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MINI-REVIEW

Fng1 is involved in crosstalk between histone acetylation and methylation

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Abstract

The histone modifications usually form complicated networks to regulate accessibility of DNA and transcription. Identification of proteins that are involved in the crosstalk among different histone modifications will help to better understand the epigenetic regulatory network in eukaryotes. The Inhibitor of Growth (ING) proteins represent a tumor suppressor family were first linked to histone modification in yeast and their functions in epigenetic regulation were further characterized. This review summarizes the crosstalk of histone modification in fungi and describes recently achieved mechanistic insights into the role of Fng1 (an ING protein in filamentous ascomycetes) in this process. We conclude that Fng1 is involved in crosstalk among histone acetylation, deacetylation and methylation.

Keywords Histone acetylation · Histone deacetylation · Histone methylation · ING protein

Introduction

Chromosomal processes such as gene transcription, chromosome segregation and genome replication are influenced by a variety of posttranslational modifications to histones. ING proteins were considered to be functionally linked to histone modification and chromosomal functions. Since ING1 class II tumor suppressors were discovered 25 years ago, a total of five ING proteins have been identified in human (Unoki et al. 2009). Similar to other tumor suppressor factors, ING proteins regulate cancer and cell proliferation through many cellular pathways, such as DNA repair, cell cycle regulation, apoptosis, cellular senescence, chromatin remodeling and inhibition of angiogenesis (Ludwig et al. 2011). The ING proteins are evolutionarily conserved factors from yeast to human with highly conserved plant homeodomain (PHD) motifs (He et al. 2005). In budding yeast, three proteins (Yng1, Yng2 and Pho23) share significant sequence identity in their PHD finger domains with human ING1. Yng1 and Yng2 serve as components of the NuA3 and NuA4 histone acetyltransferase (HAT) complexes, respectively, to regulate histone acetylation (Loewith et al. 2000). In contrast, Pho23 is associated with the Rpd3 histone deacetylase (HDAC) complex (Ludwig et al. 2011) and responsible for the deacetylation of lysine residues on the N-terminal part of the histones. Most of the filamentous ascomycetes have one Yng1/Yng2 ortholog, but the functions in fungal development and pathogenesis are largely unknown.

Functions of the *FNG1* orthologs in fungi

Recently, an ING protein named Fng1 was characterized in the wheat scab fungus, *Fusarium graminearum* (Jiang et al. 2020). By interacting with the FgEsa1 HAT of the NuA4 complex, Fng1 mediates H4 acetylation and plays an important role in the regulation of fungal growth, conidiation, sexual reproduction, pathogenicity and secondary metabolism (Jiang et al. 2020). The orthologs of Fng1 in *Schizosaccharomyces pombe* and *Candida albicans* are required for cell growth regulation and hyphal morphogenesis, respectively (Chen et al. 2010; Lu et al. 2008). It is likely

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the role of *FNG1* orthologs in fungal growth is conserved, but the functions in pathogenicity and secondary metabolism were not reported in fungi except in *F. graminearum*. Further characterization of *FNG1* orthologs in fungal pathogens will provide clues to investigate the role of histone acetylation in pathogenesis.

Association between Fng1 and histone deacetylation

The *fng1* mutant had a severe growth defect and it was unstable and readily produced fast-growing sectors (Jiang et al. 2020). Those fast-growing sectors were partially recovered in vegetative growth and conidiation, but still defective in sexual development and plant infection, indicating stage-specific regulatory networks of Fng1 (Jiang et al. 2020). By genome re-sequencing of the sectors, suppressor mutations were identified in *FgRPD3*, *FgSIN3* and *FgSDS3* that encode the key subunits of the Rpd3 HDAC complex (Jiang et al. 2020). Mutations in these three genes resulted in an elevated H4 acetylation in the *fng1* mutant (Fig. 1a). Although other HDAC genes, including *SET3*, *SNT2* and *HDA1*, are conserved in *F. graminearum*, no suppressor mutations were identified in any of them. Therefore, Fng1 appears to specifically associate with Rpd3 HDAC complex and affect H4 acetylation.

In yeast, Rpd3 resides in two different complexes called Rpd3L (large) and Rpd3S (small). Rpd3 and Sin3 are common subunits of Rpd3S and Rpd3L whereas Sds3 is a Rpd3L-specific component required for its integrity and activity (Yeheskely-Hayon et al. 2013). Although most of the Rpd3L and Rpd3S components are well conserved in *F. graminearum*, suppressor sites were identified in FgSDS3 but not in any of Rpd3S-specific components, suggesting a specific relationship between the NuA4 subunit Fng1 and the Rpd3L complex (Fig. 1a). In yeast, the NuA3 HAT complex that mediates H3 acetylation is also related to the Rpd3 HDAC complex (Kim et al. 2020). The relationship between NuA3 and Rpd3 complexes in filamentous fungi remains to be revealed. In *F. graminearum*, the interaction of Fng1 with FgSas3, a NuA3 component, was not detected and H3 acetylation was not significantly affected in the *fng1* mutant (Jiang et al. 2020).

Roles of *FNG1* in histone methylation and gene expression

Many protein motifs characteristically associated with chromatin have been shown to have affinity for modified histone tails. The PHD finger domain is known to bind with trimethylated lysines on histone tails (He et al. 2005). As an ING protein with a PHD finger domain in its C-terminus, Fng1 was targeted to chromosomal regions enriched with

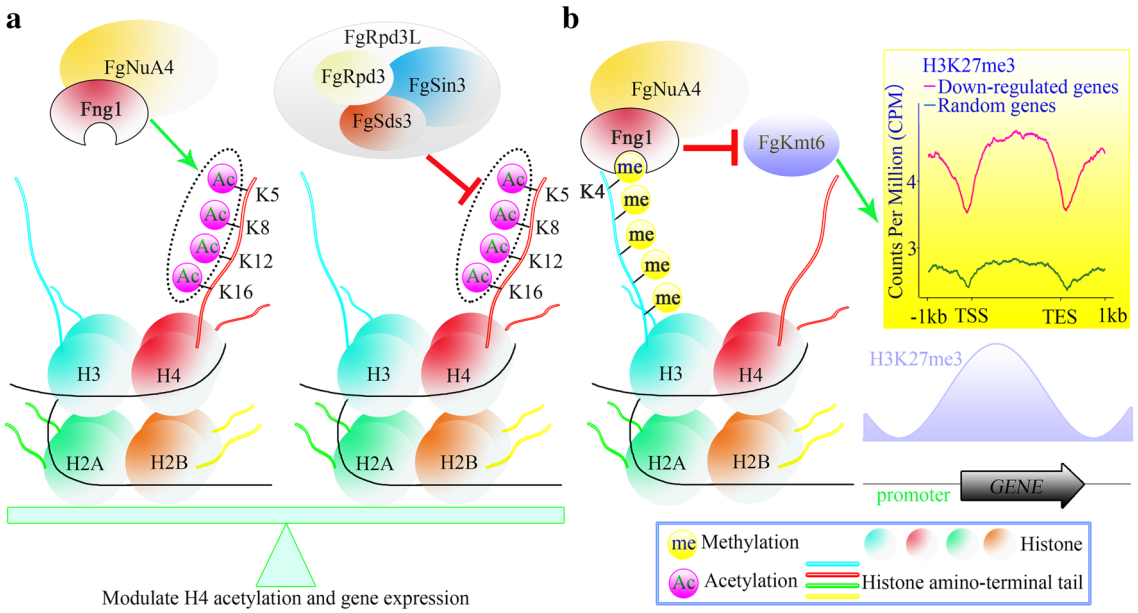

Fig. 1 Models for regulation of histone modification and gene expression by Fng1. **a** Opposing roles of NuA4 HAT and Rpd3L HDAC in regulation of histone acetylation. **b** Fng1 binds to H3K4me3 to maintain the function of the Nua4 HAT complex through the PHD domain and negatively regulates the transcription level of *FgKMT6* to affect the H3K27me3 level, and is, therefore, involved in regulating gene expression in H3K27me3-enriched chromatin regions

the H3K4me3 mark based on ChIP-seq data (Jiang et al. 2020). Deletion of the PHD finger domain in Fng1 resulted in a partial loss of function in fungal growth, development and pathogenesis (Jiang et al. 2020), indicating that a specific interaction between the PHD finger and H3K4me3 is important but not essential for the function of Fng1.

ChIP-seq analysis revealed that H4ac was highly enriched in the transcriptionally active region around the transcription start site. With a reduced H4 acetylation level, much weaker 5′-biased signals for H4ac were detected in the *fng1* mutant (Jiang et al. 2020). Based on RNA-seq data, deletion of *FNG1* affects the transcription level of over 3000 genes. Surprisingly, the distribution of Fng1-regulated genes did not fully correlate with Fng1-dependent H4 acetylation. Although Fng1 mainly localizes to euchromatin, over 50% of the genes down-regulated in the *fng1* mutant in chromosomal regions are enriched for H3K27me3. H3K27me3 is a histone modification associated with silent chromatin exists predominantly in regions that lack synteny with other Fusarium species (Connolly et al. 2013). Interestingly, the transcript level of Kmt6, the histone methyltransferase responsible for the methylation of H3K27, was increased in the *fng1* mutant. Furthermore, a strong correlation was identified between Fng1-regulated gene expression and H3K27me3 enrichment, suggesting that the increased H3K27me3 level was responsible for altered gene expression in the *fng1* mutant. These results indicate that Fng1, a crucial regulator of histone acetylation, is also involved in the regulation of histone methylation and gene expression via *KMT6* (Fig. 1b). Although ING proteins with PHD motif were known as "readers" of histone methylation marks, the role of them in regulating histone methylation has not been reported. These will extend our knowledge of the crosstalk between histone acetylation and methylation (Gong et al. 2020; Valencia-Sanchez et al. 2021; Vlaming et al. 2019).

Final thoughts

ESA1, which encodes the core subunit of NuA4 complex, is an essential gene in yeast, *F. graminearum* and other fungi (Chittuluru et al. 2011; Jiang et al. 2020). As an ING protein interacting with Esa1, *FNG1* orthologs are closely related to NuA4 complex in fungi and functional analysis of them will provide an effective means to reveal the role of NuA4 complex. Deletion of *FNG1* in *F. graminearum* resulted in a severe growth defect and fast-growing sectors of the *fng1* mutant were readily produced (Jiang et al. 2020). Because the *fng1* mutant was blocked in sexual development and pathogenicity, it is also possible to identify spontaneous suppressors associated with them. Functional characterization of the novel spontaneous suppressors in other stages, such as sexual reproduction and plant infection, may enable us to understand the regulatory networks of Fng1 more comprehensively. *PHO23*, encodes a subunit of Rpd3L complex, is paralogous to *FNG1*. Competition and association between *PHO23* and *FNG1* may mediates the genetic interaction between HAT and HDAC complexes, and sequentially balances acetylation and deacetylation. Further characterization of ING proteins will help to better understand the crosstalk among histone modification and provide insights into the study of chromosomal functions.

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Declarations

Conflict of interest The authors have declared that no competing interests exist.

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