Fungal Genetics and Biology 107 (2017) 24–30

Contents lists available at ScienceDirect

Fungal Genetics and Biology

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

Regular Articles

FvSet2 regulates fungal growth, pathogenicity, and secondary metabolism in *Fusarium verticillioides*

Qin Gu[a], Zhenzhong Wang[b], Xiao Sun[a], Tiantian Ji[a], Hai Huang[a], Yang Yang[a], Hao Zhang[a], Hafiz Abdul Samad Tahir[a], Liming Wu[a], Huijun Wu[a,*], Xuewen Gao[a]

[a] *Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Monitoring and Management of Crop Diseases and Pest Insects, Ministry of Education, Nanjing, China*
[b] *China Rural Technology Development Center, China*

ARTICLE INFO

Keywords:
Fusarium verticillioides
FvSet2
H3K36 trimethylation
FB_1
Fungal virulence

ABSTRACT

Histone H3 lysine 36 methylation (H3K36me) is generally associated with activation of gene expression in most eukaryotic cells. However, the function of H3K36me in filamentous fungi is largely unknown. Set2 is the sole lysine histone methyltransferase (KHMTase) enzyme responsible for the methylation of H3K36 in *Saccharomyces cerevisiae*. In the current study, we identified a single ortholog of *S. cerevisiae* Set2 in *Fusarium verticillioides*. We report that FvSet2 is responsible for the trimethylation of H3K36 (H3K36me3). The *FvSET2* deletion mutant (ΔFvSet2) showed significant defects in vegetative growth, FB_1 biosynthesis, pigmentation, and fungal virulence. Furthermore, trimethylation of H3K36 was found to be important for active transcription of genes involved in FB_1 and bikaverin biosyntheses. These data indicate that FvSet2 plays an important role in the regulation of secondary metabolism, vegetative growth and fungal virulence in *F. verticillioides*.

1. Introduction

Fusarium verticillioides (synonym, *Fusarium moniliforme* Sheldon; teleomorph *Gibberella moniliformis*) is a fungal agent that causes ear and stalk rot in maize (*Zea mays*) (Nelson, 1992). During infection, *F. verticillioides* produces various myotoxins, in particular fumonisins, which are extremely harmful to human and animal health (Howard et al., 2001; Munkvold, 2003; Park and Troxell, 2002; Woloshuk and Shim, 2013). Fumonisin B_1 (FB_1) is the most prevalent and most well-characterized of the fumonsin derivatives (Yin et al., 2016). A *FUM* gene cluster has been found to encode the proteins and enzymes responsible for FB_1 biosynthesis and regulation (Alexander et al., 2009; Robert et al., 2003; Rosler et al., 2016). However, the molecular mechanisms underlying the regulation of *FUM* gene transcription in *F. verticillioides* remain poorly understood.

In eukaryotes, histones are subjected to a variety of covalent modifications, such as methylation, phosphorylation, adenosine diphosphate (ADP)-ribosylation, biotinylation, acetylation, and ubiquitination (Strahl and Allis, 2000). Post-translational histone lysine methylation is widely associated with regulation of gene transcription (Brosch et al., 2008; Martin and Zhang, 2005; Peterson and Laniel, 2004; Steinfeld et al., 2007; Strahl and Allis, 2000). For example, some histone methylation events are associated with gene silencing, such as methylation of lysines 9 and 27 on histone H3, while others, including methylation of lysines 4 and 36 on histone H3, have been shown to be correlated with transcriptional activation (Black et al., 2012; Rivera et al., 2014). Specific lysine histone methyltransferases (KHMTase) can methylate the lysine residues on histone H3 (Lachner et al., 2003; Schneider et al., 2002; Shilatifard, 2006). For example, *S. cerevisiae* Set1/COMPASS catalyzes the mono-, di-, and trimethylation of lysine 4 on histone H3 (Krogan et al., 2002; Roguev et al., 2001). The methylation of lysine 9 on histone H3 (H3K9me) is catalyzed by the methyltransferase Dim5 in *Neurospora crassa* (Tamaru and Selker, 2001; Tamaru et al., 2003). Recently, Set2 was identified as the KHMTase responsible for methylation of H3K36 (H3K36me), and was found to interact with RNA polymerase II (RNAPolII) during the later stages of RNA elongation (Adhvaryu et al., 2005; Grewal and Moazed, 2003; Hampsey and Reinberg, 2003; Maltby et al., 2012; Morris et al., 2005; Shilatifard, 2004; Strahl et al., 2002).

Previous studies have shown that the methylation of histone H3 lysine plays important roles in secondary metabolism, vegetative growth, and fungal virulence in some filamentous fungi. Deletion of *SET1* in *Fusarium graminearum* and *Magnaporthe oryzae* blocked the H3K4me and led to significant defects in fungal virulence and mycotoxin biosynthesis (Liu et al., 2015; Pham et al., 2015). The trimethylation of H3K27 was found to repress the genes involved in secondary

* Corresponding author.
E-mail address: hjwu@njau.edu.cn (H. Wu).

http://dx.doi.org/10.1016/j.fgb.2017.07.007
Received 14 February 2017; Received in revised form 26 July 2017; Accepted 31 July 2017
Available online 01 August 2017

metabolite pathways in *Fusarium fujikuroi* and *F. graminearum* (Connolly et al., 2013; Studt et al., 2016). Recently, the trimethylation status of H3K9 and H3K27 in *Epichloë festucae* was reported to be important in regulating the symbiosis-specific biosynthesis of bioprotective alkaloid metabolites (Chujo and Scott, 2014). In *N. crassa*, H3K36me is associated with fungal development and adaptive response to high temperatures (Adhvaryu et al., 2005). Nevertheless, the mechanisms of H3K36me in regulation of secondary metabolism and fungal virulence in filamentous fungi remain unclear.

In the current study, we investigated the biological and genetic functions of FvSet2, the Set2 ortholog in *F. verticillioides*, which is a KHMTase for H3K36me. We report that FvSet2 controls trimethylation of H3K36 and regulates vegetative growth, fungal virulence, and secondary metabolism in *F. verticillioides*.

2. Materials and methods

2.1. Fungal strains and growth conditions

For mycelial growth tests, the wild-type *F. verticillioides* strain 7600, the *FvSET2* deletion mutants, and the complementation strains were incubated at 25 °C on potato dextrose agar (PDA; 200 g potato, 20 g glucose, 10 g agar and 1 L water), complete medium (CM) agar (CM; 10 g glucose, 2 g peptone, 1 g yeast extract, 1 g casamino acids, nitrate salts, trace elements, 0.01% vitamins, 10 g agar and 1 L water, pH 6.5) (Klittich and Leslie, 1989), and minimal medium (MM) agar (MM; 10 mM K_2HPO_4, 10 mM KH_2PO_4, 4 mM $(NH_4)_2SO_4$, 2.5 mM NaCl, 2 mM $MgSO_4$, 0.45 mM $CaCl_2$, 9 mM $FeSO_4$, 10 mM glucose, 1% agar, pH 6.9). To determine the effect of *FvSET2* deletion on conidiation, 50 mg of mycelia from each strain were added into 20 ml liquid carboxymethyl cellulose (CMC, average M_w ~ 90,000) medium (1 g NH_4NO_3, 1 g KH_2PO_3, 0.5 g $MgSO_4{\cdot}7H_2O$, 1 g yeast extract, 15 g CMC and 1 L water) and were cultured at 25 °C for 4 days in a 180 rpm shaker (Iida et al., 2008). The experiment was repeated on three independent occasions.

2.2. Generation of FvSET2 deletion and the complementation strains

The *FvSET2* gene disruption vector was constructed using the double-joint PCR method (Yu et al., 2004). The primers used to amplify the sequences are listed in Table S1. The resulting constructs were transformed into the protoplasts of the wild-type strain 7600 using the protocol described by Gu et al. (2015a,b). To confirm the phenotypic changes observed in the *FvSET2* deletion mutants were due to loss of FvSet2 functions, complementation strains were created by transforming a DNA fragment containing the full-length *FvSET2* gene, the native *FvSET2* promoter, and the putative terminator into protoplasts from the ΔFvSet2 strain (Gu et al., 2015a,b).

2.3. Pathogenicity assays

The conidia of each strain were collected and were re-suspended in sterilized water at a concentration of 10^6 conidia/ml. Subsequently, we injected a 10-μl sample of the conidial suspension into the maize cultivar B73 via a hole punched in the stem. Injected cultivars were incubated at 25 °C and 80% humidity for 15 days. Six repetitions were performed for each strain. The infected maize plants were longitudinally dissected 15 days past injection (dpi) and subsequently characterized.

2.4. Determination of FB_1 production in each strain

To determine the role of FvSet2 in FB_1 production, 25 g of maize kernels were sterilized and then were inoculated with 50 mg of fresh mycelia from the wild type strain, the ΔFvSet2 strain, or the complementation strain. After 21 dpi, the production of FB_1 by each strain was analyzed according to a previously described method (Shim and Woloshuk, 2001). Fungal ergosterol levels produced by each strain in maize kernels were measured using a HPLC system (Waters 1525) (Gu et al., 2015b). The experiment was repeated three times.

2.5. RNA extraction, CHIP, and quantitative real-time PCR analyses

Chromatin immunoprecipitation (ChIP) was performed as previously described (Liu et al., 2015). Fresh mycelia were harvested and then cross-linked with 1% formaldehyde for 15 min. Fixation was stopped with the addition of 125 mM glycine. Fixed culture samples were filtered, frozen, pulverized in liquid nitrogen, and re-suspended with 10 ml of nuclear extraction buffer I (0.4 M sucrose, 10 mM Tris–HCl, pH 8.0, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.1 mM PMSF, 1 × protease inhibitor). Samples were centrifuged at 4000*g* for 20 min at 4 °C, and the pellets were re-suspended in 4 ml of nuclear extraction buffer II (1.7 M sucrose, 10 mM Tris–HCl, pH 8.0, 10 mM MgCl2, 1% Triton X-100, 5 mM β-mercaptoethanol and 1 × protease inhibitor). Re-suspended samples were then centrifuged at 13,000*g* for 15 min at 4 °C. The resulting pellets were re-suspended in 300 μl of nuclear lysis buffer (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 1% SDS and 1 × protease inhibitor). Samples were then sonicated by two pulses of 30 s sonication and 1 min rest. After centrifugation at 4000*g* for 5 min at 4 °C, the supernatant was transformed into a clear tube, and was diluted with 10 × ChIP dilution buffer (1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris–HCl, pH 8.0 and 167 mM NaCl). Immunoprecipitation was performed using a H3K36me3 specific antibody (Ab9050; Abcam, Cambridge, MA, USA) along with protein A agarose (Santa Cruz, CA, USA). After washing, eluting, reversing the cross-linking, and removing all proteins, the resulting pellets were re-suspended in 50 μl of distilled water. The cross-linked immunoprecipitated DNA was extracted by phenol/chloroform. Finally, quantitative PCR amplifications were performed with the DNA Engine Opticons 4 system (MJ Research, Waltham, MA), using SYBR green I fluorescent dye detection. The sequences of PCR primers used for ChIP-qPCR analysis are provided in Table S1. Relative enrichment values were calculated by dividing the amount of immunoprecipitated DNA by the amount of input DNA. The experiments were independently repeated three times.

To determine the impact of *FvSet2* deletion on expression of *FUM* and *BIK* genes, mycelia of each strain were inoculated into liquid GYAM (0.24 M glucose, 0.05% yeast extract, 8 mM L-asparagine, 5 mM malic acid, 1.7 mM NaCl, 4.4 mM K_2HPO_4, 2 mM $MgSO_4$, and 8.8 mM $CaCl_2$, pH 3.0) or PDB grown for 36 h at 25 °C in a shaking incubator (180 rpm). Two layers of miracloth were used to harvest the mycelia. The harvested mycelia were lyophilized and ground in liquid nitrogen. Total RNA was extracted from each sample using the RNAiso Reagent (TaKaRa). Reverse transcription was performed on 10 mg of each RNA sample, using the RevertAid H Minus First Strand cDNA Synthesis Kit employing the oligo$(dT)_{18}$ primer (Fermentas Life Sciences, Burlington, Canada). The expression of each gene was determined by quantitative real-time PCR with the primers listed in Table S1. The *FvACTIN* gene was amplified as a reference gene using the FvActin-F + FvActin-R primer pair (Supporting information Table S1). The experiment was repeated on three independent occasions.

2.6. Western blotting assay

Fresh mycelia from each strain were inoculated into 150 ml liquid CM and grown in a shaker (200 rpm) at 25 °C for 36 h. Mycelia were harvested and washed with deionized water. The harvested mycelia were subsequently ground in liquid nitrogen. Protein was extracted according to a previously established protocol (Gu et al., 2015a). Proteins were separated by SDS polyacrylamide gel electrophoresis on a 10% denaturating gel. Proteins were transferred to an Immobilon-P transfer membrane (Millipore, Billerica, MA, USA) using a Bio-Rad electroblotting apparatus. The Ab9050 antibody (Abcam, Cambridge,