed during the asexual life cycle [32,33]. Genetic evidence shows that CAT-3 is the key catalase in growing hyphae whose function could not be replaced by other catalases, even under oxidative stress conditions [34]. CAT-1 mainly exists in conidia [35], whereas the CAT-2's function is unclear. Precise regulation of responsive gene expression to the environmental or intercellular stimuli is thought to be crucial for the development of an organism and its ability to respond to environmental stress. In *N. crassa*, the strictly regulated expression of *cat-3* provides a strong model to study the mechanisms of gene expression controlled by chromatin modifications. Even though a lot of studies have revealed that H2A.Z mediated gene expression responds to environmental stimuli, its function on regulation of gene responsible for oxidative stress is still unclear.

Here, we demonstrated that H2A.Z represses *cat-3* gene expression in a SWR1 complex dependent fashion, counteracting positive effect mediated by a transcriptional activator CPC1 (cross pathway control-1). H2A.Z maintains *cat-3* gene expression at a low level under normal circumstances via antagonizing the effects of CPC1. Deletion of H2A.Z gene results in high resistance to H_2O_2 as well as the robust expression of CAT-3. CPC1 is the *Neurospora* homologue of GCN4 in yeast and our laboratory has previously proved that it positively regulates *cat-3* expression [36]. We observed increased binding of CPC1 to *cat-3* locus in *H2A.Z*^{KO} mutants, suggesting that the deposition of H2A.Z on nucleosomes at *cat-3* locus may inhibit excessive CPC1 binding to the chromatin in wild-type strain. Furthermore, H2A.Z disassociated from *cat-3* locus under oxidative stress, along with elevated CPC1 recruitment, supporting the idea that H2A.Z poises for responsive gene full activation.

2. Results

2.1. *H2A.Z*^{KO} *mutants confer a strong* H_2O_2*-resistance phenotype*

In fungi, the histone variant H2A.Z is highly conserved during eukaryotic evolution (Fig. S1A and B). Although H2A.Z is essential in many organisms [37–39], deletion of H2A.Z (also known as Htz1) gene from *S. cerevisiae* genome is achievable [15]. To elucidate the role of H2A.Z in *cat-3* gene transcription, we first deleted the gene encoding the histone variant H2A.Z (NCU05347) from *N. crassa* genome. Similar to that in budding yeast, the *H2A.Z*^{KO} homokaryotic mutants were obtained by gene replacement, indicating that H2A.Z gene is dispensable for the survival of *N. crassa* cells. As depicted in Fig. 1A, *H2A.Z*^{KO} mutants exhibited a deficiency in growth rates and in conidial development compared with wild-type. Given that CAT-3 is the key catalase in exponential mycelia and responses to different stimuli [35], we examined the H_2O_2 sensitivity in *H2A.Z*^{KO} strains using race tube experiments to test whether histone variant H2A.Z potentially participates in the response to the oxidative stress by regulating *cat-3* gene transcription. Conidia of wild-type or mutants were inoculated at one end of each race tube with the medium containing 0, 10 or 20 mM H_2O_2. The position of the advancing mycelia front was marked at 24 h intervals on the tube until experiment was completed. Then the relative growth rate of each strain was measured by calculating the average growth rate of each strain on the medium containing H_2O_2 relative to that on the medium without H_2O_2. The results illustrated in Fig. 1B and C depict that the relative growth rate of *H2A.Z*^{KO} mutants was significantly faster on medium with H_2O_2 than those of wild-type strains, indicating that *H2A.Z*^{KO} strains have strong H_2O_2 resistance compared with wild-type strain.

To further confirm the function of H2A.Z in H_2O_2 resistance, a plasmid in which *H2A.Z* gene is driven by *ccg-1* promoter was reintroduced to the *H2A.Z*^{KO} strain. As shown in Fig. 1D and E, expression of wild-type H2A.Z-3Myc can complement the growth deficiency of *H2A.Z*^{KO} strains and the sensitivity to H_2O_2, indicating that the observed phenotypes of mutants were due to the deletion of H2A.Z. Taken together, these results suggest that H2A.Z may play a role in the regulation of *cat-3* transcription in response to H_2O_2 treatment.

2.2. *H2A.Z functions to suppress cat-3 gene expression*

To test whether H2A.Z affects the activities of catalases, we measured the zymogram of catalases using an in-gel experiment both in wild-type and *H2A.Z*^{KO} strains [40]. As shown in Fig. 2A, the bands corresponding to CAT-3 activity were robustly higher in mutants than