Régulation épigénétique de la production de mycotoxines chez Fusarium graminearum

Zhenhui Chen

THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE Sciences de la Vie et de la Santé

SPÉCIALITÉ Génétique

Par Zhenhui CHEN

Régulation épigénétique de la production de mycotoxines chez *Fusarium graminearum*

Sous la direction de : Nadia PONTS

Soutenue le 29 Novembre 2019

Membres du jury :

M. GALLUSCI Philippe	Professeur Université de Bordeaux	Président
Mme. MALAGNAC Fabienne	Professeure Université Paris Saclay	Rapporteure
M. LE TRIONNAIRE Gaël	Chargé de Recherche INRA	Rapporteur
Mme PONTS Nadia	Chargée de Recherche INRA	Directrice de Thèse
Mme. FOULONGNE-ORIOL Marie	Chargée de Recherche INRA	Invitée

Acknowledgements

Upon the completion of this thesis, I am grateful to those who have offered me encouragements and supports during my PhD study.

First and foremost, the profound gratitude should go to my supervisor, Nadia Ponts. In China, there is an old saying: a teacher is the one who could propagate the doctrine, impart professional knowledge, and resolve doubts. Nadia did it. She has given me great guidance and encouragement throughout the process of selecting my research topic, designing and doing experiments, developing and solving questions, and writing the thesis. When I got puzzled, her valuable suggestions always make me enlightened. She also encouraged me to put forward my own ideas and try to think as a researcher. I really enjoy discussing scientific questions with her. Besides, I want to give my thanks for her thoughtful kindness in life. When I was sick, when I was unhappy, when I was stressed, she was always there to help me. Her support and comfort gave me much strength. Sometimes, I even think she knows me better than myself. I want to tell her again, it is lucky to be her student.

Secondly, I want to express my heartfelt thanks to all the colleagues who have helped and taught me during the study. I am particularly grateful to Enric Zehraoui, Christine Ducos and Laetitia Pinson-Gadais. Enric was the one who worked with me as soon as when I arrived in our lab. He showed me the principle and manipulation of the creation of mutants with incomparable patience, and helped me to adapt to our lab. Christine is a warm-hearted and cute colleague, who taught me how to exact DNA & RNA, how to do qPCR. Together with Fabien Dumetz, she also helped me to do PCR for Sanger sequencing when I was writing, which really lightened my pressure. Laetitia is a specialist of fungal morphology, she helped me to identify species when I have difficulties in recognizing. We also shared one office for almost 3 years, as soon as I need help, she would come immediately no matter how busy she was.

Moreover, I would like to extend my gratitude to Sylvain Chereau, Vessela Atanasova-Penichon and Marie-Noelle Verdal-Bonnin, the biochemists, who explained and taught me the extraction and analysis of mycotoxins; Magalie Moinard, who showed me the methods to induce sexual reproduction of Fusarium graminearum; Marie Foulongne Oriol, we worked together to prepare the bank for whole genome sequencing; Thierry Gibard, our informatician, who solved many problems related with my computer; Corine Grimaldi and Marie-France Neveux, our secretaries, who helped me to deal with a lot of paperwork. I also want to thank all the other members and the trainees in our lab, especially Aurelie Etier, Manon Yager, Anastasia Lamaison, who helped me work on the side project. And my sweet hearts, Saranyaphat Boonmee, Yasmine Chakroun, Saoussen El Ghoul and Diane Dibi who gave me a lot of warmth.

Also, I owe many thanks to my intimate Chinese friends in Bordeaux, Junhua Kong, Lina Wang, Zaicheng Zhang, Yulin Cai, Jiaojiao Wang, Zhengyan Shen, Wencan He. Before we arrived here, we did not know each other at all. But during the three years, we get along like a family. As I am the youngest 'sister' in the family, they always give me more care and tolerate my caprice, which touched me again and again. We share happiness, talk our dreams, complain annoyance together and also encourage each other. I will appreciate the precious and special friendship forever.

Special thanks would go to my beloved family for their loving considerations and great confidence in me all through these years and their supporting without a word of complaint. I have the best parents, sister and brother in the world. I miss them so much.

Finally, I want to say thanks to my dear motherland, People's Republic of China. I love her deeply and I am proud of being a Chinese.

Abbreviations

5mC	5-Methylcytosine
6mA	N6-methyladenine
ADON	AcetylDeoxynivalenol
AFB1	Aflatoxin B1
ANIV	AcetylNivalenol
ANP32E	Acidic leucine-rich nuclear phosphoprotein 32 family member E
ATP	Adenosine Triphosphate
AUR	Aurofusarin
bp	Base pair
CCR	Carbon Catabolite Repression
cDNA	Complementary Deoxyribonucleic acid
CHD	Chromodomain Helicase Deoxyribonucleic acid-binding
ChIP-seq	Chromatin Immunoprecipitation coupled to Deep Sequencing
CM	Complete Medium
CMC	Carboxymethyl Cellulose
Comp.	Complementation
COMPASS	Complex of Proteins Associated with Set1
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DNMT	DNA methyltransferases
dNTP	deoxyNucleoside Triphosphate
DON	Deoxynivalenol
DSB	DNA double–strand breaks
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of United Nations
FB1	Fumonisin B1
FHB	Fusarium Head Blight
FLC	Flowering Locus C
Gcn5	General control protein5
GCR	Genetic Compensation Response
gDNA	genomic Deoxyribonucleic acid
GER	Gibberella Ear Rot
HAT	Histone Acetyltransferase

HDAC	Histone Deacetylase
HFD	Histone-fold Domain
HMT	Histone methyltransferases
HOG	High Osmolarity Glycerol
HP1	Heterochromatin Protein 1
HPLC-DAD	High Performance Liquid Chromatography coupled to a Diode Array Detector
HR	Homologous Recombination
HXKYme(1, 2, 3)	(1 mono-, 2 di-, 3 tri-) methylation of lysine (Y) on Histone (X)
I156	*Fusarium graminearum* Strain INRA156
I171	*Fusarium graminearum* Strain INRA171
I349	*Fusarium graminearum* Strain INRA349
I605	*Fusarium graminearum* Strain INRA605
I812	*Fusarium graminearum* Strain INRA812, identical to the reference strain PH-1 FGSC9075
INO80	Swr1-related Inositol requiring 80
ISWI	Imitation SWI
KMT	Lysine Methyltransferase
me5C	methylated carbon 5 of the pyrimidine ring on cytosine
MIP	Methylation induced premeiotically
mRNA	Messenger Ribonucleic acid
MS	Synthetic Medium
NCP	Nucleosome Core Particle
NHEJ	Non-Homologous End Joining
NIV	Nivalenol
NMD	Nonsense-mediated mRNA decay
NMR	Nitrogen Metabolite Repression
OE	Overexpression
OSR	Oxidative Stress Response
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PRC	Polycomb Repressive Complex
PTC	Premature Termination Codons
PTM	Post-Translational Modification
qPCR	quantitative Polymerase Chain Reaction
RIP	Repeat-induced Point Mutation

RNA	Ribonucleic acid
RNAPII	Ribonucleic acid polymerase II
RNase	Ribonuclease
ROS	Reactive Oxygen Species
SAGA	Spt-Ada-Gcn5 Acetyltransferase
SAM	S-adenyl methionine
SM	Secondary Metabolites
SNP	Single-nucleotide Polymorphism
SRCAP	Snf2-related CREBBP activator protein
SRI	Set2 Rpb1 interacting
SUMO	Small Ubiquitin-like MOdifier
SWI/SNF	Switch/Sucrose Non-fermentable
TBP	TATA-binding protein
TCT	Trichothecene
TCTA	Type A Trichothecene
TCTB	Type B Trichothecene
TE	Transposable Elements
Tip60 (KAT5)	Histone acetyltransferase KAT5
Tri cluster	Trichothecene biosynthetic gene (*Tri*) cluster
TSS	Transcriptional Start Site
TWEEN	Polysorbate
Vel	Velvet-like
WGS	Whole Genome Sequencing
ZEA	Zearalenone

Table of Contents

Chapter 1
Introduction

Foreword

This introduction is composed of five sections.

In **Section 1.1**, a general introduction was done for the filamentous fungus *Fusarium graminearum*, including its taxonomic status, morphological characteristics and importance in agriculture.

In **Section 1.2**, we summarized the potential roles of secondary metabolites (SMs) of filamentous fungi with a focus on mycotoxins, especially trichothecenes, which are mainly produced by *F. graminearum* and *Fusarium culmorum* on wheat.

In **Section 1.3**, we explained how environmental factors, such as nutrient source, pH, H_2O_2 and light, impact the production of SM in filamentous fungi.

Recent studies shown that chromatin structure changes also play key role on the regulation of SM biosynthesis by filamentous fungi. Therefore, in **Section 1.4**, we described the dynamic structure of chromatin in eukaryotes and how it controls gene expression in filamentous fungi.

As our project mainly focus on the histone variant H2A.Z, a **Review Paper** is presented to summarize the already known and potential functions of H2A.Z, with a focus on fungi.

Finally, the hypothesis and objectives pursued during my PhD work are presented in the **Synopsis**.

1.1 The cereal killer: *Fusarium graminearum*

1.1.1 What are filamentous fungi

The kingdom of Fungi, is a very diverse group of eukaryotic organisms, distinct from plants and animals. As an estimate, there may be as many as 3.8 million species of fungi worldwide (Hawksworth and Lücking 2017). The vast majority of these little-understood organisms are 'filamentous fungi', named because they are composed of a web of filaments called 'hyphae'. Hyphae are multicellular structures with branches growing at the tip and extend in different dimensions to form a network of threads known as mycelium. Aerial mycelium allow vegetative (non-sexual) reproduction by producing spores, or conidia, which are specialized structures with a protective coat that shields them from harsh environmental conditions such as drying out and high temperatures **(Figure 1)**. Additionally, some highly specialized sexual reproductive or protective structures could also be formed by many species under specific conditions, such as ascospores. With the help of wind, rain or insects, spores spread to new habitats and start to produce new hyphae (Reinhard and Meritxell 2017; "About Microbiology – Fungi").

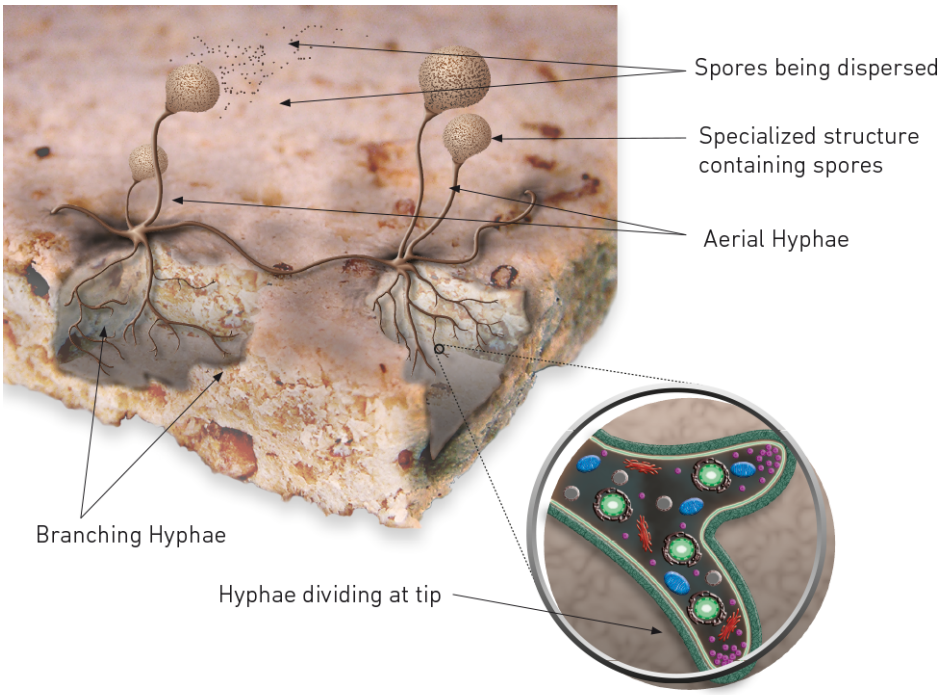
Due to the ability of filamentous fungi to grow on almost any substrate and under harsh conditions, they are ubiquitous in various environments (More *et al*. 2010) with *Aspergillus, Fusarium, Penicillium, Alternaria*, and *Acremonium* genera being the most widely distributed and reported genera belong to them **(Figure 2)** (Pitt and Hocking 2009).

1.1.2 The genus *Fusarium*

The genus *Fusarium* is classified as belonging to the Class Sordariomycetes of Phylum Ascomycota **(Figure 2)** (van Diepeningen *et al*. 2014). Up to now, around 1,000 species have been identified (Nelson, Toussoun, and Marasas 1983; Moretti 2009). Morphologies inter-species is highly variable and can be strongly influenced by environmental factors. Usually, *Fusarium* species are fast-growing at 25°C in culture medium (Guarro 2013). They grow with a velvety or cottony, flat surface whose color may be white, yellow, pink, purple, salmon or grey , with tan, red, violet or brown on the reverse (Nucci and Anaissie 2007).

Several *Fusarium* species undergo both asexual and sexual reproductions, even though only fewer than 20 % fusaria have a known sexual cycle (Ma *et al*. 2013). Typically, *Fusarium*

A



B

Figure 1. Fungal mycelium. **(A)** Representation of mycelium formation and distribution; **(B)** *Rhizopus nigricans* growing on bread left in a moist plastic bag for seven days. Tangled mycelium is visible (white filaments) as well as sporangia bearing spores (brownish/black spheres) ("About Microbiology – Fungi")

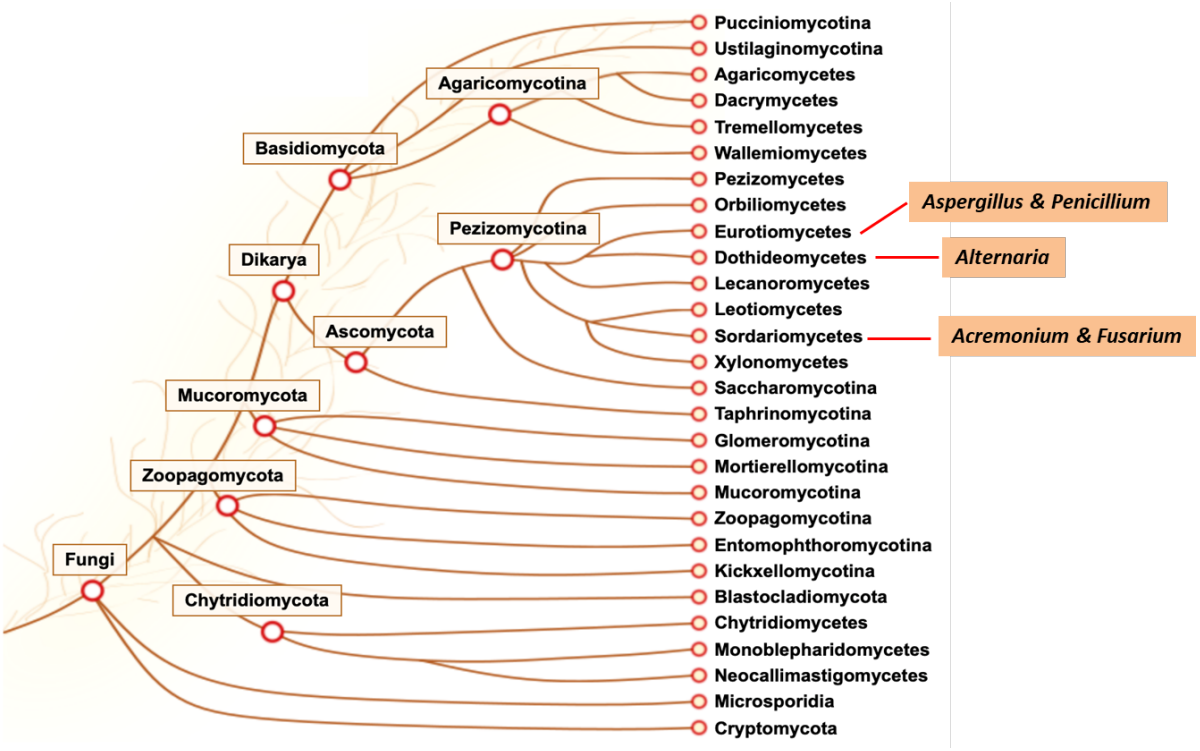


Figure 2. Phylogenetic tree of Fungi kingdom. Among them, *Aspergillus* and *Penicillium* belong to class Eurotiomycetes, *Alternaria* belongs to class Dothideomycetes, *Fusarium* and *Acremonium* belong to class Sordariomycetes. Those are the most widely distributed and reported genera of filamentous fungi (Grigoriev *et al.* 2011; 2014).

produces three types of spores during the asexual stage, including macroconidia, microconidia and chlamydospores as represented in **Figure 3** (Tupaki-Sreepurna and Kindo 2018). Macroconidia can be produced on monophialides and polyphialides in the aerial mycelium, but also on short monophialides in specialized structures called sporodochia. They are hyaline, formed holoblastically and singly, banana / canoe-shaped, multicellular / multiseptate with a foot cell at the base (Nelson, Dignani, and Anaissie 1994). Microconidia are produced in the aerial mycelium in clumps or chains on both monophialides and polyphialides. Under unfavorable conditions, such as dry or hot seasons, the thick-walled large resting spores called chlamydospores are produced to ensure the survival of fungi (Moretti 2009). During sexual reproduction, ascospores are produced in groups of eight in an ascus contained within a flask-shaped structure known as perithecia. Warm, moist condition promotes the maturation of perithecia and the liberation of ascospores from them. Homothallic species are capable of self-fertilization, while heterothallic species can only produce ascospores through outcross (Ma *et al*. 2013; Prussin II *et al*. 2014). Spore types and morphology may vary between species, which contributes to distinguish species of *Fusarium* (Leslie and Summerell 2006).

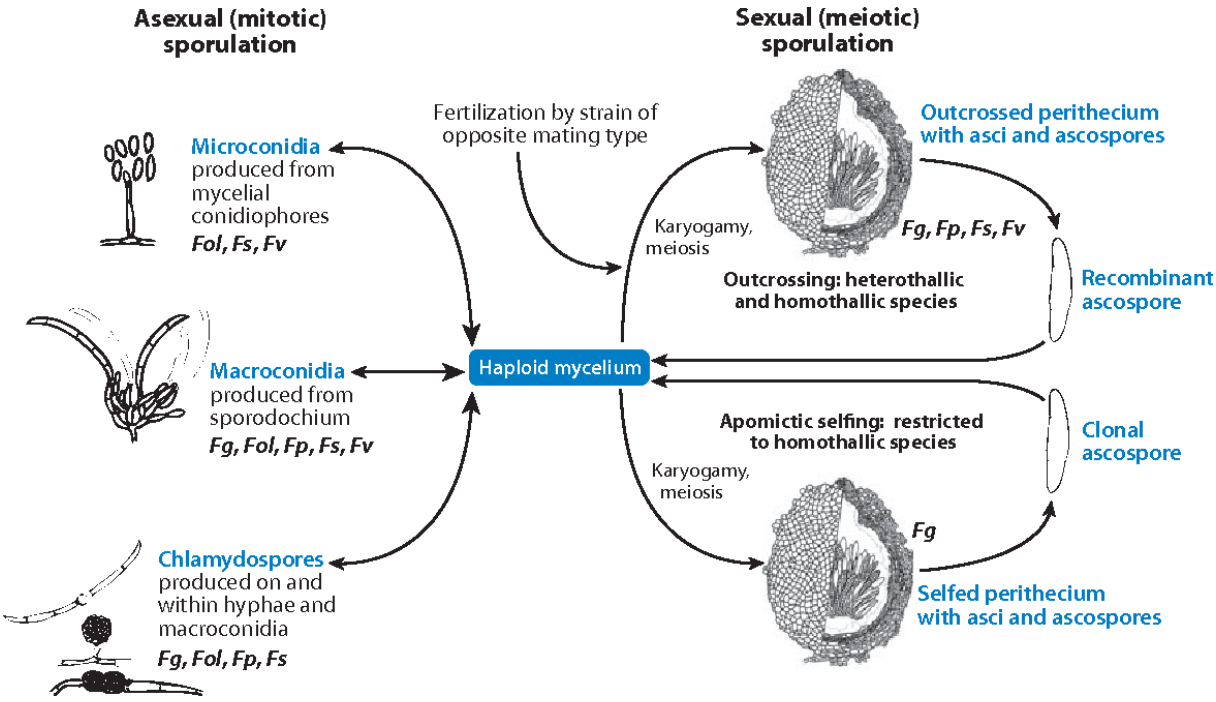


Figure 3. Generalized life cycle of *Fusarium*. During sexual reproduction, ascospores are produced in ascus contained within the flask-shaped perithecium through outcross (heterothallic species) or self-fertilization (homothallic species). Abbreviations: *Fg, F. graminearum*; *Fol*, *F. oxysporum* f. sp. *lycopersici*; *Fp*, *F. pseudograminearum*; *Fs, F. 'solani'* f. sp. pisi; *Fv, F. verticillioides* (Ma *et al*. 2013).

1.1.3 *F. graminearum*-specific features

Even though many *Fusarium* species appear to be harmless, several of them are among the known most important plant-pathogenic fungi, causing diseases in a large number of crops, such as wheat, rice, bean, soybean, and can also be harmful to humans and animals (Nucci and Anaissie 2007; Moretti 2009). One example is the plant pathogen *F. graminearum* **(Figure 4)**. It is the primary causal agent of Fusarium Head Blight (FHB) in barley and wheat, which is considered as one of the most devastating plant disease in the world (Windels 2000; Gilbert and Haber 2013). *F. graminearum* is a haploid ascomycete contains four chromosomes. Its whole genome (strain PH-1) has been sequenced with a total size of 48.4 Mb and a set of 14164 predicted genes (King et al. 2015). It is able to undergo asexual reproduction by producing macroconidia and chlamydospores or sexual reproduction by producing ascospores. Macroconidia are slender, thick-walled, 5 to 6 septate and sickle-shaped to almost straight, while ascospores are fusoid-shaped with 1-3 septate. *F. graminearum* is homothallic and thus during the teleomorph stage, perithecia **(Figure 4B)** can be formed without the need of out-cross, which makes it differs from other filamentous fungi (Leslie and Summerell 2006).

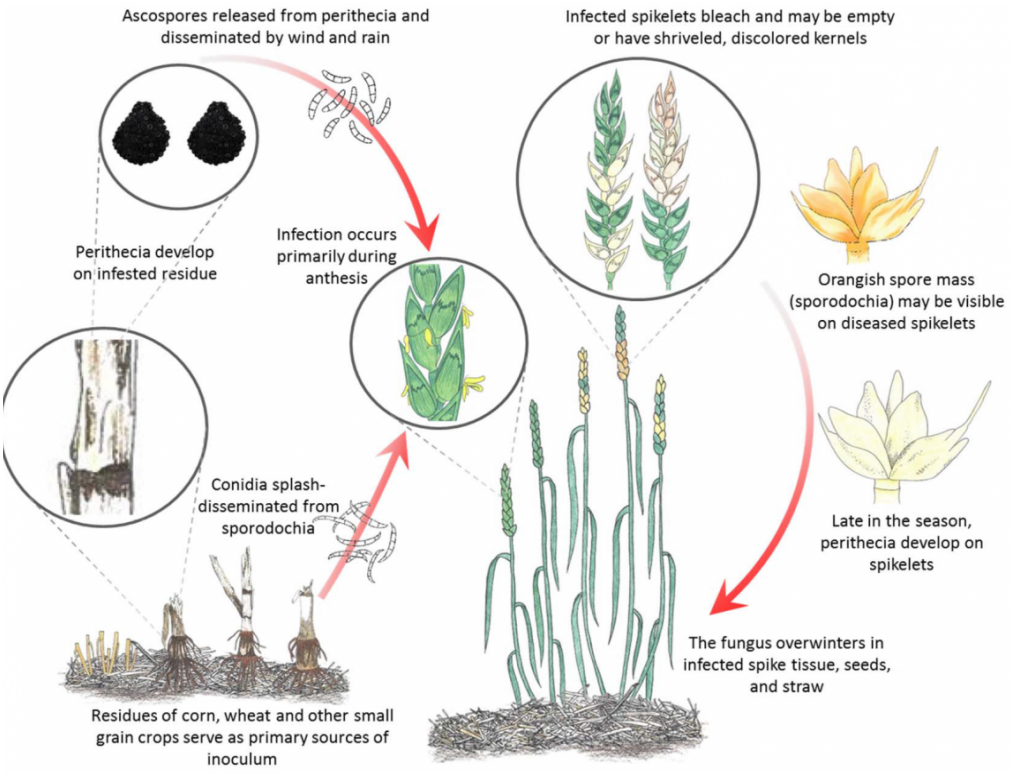
A **B**

Figure 4. Morphological features of *F. graminearum* (strain INRA812, PH-1). **(A)** 100 spores were incubated on agar complete medium at 25°C in the dark for five days; **(B)** Morphology of perithecia on carrot agar medium.

Infected plant debris on which the fungus overwinters as saprophytic mycelia is thought to be the primary inoculum for infection (Goswami and Kistler 2004). The development and maturation of *F. graminearum* conidia and perithecia occurs concurrently with the anthesis stage of cereal crops during spring, when the warm and moist weather is favorable for them (Markell and Francl 2003). Deposition of the sticky spores (primarily ascospores) by wind, rain or insects on or inside flowering spikes of the host plants initiates infection (Bushnell *et al*. 2003). Instead of direct penetration through the epidermis, fungal hyphae develop on the exterior surfaces of florets and glumes, allowing them to grow toward stomata and other susceptible sites within the inflorescence including anthers and stigmas. Through the vascular tissue in the rachis and rachilla, the fungus spreads from spikelet to spikelet **(Figure 5A)** (Bushnell *et al*. 2003; Yang *et al*. 2013).

Typical symptoms of disease are brown, dark purple to black necrotic lesions formed on the exterior surface of wheat florets and glumes as well as the bleaching of inflorescence tissue **(Figure 5B and 5C)**. In barley, symptoms are not as visible as in wheat. Infected spikelets may show a browning or water-soaked appearance, while the kernels show a tan to dark brown discoloration. In both wheat and barley, pink to salmon-orange spores of the fungus could be

A



B

C

Figure 5. FHB disease cycle and symptoms. **(A)** Disease cycle of FHB; **(B)** Wheat head infection with bleached spikelets ("Fusarium Head Blight (Scab) of Wheat") **(C)** Infected wheat kernels showing pink and white discolorations ("LSU Small Grains Data").

observed on infected spikelets, glumes and kernels during prolonged wet periods (Goswami and Kistler 2004). In addition to the losses of yield and quality, during the infection stages, *F. graminearum* possesses great potential to produce significant levels of mycotoxins such as trichothecenes (TCT) and zearalenones (ZEA) **(See details in Section 1.2)**, which are toxic to human and other animals (McMullen, Jones, and Gallenberg 1997).

Deoxynivalenol (DON), a type B trichothecene (TCTB) produced by *F. graminearum* is essential virulence factor associated with disease progression. In wheat, it not only leads to the inhibition of protein synthesis and mitochondrial function of host cells, but also apoptosis. It seems that DON production is tightly linked with fungal conidia and ascospore formation (Gunupuru, Perochon, and Doohan 2017). In addition, DON could be used by *Fusarium* to disturb the plant defense pathway at several critical time points of infection to assure successful colonization and symptom development (Calvo *et al*. 2002; Audenaert *et al*. 2013).

1.2 Potential roles of natural products of filamentous fungi with a focus on mycotoxins

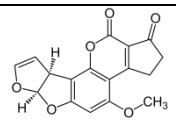
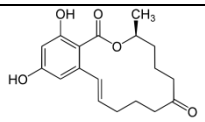
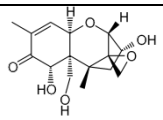
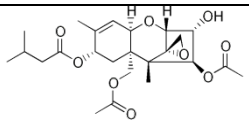
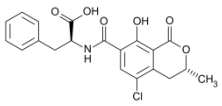
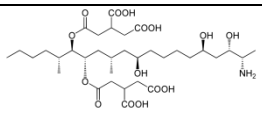
SMs are low molecular weight molecules that are not essential for organism growth and development, but they play roles in important functions such as protection, competition, and species interactions. Filamentous fungi are an invaluable source of natural SMs which are difficult to mimic through chemical synthesis (Baral, Akhgari, and Metsä-Ketelä 2018). Among them, particular interest is given to antimicrobials because of their functions in pharmaceutical industries to treat bacterial infections (Aiken *et al*. 2014). For example, the β-lactam antibiotics such as penicillins and cephalosporins, produced by certain species of *Penicillium* and *Acremonium*, are the most widely applied antibiotics in the world (Hamad 2010). Lovastatin and mevastatin are another compounds with clinical importance used as cholesterol-lowering agents which are primarily derived from *Aspergillus terreus* and *Penicillium citrinum*, respectively (Manzoni and Rollini 2002). Meanwhile, filamentous fungi have also been exploited for wastewater treatment based on their ability to produce various enzymes (Sankaran *et al*. 2010).

In addition to these metabolites with potential benefits, filamentous fungi also produce undesirable compounds such as mycotoxins. The term 'mycotoxin' originated from the Greek word for fungus 'mykes' and the Latin word 'toxicum' meaning poison (Turner, Subrahmanyam, and Piletsky 2009). They are defined as highly toxic SMs produced by saprophytic or endophytic molds, which infect a broad range of crops during their growth in the field or during storage (Niu 2010; Gruber-Dorninger, Jenkins, and Schatzmayr 2019). According to Food and Agriculture Organization of United Nations (FAO) surveys, about half of crops in the world may be contaminated with mycotoxins (FAO and Collette 2011).

Mycotoxins are of low molecular weight (<700 Da), with highly variable structure. Between 300 and 400 mycotoxins have been isolated and identified (Berthiller *et al*. 2007), those with most concern are aflatoxins (*e.g.,* Aflatoxin B1, or AFB1), fumonisins, ZEA, TCTB (*e.g.,* DON), type A trichothecene, or TCTA (*e.g.,* T-2 toxin), and ochratoxin (OTA) (Gruber-Dorninger, Jenkins, and Schatzmayr 2019; Alberti, Foster, and Bailey 2017; Hussein and Brasel 2001). Most of these toxins are released by several species of fungi and each strain can produce more than one mycotoxin as listed in **Table 1** (Alshannaq and Yu 2017; Weidenbörner 2007). Furthermore, each plant may be infected by different species at the same time, thus leading

to the interaction between mycotoxins and the possible occurrence of synergistic effects (Kubena *et al*. 1997; Grenier and Oswald 2011). Once ingested by humans and other animals, mycotoxins can cause diseases or toxic syndromes that may lead to death (Bennett and Klich 2003b). The main toxic effects include carcinogenicity, genotoxicity, nephrotoxicity, hepatotoxicity, estrogenicity, reproductive and digestive disorders, immunosuppression and dermal effects **(Table 1)** (Bryden 2012; Grenier and Oswald 2011)

Table 1. Chemical Structures, major genera of producing fungi and health effects of common mycotoxins.

Mycotoxins	**Chemical Structure**	**Producing Fungi**	**Commodities Affected**	**Mycotoxicosis**
Aflatoxins		*A. flavus* *A. parasiticus*	Corn, cotton, seed, peanuts, soy	Hepatotoxic, carcinogenic, teratogenic
Zearalenone		*F. graminearum* *F. verticillioides* *F. culmorum*	Corn, wheat, barley	Estrogenic effects, male infertility
Deoxynivalenol		*F. graminearum* *F. culmorum*	Wheat, barley, oats, rye, corn, rice	Digestive and blood disorders, infertility
T-2 toxin		*F. sporotrichioides,* *F. poae* *F. acuinatum*	Corn, wheat, barley	Digestive and blood disorders, infertility
Ochratoxin A		*A. ochraceus* *A. nigri* *P. verrucosum*	Wheat, barley, oats, corn, others	Nephrotoxic, carcinogenic, immune suppression
Fumonisins		*F. verticillioides* *F. proliferatum*	Corn	Nephro- and hepatotoxic, immune suppression

As mycotoxins are invisible, tasteless and chemically stable, they are difficult to avoid. To minimize their harmful effects on both humans and animals, strict regulations have been established worldwide to control the maximum amount of ingestion (Niu 2010). For example,

in China, in formula feeds for piglets and young poultry, concentration of fumonisin B1 (FB1) should not exceed 10 ug/kg, while the concentration of DON should be lower than 1mg/kg ("Regulations" 2019) Cereals such as corn, wheat, barley are main sources for food by-product and feed production. Therefore, in addition to direct health risks, contamination of cereals with mycotoxins also cause economic losses at all levels including crop and animal production, processing and distribution (Wu 2007; Robens and Cardwell 2003). Thus, it is of great importance to control the production of mycotoxins by filamentous fungi.

1.2.1 Fumonisins

Fumonisins, a group of mycotoxins that were first discovered in 1988 (Bennett and Klich 2003b) and are mainly produced by *Fusarium verticillioides* and *Fusarium proliferatum*. They occur worldwide primarily in maize, causing the so-called Gibberella Ear Rot (GER), especially when cultivated in warm regions (Glenn 2007; Voss, Smith, and Haschek 2007). For example, in 2014, fumonisins were detected in more than 98% of corn product samples collected from Shandong Province in China (F. Li et al. 2015). They can also contaminate sorghum, wheat, barley, soybean, figs, black tea, and medicinal plants (Alshannaq and Yu 2017).

As illustrated in **table 1**, fumonisins are structurally different from most other mycotoxins and totally soluble in organic solvent (Blackwell *et al*. 1996). Among them, FB1 is the most commonly found and toxic. Fumonisins affect animals in different ways by disrupting sphingolipid metabolism, which are important components of cellular membrane and neural tubes (Dutton 1996; Marasas 1995; E. Wang *et al*. 1991). In humans, they may be cancer promoters (Eric W. Sydenham *et al*. 1991). In China, northeast Italy and South Africa, studies showed that the occurrence of FB1 is correlated with the occurrence of a higher incidence of esophageal cancer (Peraica *et al*. 1999; H. Wang *et al*. 2000; Persson *et al*. 2012). Meanwhile, they lead to leukoencephalomalacia in equines (Marasas *et al*. 1988) and rabbits (Bucci, Hansen, and LaBorde 1996), and cause hepatotoxic and carcinogenic effects as well as apoptosis in the liver of rats (Gelderblom *et al*. 1996; Pozzi *et al*. 2001).

1.2.2 Zearalenone

ZEA and derivatives are macrocyclic β-resorcyclic acid lactones which are biosynthesized through polyketide pathway by *F. graminearum*, *F. culmorum* mostly, as well as other *Fusarium* species (Hueza *et al*. 2014). These fungi can be found on almost all continents, and

they are able to infect wheat, barley, rice, maize, and other crops during both pre- and post-harvested stages, resulting in the contamination of human foods and animal feed worldwide (Zinedine *et al*. 2007).

Once ingested by human, swine or other animals, ZEA is absorbed rapidly by the gastrointestinal tract and bio-transformed into α- and β-zearalenol, which are its major biologically active reductive metabolites. The binding affinity of both ZEA and its metabolites to estrogen receptors makes them become highly estrogenic, especially α-zearalenol (Bertrand Grenier and Applegate 2013; Zinedine *et al*. 2007; Binder and Krska 2012). It is reported that their hormonal action is higher than that of most other naturally occurring non-steroidal estrogens, such as soy and clover isoflavones (Bennett and Klich 2003). In pigs, hyperestrogenic syndrome appears with an dietary concentration of ZEA as low as 1 ppm; higher dosage can lead to disrupted conception, abortion and other reproductive problems (Kurtz and Mirocha 1978).

1.2.3 Trichothecenes

TCT constitute a family of more than 200 sesquiterpenoid metabolites. Each TCT contain a common 12,13-epoxytrichothene skeleton and a double bond at the 9, 10 carbon positions with various side chain substitutions of oxygen containing functional groups at possible sites on carbons 3, 4, 7, 8, and 15 (*Protection Against Trichothecene Mycotoxins* 1983). According to the types of these functional groups, TCT family can be divided into four groups: type A, B, C, and D. TCTA have a hydrogen or ester at the C-8 position such as T-2 toxin, while TCTB contains a ketone at the same position. Type C trichothecenes have an extra carbon 7, carbon 8 epoxide group such as crotocin. Concerning the type D trichothecenes, additional rings with diverse functional groups between carbon 4 and carbon 15 appear, for example, the roridin A and satratoxin H (McCormick *et al*. 2011).

Among the four types of TCTs, TCTA and TCTB are the most common and well-studied (Bennett and Klich 2003a). Exposure to these toxins induce feed refusal, immunological problems, vomiting, skin dermatitis, and hemorrhagic lesions (Ueno 1985; Pestka 2008). They are also phytotoxic and act as virulence factors in wheat head scab (Proctor, Hohn, and McCormick 1995).

1.2.4 The trichothecene biosynthetic pathway

My project focuses on TCTB. They are mainly produced by *F. graminearum* and *F. culmorum*. TCTB can be classified into two chemotypes: chemotype I, including DON and its acetylated derivatives 3-acetylDeoxynivalenol (3-ADON) and 15-acetylDeoxynivalenol (15-ADON); chemotype II, including nivalenol (NIV) and its acetylated derivative 4-acetylNivalenol (4-ANIV) (*syn*. fusarenone-X) (E. W. Sydenham *et al*. 1991).

Most genes involved in the TCT biosynthetic pathway have been identified. They are located at three different *loci* on different chromosomes **(Figure 6):** a 35-kb long trichothecene biosynthetic gene cluster (*Tri* cluster) containing 12 genes that control the biosynthesis on chromosome 2 (Brown *et al*. 2004); a two-gene *Tri1-Tri16* cluster on chromosome 1; and a single gene *Tri101* locus that on chromosome 4 (Kimura, Matsumoto, *et al*. 1998; W Peplow *et al*. 2003). Besides, a *Tri15* gene is also located outside the core cluster on chromosome 3 and involves in the negative control of trichothecene biosynthesis (Alexander *et al*. 2004).

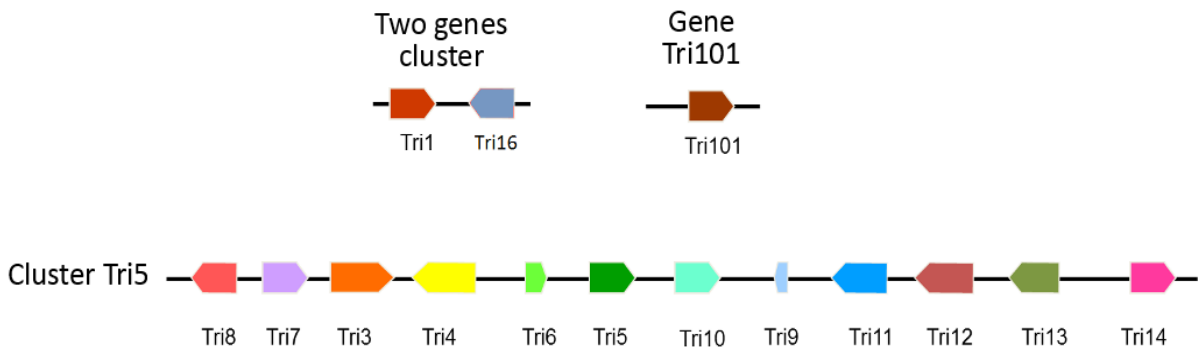


Figure 6. Gene clusters involved in trichothecene biosynthetic pathway in *Fusarium*, including a *Tri* cluster containing 12 genes, a two-gene *Tri1-Tri16* cluster and a single gene *Tri101.*

The pathway begins with the cyclization of farnesyl pyrophosphate, a primary common metabolic intermediate of different secondary metabolism, to form trichodiene **(Figure 7)**. The reaction is mediated by the terpene cyclase trichodiene synthase which is encoded by *Tri5* (T. M. Hohn and Vanmiddlesworth 1986; T. M. Hohn and Beremand 1989). Trichodiene undergoes six steps of oxygenations catalyzed by a cytochrome P450 monooxygenase encoded by *Tri4* (Thomas M. Hohn, Desjardins, and McCormick 1995) to add four oxygens at C-2, C-3, C-11, and the C-12, C-13-epoxide to synthesize the intermediate isotrichotriol (McCormick, Alexander, and Proctor 2006b). Isotrichotriol is converted to isotrichodermol by a non-enzymatic isomerization and cyclization (McCormick *et al*. 1990). This step is followed

by a reaction to form isotrichodermin with the help of acetyltransferase encoded by *Tri101*, which is involved in the self-production of the trichothecenes-producing organisms (Kimura, Kaneko, *et al*. 1998). Under the control of *Tri11*, a hydroxyl group is then added to C-15 and subsequently acetylated to form calonectrin, a central metabolite (Alexander, Hohn, and McCormick 1998; McCormick, Hohn, and Desjardins 1996). These 10 steps are common for the production of TCTB and T-2 toxin.

Afterwards, the pathway divides into three directions. In DON producers (*e.g., F. graminearum*), calonectrin is converted to either 3-ADON, 15-ADON or DON by the catalyzation of products encoded by *Tri1* and *Tri8*. Owing to polymorphisms in the sequences of the esterase-encoding gene *Tri8,* formation of 3-ADON or 15-ADON occurs in a strain specific manner in *F. graminearum* (Alexander *et al*. 2011). In NIV and T-2 toxin producers, a hydroxyl group is added at C-4 of calonectrin (controlled by *Tri13*) and subsequently acetylated (under the control of *Tri7*) to produce 3, 4, 15-triacetoxyscirpenol (Lee *et al*. 2002). In *F. graminearum*, this intermediate is converted to NIV or 4-ANIV by the metabolization of enzymes encoded by *Tri1* and *Tri8*. In *Fusarium sporotrichioides*, another hydroxyl group is added to C-8 controlled by *Tri1*, followed by the addition of an isovaleryl moiety controlled by *Tri16*. Finally, the acetyl group is removed from the C-3 position by an esterase controlled by *Tri8* to generate T-2 toxin (McCormick, Alexander, and Proctor 2006a).

In addition to these genes involved directly in the pathway, there are several specific regulators. Deletion mutants of the positive transcription factors *Tri6* and *Tri10* in *F. graminearum* were created in order to understand genome-wide regulatory control of toxin synthesis (Seong *et al*. 2009). Results showed that transcript levels for over 200 genes, including those related to housekeeping functions, secondary metabolism, and pathogenesis, were altered in the *Tri6* deleted mutant. Therefore, *Tri6* is a global regulator rather than specific to the TCT pathway. They also found that *Tri6* negatively regulated the expression of *Tri10* but deletion of *Tri10* had no obvious effect on *Tri6* expression (Seong *et al*. 2009).

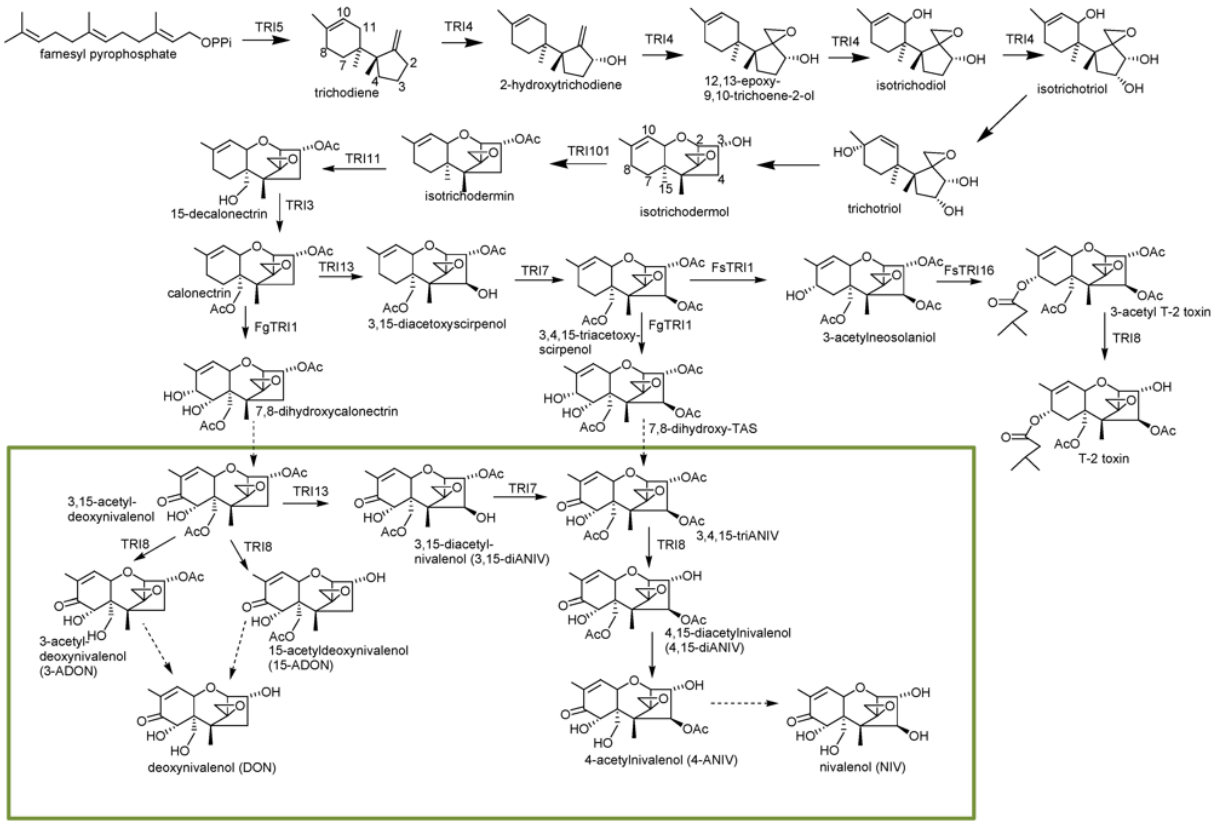


Figure 7. Proposed biosynthetic pathway of trichothecene in *Fusarium*. Genes encoding enzymatic step are indicated near each arrow, representing one step. Green box identifies TCTB (Susan P. McCormick et al. 2011).

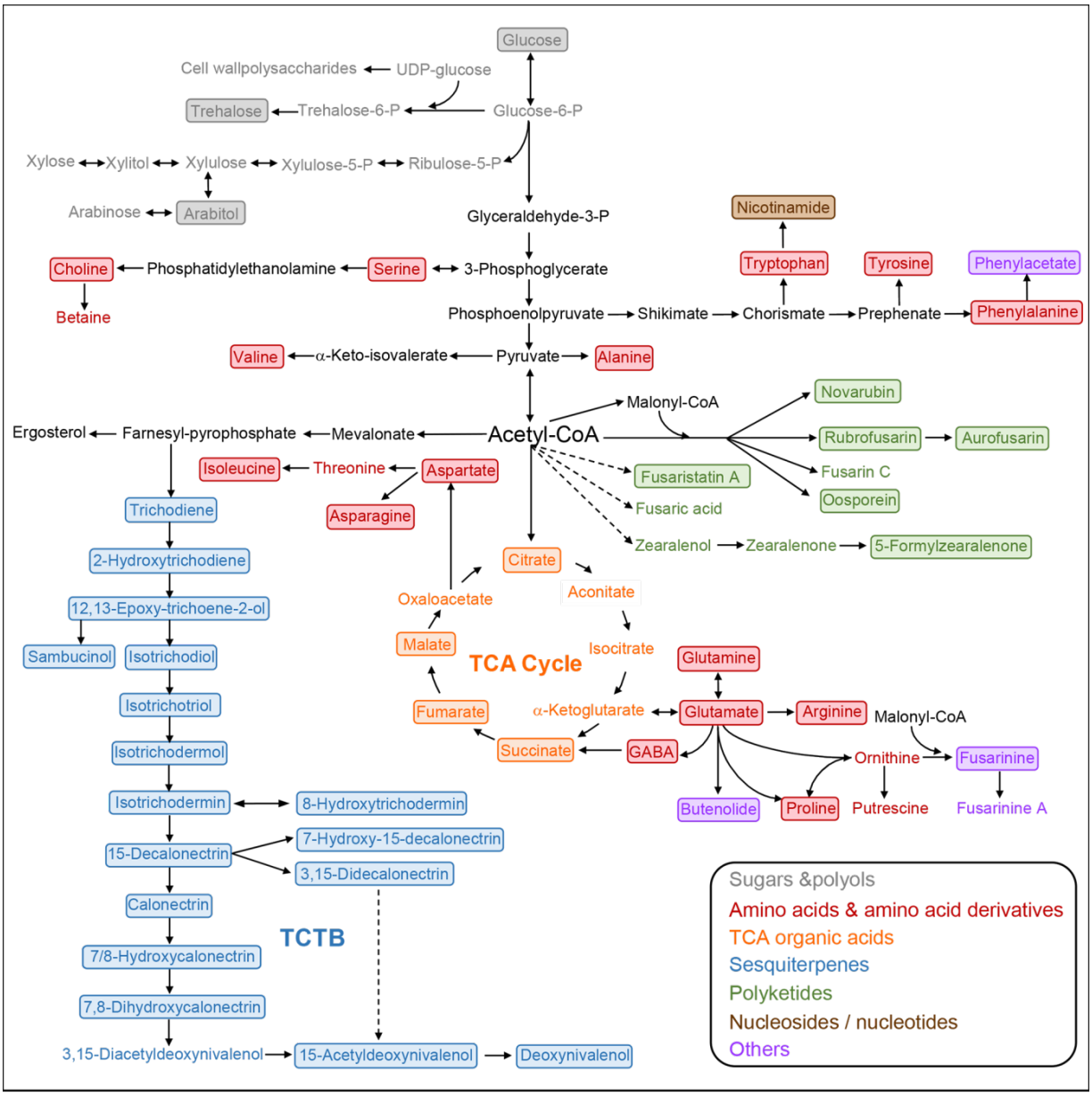


Figure 8. Overview of metabolic pathways indicating the relationship between the TCTB biosynthesis and other central or specialized processes in *F. graminearum* (Atanasova-Penichon *et al*. 2018).

Notably, a predicted transcription factor *Tri14*, which is also located in the "*Tri* cluster", is required for high virulence and DON production on wheat but not for DON synthesis in *vitro* in *Fusarium* species (Brown *et al*. 2002; Dyer *et al*. 2005). Finally, *Tri15*, a gene that represents a fourth TCT locus, is predicted to encode a Cys (2) - His (2) zinc finger protein. It is reported in *F. sporotrichioides* to act as a negative regulator of at least some of the TCT biosynthesis. However, in *F. graminearum*, *Tri15* is found to be unlinked to the main TCT biosynthetic gene cluster (Alexander *et al*. 2004).

Recently, analyses combined with ^{1}H nuclear magnetic resonance and liquid chromatography-quadrupole time of flight-mass spectrometry were performed to elucidate the association of TCTB biosynthesis with other central and specialized processes in *F. graminearum* (Atanasova-Penichon *et al*. 2018). Results demonstrated that the inhibition of TCTB production induced by caffeic acid exposure was associated with significant changes in the secondary and primary metabolism of *F. graminearum* including the accumulation of several polyketides, alteration in tricarboxylic acid cycle, and modifications in the metabolism of several amino acids and sugars, even though the fungal growth was not affected. The authors found that, as shown in **Figure 8**, the TCTB biosynthetic pathway TCTB is closely linked with other primary or secondary metabolic pathways, suggesting that TCTB plays a multifaceted role in the life cycle of *F. graminearum* (Atanasova-Penichon *et al*. 2018).

1.3 Regulations of SMs biosynthesis by environmental factors

1.3.1 Carbon and nitrogen source

In many fungi, signals or substrates from the environment play an important role in controlling the production of mycotoxins, especially carbon resources. When a more readily available carbon source is present in the medium, certain enzymes required for less-favored carbon source will be switched off to help microorganisms to precisely adapt their physiology to the environment. This mechanism is called carbon catabolite repression, or CCR. It has been studied extensively in *Aspergillus* species, and is mediated by the transcription factor CreAp, a C_2H_2 DNA-binding protein (Katoh *et al*. 2007; Ries *et al*. 2016). In *A. nidulans*, expression of isopenicillin-N-synthetase gene is repressed in glucose-containing medium and penicillin biosynthesis is inhibited (Espeso and Peñalva 1992). In order to see the effects of different types of carbon resources on TCT production in *F. graminearum*, nine strains of *F. graminearum* were cultivated in media containing 12 various carbon sources (Jiao, Kawakami, and Nakajima 2008). Significantly higher levels of TCT (DON and 3ADON) production were commonly observed in the presence of sucrose, 1-kestose and nystose for all of the strains tested. Meanwhile, *Tri4* and *Tri5* expressions were upregulated in sucrose-containing medium, but not with glucose. To further investigate whether trichothecene biosynthesis was subject to CCR, glucose was added into the sucrose-containing medium. However, TCT accumulation was not repressed, indicating that there may be other factors involved in the regulation of trichothecene production rather than CCR (Jiao, Kawakami, and Nakajima 2008).

Nitrogen is an essential requirement for fungal growth, and fungi are able to use a wide variety of compounds as nitrogen sources. Utilization of different nitrogen source is selective. Generally, favored nitrogen sources, such as glutamine and ammonium, are used preferentially (Wong, Hynes, and Davis 2008). The global regulation of nitrogen source utilization has been widely investigated in *A. nidulans*, a process known as nitrogen metabolite repression (NMR). Up to now, major transcription factors that have been identified underlying this regulation are GATA factors. These factors share a common DNA binding motif (Cys-X2-Cys-X17-Cys-X2-Cys) that recognizes a core 5'-GATA-3' sequence (Scazzocchio 2000). In *A. nidulans*, AreA protein is a positively acting GATA factor. The AreAp deleted mutants lose the ability to use any nitrogen source other than glutamine and ammonium (Arst and Cove 1973; Marzluf 1997). The AreB protein contains an N-terminal

GATA zinc finger and a C-terminal leucine zipper domain, and is generally regarded as the negative counterpart to AreAp even though the function of AreBp seems to be more complex (Conlon *et al*. 2001; Dzikowska *et al*. 2003). Orthologues of AreAp or AreBp have been identified in *F. graminearum*, *F. fujikuroi*, *A. flavus*, and *Penicillium chrysogenum*. They were shown to be involved in various secondary metabolism pathways including DON, bikaverin, aflatoxin and penicillium biosynthesis (Haas and Marzluf 1995; Ehrlich and Cotty 2002; Mihlan *et al*. 2003). It was further reported that AreAp regulates the production of mycotoxins directly or indirectly, independently from nitrogen status, and plays a role in the utilization of certain amino acids in *F. graminearum* (Giese, Sondergaard, and Sørensen 2013). Besides, a study identified particular amine compounds (*e.g.,* ornithine, agmatine, and putrescine) that could support, in defined media, levels of DON production and *Tri5* expression equivalent to those observed during infection of *F. graminearum*. All these compounds belong to the arginine-polyamine biosynthetic pathway in plants, which means that metabolites produced in this pathway may promote TCT production (Gardiner, Kazan, and Manners 2009a). These authors also performed a global analysis of fungal gene expression using the Affymetrix *Fusarium* GeneChip during culture under DON-inducing conditions (with agmatine) compared with non-inducing conditions. Results indicated that agmatine differentially regulates a large number of fungal genes, including both known and previously uncharacterized putative SM biosynthetic gene clusters. Further study found three of the differentially regulated genes were under the control of *Tri6* transcriptional regulator (Gardiner, Kazan, and Manners 2009b). All in all, even though nitrogen source selection and DON production in *F. graminearum* are linked, further studies should be carried out to investigate and address the underlying mechanisms.

1.3.2 pH

pH is a critical host-related environmental signal that can limit or enhance growth, reproduction, toxin production, and virulence of phytopathogenic fungi (Caracuel *et al*. 2003; Sánchez-Rangel *et al*. 2018). Studies showed that filamentous fungi have the ability to sense pH and respond with regulatory system (Peñalva and Arst 2002). For example, *A. nidulans* adapts to pH changes by adjusting the secretion of enzymes with optimal activity accordingly, which is mediated by a mechanism that tailors genes expression to ambient pH (Caracuel et al. 2003). It involves a highly conserved zinc finger transcription factor PacCp

that is activated by at least six components encoded by *palA, palB, palC, palF, palH, palI* (Nahas, F. Terenzi, and Rossi 1982; Tilburn *et al*. 1995). Once active, PacCp is translocated to the nucleus where it can recognize the consensus DNA site 5'-GCCAAG-3' and consequently up-regulate the transcription of alkaline-pH responsive genes, and down-regulate acid responsive genes (Tilburn *et al*. 1995; Espeso and Peñalva 1996).

Homologues of *A.nidulans* PacCp have been identified in various filamentous ascomycetes, which were found involved in the production of penicillin, cephalosporin C and fumonisin in *P. chrysogenum* (Suárez and Peñalva 1996), *Acremonium chrysogenum* (Schmitt, Kempken, and Kück 2001) and *F. verticillioides* (Flaherty *et al*. 2003), respectively. In *F. graminearum* and *F. culmorum*, neither the toxin nor *Tri* transcripts were detected when the pH was maintained neutral (Merhej *et al*. 2010). However, shifting from neutral to acidic pH by mycelium transfer induced *Tri* genes expression and toxin accumulation, especially *Tri5* and *Tri101*. Meanwhile, the induction of toxin production seems not to depend on strains or chemotype (Merhej *et al*. 2010). By constructing Pac1p (ortholog of PacCp) deleted mutant of *F. graminearum*, further study revealed that Pac1p negatively regulates *Tri* gene expression and toxin production, as the mutant exhibited an over-expression of *Tri* genes and an increase in the toxin production under acidic pH but showed a reduced development under neutral and alkaline pH. In fact, in *F. graminearum*, promoters of various *Tri* genes including *Tri6* and *Tri10* contain the sequence "GCCARG", which corresponds to the consensus binding sequence for PacCp in *A. nidulans*. Therefore, the repression observed here could be exerted either directly by binding to the promoters of various *Tri* genes or through the regulation of a common regulator of *Tri* genes such as *Tri6* which is quickly and strongly repressed upon pH change (Jawad Merhej, Richard-Forget, and Barreau 2011). Finally, the combination of low pH and supplementation with amines results in significantly enhanced expression of the *Tri5* gene and increased DON production during axenic growth of *F. graminearum*. It was hypothesized that nitrogen source may, at least partially, act through triggering an acidification of the extracellular environment (Gardiner *et al*. 2009).

1.3.3 H_2O_2

During plant-pathogen interactions, various ways have been developed by the hosts to prevent infections. The earliest and most widely studied defense event is that plants will release reactive oxygen species (ROS) such as H_2O_2 as an early response to pathogens. In

response to such an oxidative burst, pathogens could induce mechanisms, broadly called oxidative stress response (OSR), to scavenge elevated ROS levels (Shetty *et al*. 2008; Heller and Tudzynski 2011). It was found that, in *F. graminearum*, daily exogenous supplementation of H_2O_2 enhanced DON accumulation (Ponts *et al*. 2006). On the other hand, adding catalase to the culture medium leads to the degradation of H_2O_2, and meanwhile, a strong decrease in the accumulation of DON and 15-ADON. The induction or suppression of DON production by adding H_2O_2 or catalase were accompanied by a up or down regulation of *Tri* genes expression, respectively, in particularly *Tri4, Tri5*, and *Tri12* (Ponts *et al*. 2007). In addition, induction of trichothecene by H_2O_2 could be chemotype-dependent. Indeed, accumulation of toxins were less affected by adding H_2O_2 in NIV-producing strains compared to DON-producting strains, which may relate to a H_2O_2-metabolizong capacity in NIV isolates (Ponts *et al*. 2009). However, an opposite result was observed for the oxidative stress caused by diamide: the global level of TCTB production was drastically increased by the treatment with diamide regardless of the chemotype, suggesting the intervention of different response mechanisms (Ponts *et al*. 2009).

Yap1p is a well-known transcription factor activated by oxidative stress in the yeast *Saccharomyces cerevisiae* (Moye-Rowley 2003; Rodrigues-Pousada, Menezes, and Pimentel 2010). Briefly, upon exposure to oxidative stress, Yap1p could activate the transcription of target detoxification genes, such as *cta1* and *ctt1*, coding respectively for peroxisomal and cytosolic catalase (Yan, Lee, and Davis 1998; Jamieson 1998). In the Fgap1p (ortholog of yeast Yap1p) deleted mutant of *F. graminearum*, activation of OSR gene expression and toxin accumulation in response to oxidative stress was no longer observed (Montibus *et al*. 2013). This result indicated that the TCTB pathway is linked to the metabolism of H_2O_2 in *F. graminearum* with Fgap1p playing a central role. Conversely, antioxidant compounds were shown to inhibit toxin production in *F. graminearum*.

1.3.4 Light

Fungi use light as a source of information rather than a source of energy like plants do. They respond to illumination in various ways and develop considerable adaptations in their metabolic pathways upon growth in light or after perception of a light pulse (Tisch and Schmoll 2010). Most of current knowledge derives from the model *A. nidulans*. Formation of sexual fruiting bodies and production of certain SMs occur preferentially in darkness and are

coordinately inhibited by light as an external signal. In contrast, formation of asexual spores is promoted by light (Bayram *et al*. 2008; Sarikaya Bayram *et al*. 2010). This regulation is known to be mediated by the VeA-VelB-LaeA complex. VeAp is encoded by *velvet* identified in 1965 (Käfer 1965). VelBp interacts with the N-terminus of VeAp. They can enter the nucleus in darkness and act as activation of sexual development and inhibition of asexual development (Calvo 2008). Illumination reduces the cellular amounts of VeAp and VelBp. In nucleus, they assemble with the non-velvet protein LaeA, a master epigenetic regulator of secondary metabolism. Therefore, the VeA-VelB-LaeA complex regulates not only light-responding development but also SM production including sterigmatocystin, penicillin and many other compounds (Tisch and Schmoll 2010). Besides, VeAp could interact with the phytochrome-like red light receptor FphAp, therefore forming a complex which also comprises LreAp and LreBp, the *A. nidulans* orthologs of the *N. crassa* photoreceptors WC-1 (for white collar) and WC-2 (Purschwitz *et al*. 2008; Purschwitz, Müller, and Fischer 2009).

The velvet and LaeA proteins are conserved in various genera of fungi. Homologues of VeA act as positive regulators of genes involved in biosynthesis of cephalosporin C in *A. chrysogenum* (Dreyer *et al*. 2007), or production of the deleterious mycotoxins fumonisin, fusarin C, TCT or DON, beauvericin in the plant pathogens *F. verticillioides*, *F. fujikuroi*, *F. graminearum* and *F. oxysporum*, respectively (Myung *et al*. 2009; Wiemann *et al*. 2010; Jiang *et al*. 2011; Jawad Merhej *et al*. 2011; López-Berges *et al*. 2013).

1.4 Dynamics of chromatin structure and SMs production

The complex between DNA and proteins in eukaryotes called chromatin, whose basic unit is nucleosome. A nucleosome core particle (NCP) consists of two copies of each of the core histones H2A, H2B, H3, and H4, to form the histone octamer around which approximately 146 bp of DNA wrap in 1.67 left-handed super helical turns (Hanson *et al*. 2003; Bargaje *et al*. 2012). The steps of nucleosome assembly are as following: first, a tetrasome consisting of an $(H3{\cdot}H4)_2$ tetramer is formed on the DNA followed by the addition of two H2A-H2B dimers. (Krude 1995; Mazurkiewicz, Kepert, and Rippe 2006). Finally, NCPs are held together by stretches of linker DNA to form a 10-nm chromatin fiber, in association with linker histones such as H1 and its isoforms (Alberts *et al*. 2008). The chromatin can then be further condensed by coiling into 30-nm fibers **(Figure 9)**.

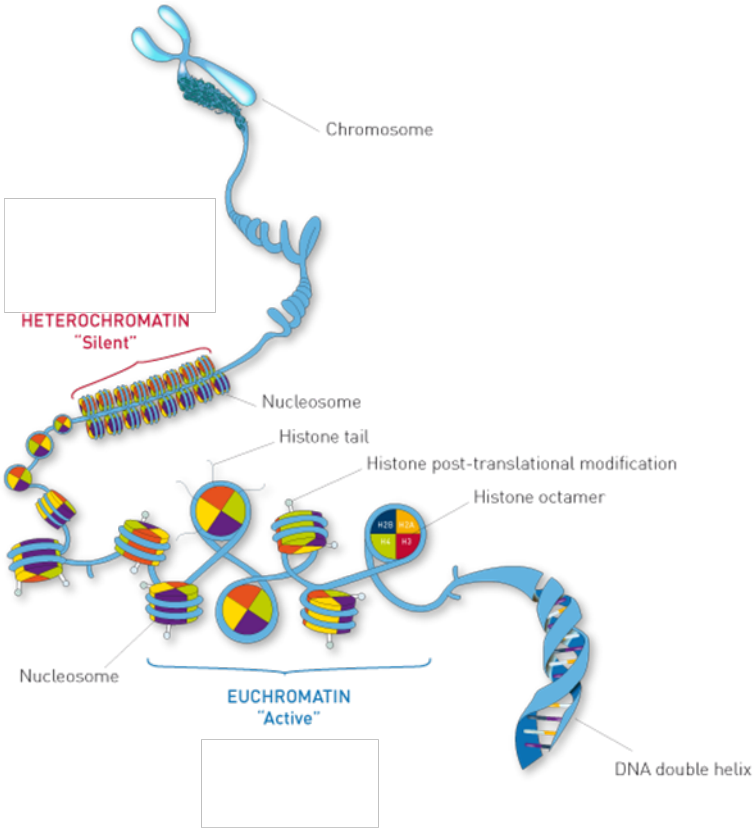


Figure 9. Typical structure of chromatin. Each nucleosome consists of eight histones (purple: H2B; orange: H2A; green: H4; red: H3) with DNA wrapped around, which helps its packaging into a compact form that fits inside the cell nucleus. Changes in chromatin structure are associated with DNA replication and transcription ("Chromatin Immunoprecipitation Sequencing (ChIP-Seq) Analyisis & Services | Diagenode").

According to the level of condensation, chromatin can be divided into two types: euchromatin and heterochromatin. During the interphase of cell life cycle, most chromatin is

relatively decondensed – it is called euchromatin – especially in genomic regions containing genes actively transcribed (Cooper 2000; International Human Genome Sequencing Consortium 2004). In contrast, there is about 10% of interphase chromatin that is in a super condensed state called heterochromatin, which is transcriptionally inactive and typically contains highly repeated DNA sequences, such as those present at centromeres and telomeres. Heterochromatin is enriched during mitosis, to facility their distribution to daughter cells. Such condensed chromatin can no longer be used as a template for RNA synthesis, thus transcription stops during mitosis (Volpe *et al*. 2002; Ou *et al*. 2017).

Chromatin is a highly dynamic architecture intimately linked to the control of gene expression in eukaryotes. This property mainly results from several parameters: (1) ATP-dependent chromatin remodeling complexes that allow reposition (slide, twist or loop) of nucleosomes, eviction of histones away from DNA or exchange of histone variants (Lusser and Kadonaga 2003a; Wang, Allis, and Chi 2007); (2) Histone PTMs which leads to structure and function alteration (Fan *et al*. 2015); and (3) DNA methylation which affects gene expression (Tost 2010).

1.4.1 Chromatin remodeling

- **Protein complexes that remodel chromatin**

The process 'chromatin remodeling' is mediated by several chromatin remodeling complexes that utilize energy derived from adenosine triphosphate (ATP) hydrolysis to alter nucleosome structure or conformation and, thereby, regulate the access of transcription factors to their cognate DNA binding sites (Lusser and Kadonaga 2003b; Hota and Bruneau 2016). All chromatin remodeling complexes are made up of a single highly conserved ATPase, which belongs to SNF2 family, and multiple associated subunits. Generally, ATPase subunit binds and hydrolyzes ATP, while the associated subunits modulate the catalytic activity of the ATPase subunit and provide specificity to genome binding (Eisen, Sweder, and Hanawalt 1995). Based on the sequence similarity between their ATPase domains and the presence of other subunits, chromatin remodeling complexes can be divided into four major subfamilies including Switch/Sucrose Non-fermentable (SWI/SNF), Imitation SWI (ISWI), Chromodomain Helicase Deoxyribonucleic acid-binding (CHD), and Swr1-related Inositol requiring 80 (INO80) (Becker and Hörz 2002; Bao and Shen 2007). As showed in **Figure 10**, for each subfamily, the ATPase domain comprises an N-terminal DEXDc and a C-terminal HELICc subdomain,

separated by an insert region (Xu, Kanagaratham, and Radzioch 2013). The SWI/SNF family contains an HSA domain for actin binding, and a bromodomain which recognizes and binds to the acetylated histone tails. SWI/SNF regulates transcription, mitotic exit, and activation of weak replication origins. *In vitro*, it binds hyperacetylated nucleosomes, mobilizes nucleosomes, and acts as a directional DNA translocase. The ISWI family is characterized by SANT and SLIDE domains, which are important for histone binding. It is involved in transcription, DNA replication, and chromatin assembly. The CHD family contains two N-terminal chromodomains that play roles in the remodeling of chromatin structure and transcriptional regulation of genes. The INO80 family, similar with the SWI/SNF family, also has an HSA domain, however the insert region between the DEXDc and HELICc subdomains is three times longer than that of other three families. INO80 family is involved in the regulation of transcription, DNA repair and exchange of histone variant H2A.Z (Lusser and Kadonaga 2003a; Bao and Shen 2007; Hota and Bruneau 2016).

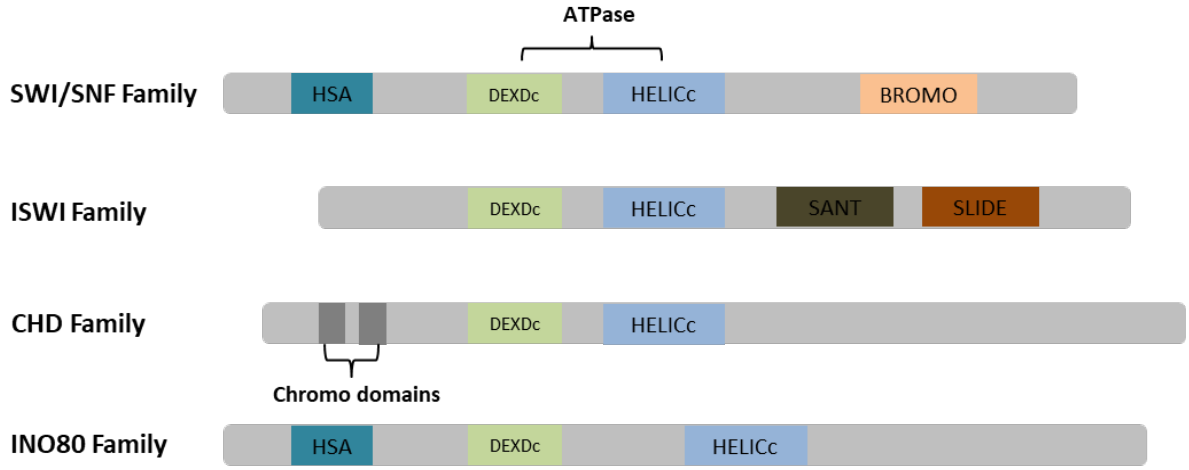


Figure 10. Domain architecture of SNF2 family of proteins. Domain organization of the catalytic subunits of SWI/SNF, ISWI, CHD and INO80/SWR subfamilies of chromatin remodelers are shown. All of these subunits are SNF2 family proteins. They all contain an ATPase domain, which consists of a DEXDc and HELICc domains, with each subfamily possessing additional domains. For example, in SWI/SNF family, there is an HSA domain (for actin binding) and a bromodomain (recognizes and binds to the acetylated histone tails); in ISWI family, there are SANT and SLIDE domains (important for histone binding); in N-terminal of CHD family, there are two chromodomains (chromatin remodeling and gene transcriptional regulation); the SWI/SNF family, also has an HSA domain.

- **Histone Variants**

In most eukaryotes, canonical histones including H2A, H2B, H3, H4 are encoded by multiple genes. They are only expressed during the S-phase of cell cycle after DNA replication (Talbert and Henikoff 2014). In metazoans, histone mRNAs are generally intronless and are not

polyadenylated. Instead, they have special stem-loop structures to trigger translation (Marzluff and Koreski 2017). However, introns exist in histone mRNAs of Basidiomycetes and filamentous Ascomycetes including *F. graminearum* (Nishida and Yun 2011). The individual paralogous (non-allelic) genes of a histone family may also encode related but distinct protein isoforms, commonly referred to as "histone variants" (Talbert *et al*. 2012). Most of them are encoded by a single gene containing one or more introns expressed throughout the cell cycle, and can thus be incorporated into nucleosomes during the whole cell cycles (Kaygun and Marzluff 2005; Talbert and Henikoff 2014; Maze *et al*. 2014). Up to now, histone variants of all canonical histones have been recognized, with the exception of H4 (M Arnaudo, Molden, and A Garcia 2011).

Table 2. List of histones including linker histone, canonical histones and histone variant in *F. graminearum* (strain PH-1) and their encoding genes.

	Name	Gene ID
Linker histone	H1	FGRAMPH1_01G20501
Canonical histone	H3	FGRAMPH1_01G14931
	H4	FGRAMPH1_01G17029
	H2B	FGRAMPH1_01G26111
	H2A	FGRAMPH1_01G26109
Histone variant	H2A.Z	FGRAMPH1_01G03973
	H3.3	FGRAMPH1_01G06247

H2A family encompasses the largest number of variants, including H2A.X, H2A.Bdb, H2A.Z and macroH2A. As shown in **Table 2**, H2A.Z is the only H2A histone variant found in *F. graminearum* and it is my focus during my PhD. The details of histone variant H2A.Z will be discussed in the **Review Paper.** H2A.X constitutes 2–25% of mammalian histone H2A depending on the organism and cell type (Dickey *et al*. 2009). It has the most similar sequence with H2A but with a divergent C-terminal tail. H2A.X can undergo both replication dependent and independent transcription as the mRNA of H2A.X can have either a polyA tail or a stem loop structure (Mannironi, Bonner, and Hatch 1989). It can be acetylated, ubiquitinated and phosphorylated (M Arnaudo, Molden, and A Garcia 2011). H2A.Bbd is the least studied histone variant. When compared to canonical H2A, the protein sequence of H2A.Bbd is shorter and arginine enriched. It is reported that nucleosomes containing H2A.Bbd contain 130 bp of DNA instead of the traditional 146 bp, meaning that the

incorporation of H2A.Bbd affect chromatin structure (Doyen *et al*. 2006). MacroH2A is an unusual histone variant with a bulky C-terminal non-histone domain (about 30kDa) which distinguishes it from all other histones (Sun and Bernstein 2019). Mass spectrometry analysis revealed that macroH2A can be ubiquitinated, phosphorylated and acetylated (M Arnaudo, Molden, and A Garcia 2011).

H3 has three main variants: H3.1 and H3.2 (known as the "canonical" histone H3), H3.3, and CenH3. These variants are surprising similar in sequence. For example, in mammals, H3.1 only differs from H3.2 by a change in Cysteine 96 to Serine and H3.3 differs from H3.1 by only five residues. Although the changes in amino acid sequence are subtle, they have large differences in their expression, localization in chromatin, and modification state. For example, compared to H3.1 and H3.2, H3.3 is usually localized to heterochromatin and enriched for histone modifications that are associated with gene activation (Hake et al. 2005; Schulmeister, Schmid, and Thompson 2007; M Arnaudo, Molden, and A Garcia 2011; Shi, Wen, and Shi 2017).

The variant of histone H2B mainly include H2B.1, H2B.W, subH2B and H2B.Z, among which H2B.Z is an apicomplexan specific variant that is known to interact with H2A.Z (Draizen et al. 2016). In fact, due to sequence similarity of the H2B gene family, variants of H2B are rarely encountered at least in mammals, apicomplexa and sea urchins and have not been studied as deeply as other histone variants. Variants of H1 are common, but similar with H2B, much less is known of their functional specialization (Takami, Takeda, and Nakayama 1995; Hoeijmakers *et al*. 2013).

These histone variants are involved in diverse biological processes **(summarized in Figure 11)** including transcription, chromosome segregation, DNA repair and recombination, chromatin remodeling, ADP-ribosylation, germline-specific DNA packaging and activation, and even extra-nuclear acrosomal function (Maze *et al*. 2014; Henikoff and Smith 2015).

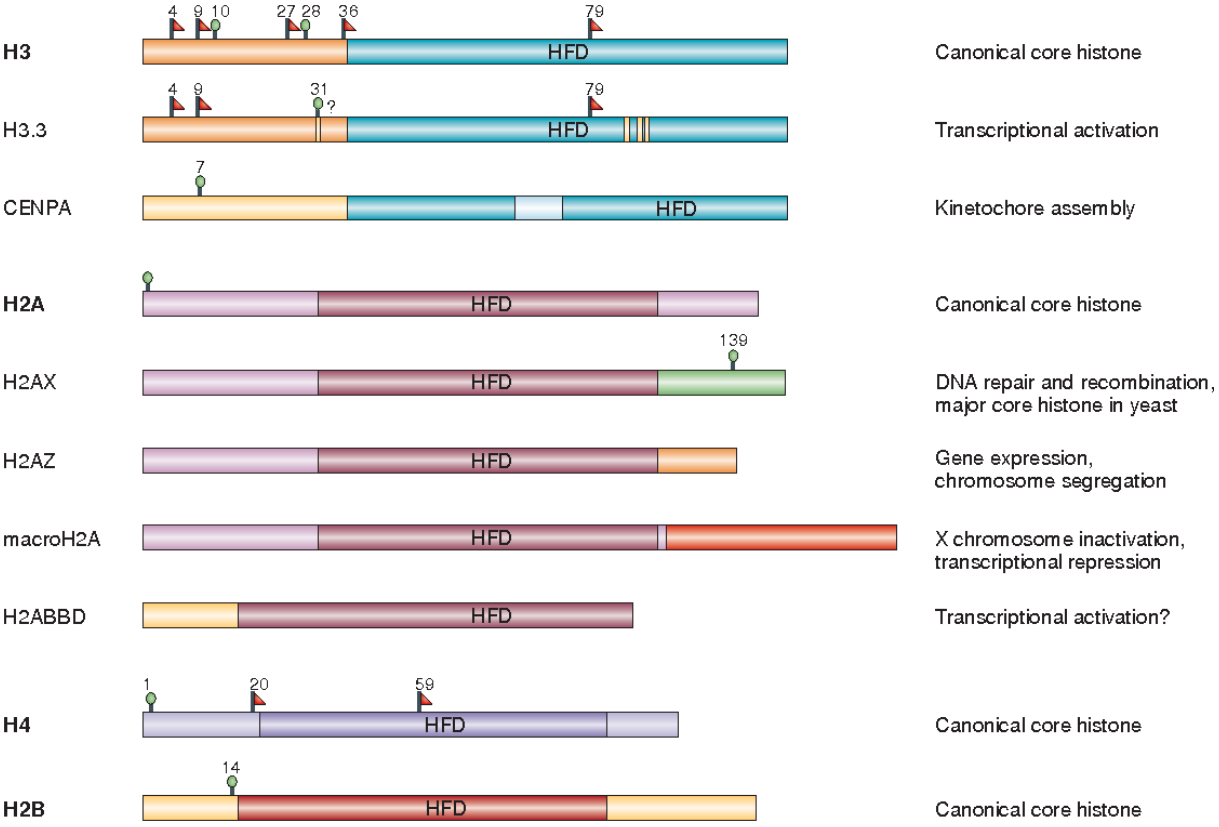


Figure 11. Canonical histones and their variants (Sarma and Reinberg 2005). The major core histones contain a conserved histone-fold domain (HFD). In addition, they contain N- and C-terminal tails that harbor sites for various post-translational modifications. For simplicity, only well-established sites for lysine methylation (red flags) and serine phosphorylation (green circles) are shown (other types of modifications, such as ubiquitylation, are not shown). The proposed functions of the variants are listed. Among them, H2A.Z is the only histone variant found in *F. graminearum.*

1.4.2 Histone post-translational modifications (PTMs)

Histone tails are extensive marked by covalent PTMs, including methylation, phosphorylation, acetylation, ubiquitylation and sumoylation. These PTMs impact gene expression by altering chromatin state or recruiting histone modifiers (Fan *et al*. 2015; Audia and Campbell 2016). Histone H3 are the most modified histone, followed by H4. In most species, histone H3 is primarily acetylated at lysines 9, 14, 18, 23, and 56, methylated at arginine 2 and lysines 4, 9, 27, 36, and 79, and phosphorylated at serine 10 and 28 as well as threonine 3 and 11. Histone H4 is primarily acetylated at lysines 5, 8, 12 and 16, methylated at arginine 3 and lysine 20, and phosphorylated at serine 1 **(Figure 12)** ("Histone Modifications" 2013). In filamentous fungi, a well-characterized system has been established in the model fungus *N. crassa* to elucidate epigenetic phenomena. More and more studies focus on the roles of chromatin change in the regulation of SMs have been carried out in *Fusarium*, highlighting the major importance of histone acetylation and methylation. Here,

we discuss histone methylation and acetylation in filamentous fungi, with a focus on *Fusarium* species.

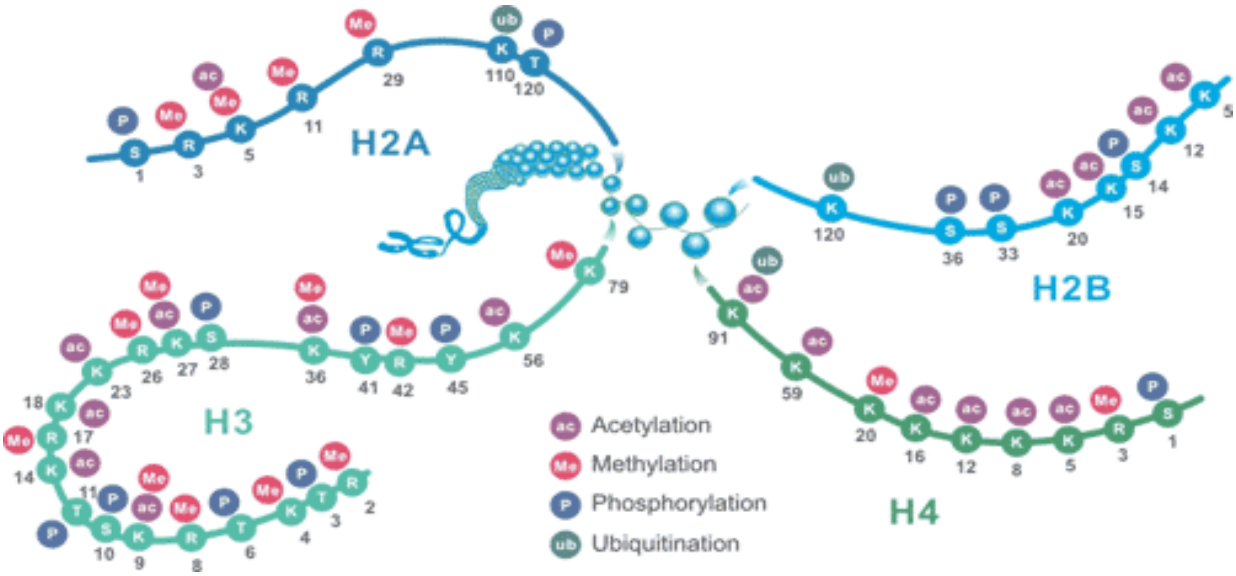


Figure 12. Schematic representation of common histone modification sites. K: lysine; R: arginine; S: serine; Y: tyrosine; T: threonine ("Four Common Histone Modifications-CUSABIO").

1.4.2.1 Histone methylation

Histone methylation is defined as the transfer of one, two, or three methyl groups from S-adenosyl-L-methionine to lysine or arginine residues of histone proteins by histone methyltransferases (HMTs). The methyl group can be removed by histone demethylases (HDACs). In nuclei, the occurrence of histone methylation is related with the activation or silencing of specific genes within the DNA complexed with the modified histone (Greer and Shi 2012). For example, H3K9 methylation is the mark of heterochromatin, which contributes to the transcriptionally inactive state of chromatin. Normally, H3K9me3 is necessary for the recruitment of heterochromatin protein 1 (HP1) by creating a binding site. HP1 is involved in the establishment and maintenance of heterochromatin (Lomberk, Wallrath, and Urrutia 2006).

In both *N. crassa* and *F. graminearum*, deletion of HP1 did not show any cytological or morphological defect. However, in *N. crassa*, there was a strong up regulation of several SM gene clusters (Smith *et al*. 2011). In *F. graminearum*, both up and down regulation of SM genes could be detected. For example, expression of genes required for the production of the pigment aurofusarin (AUR) was greatly enhanced while gene expression and metabolites

involved in the DON pathway was inhibited. However, the heterochromatic mark H3K9me3 was both enriched in these two gene clusters in HP1 deleted mutants (Reyes-Dominguez *et al*. 2012). Dim5p, or Kmt1p, a lysine histone methytransferase (HMT) enzyme, is responsible for the methylation of H3K9 in eukaryotes. In *Botrytis cinerea*, Dim5p is required for the regulation of its development and virulence (Zhang *et al*. 2016). In *F. verticillioides*, mutants lacking Dim5p showed significant defects in fungal development and pathogenicity but an increased tolerance to osmotic stress (Gu *et al*. 2017).

H3K27 methylation is a histone modification known to be linked with gene silencing. In *F. graminearum*. Anti-H3K27me3 Chromatin Immunoprecipitation coupled to Deep Sequencing (ChIP-seq) experiment showed that extensive segments were enriched with H3K27me3, covering a third of the genome. Removal of the mark by mutation of the methyltransferase subunit (Kmt6p) of the Polycomb Repressive Complex 2 (PRC2) resulted in activation of more than 1,500 genes, *i.e.,* ~11% of the genome, predominantly genes involved in the production or detoxification of SMs or predicted to play a role in pathogenicity (Connolly, Smith, and Freitag 2013). In *F. fujikuroi*, Kmt6p appears to be essential (Studt, Rösler, *et al.* 2016). Knock down of Kmt6p reduced H3K27me3 levels at usually decorated gene *loci* and induced four otherwise silent putative SM gene clusters accompanied by the accumulation of novel metabolites.

H3K36me is closely associated with euchromatic region of eukaryotic genomes (Ho *et al*. 2014). In filamentous fungi, this mark can be deposited at specific *loci* by two genes, *Set2* and *Ash1*. Similar to *S. cerevisiae*, *F. fujikuroi* Set2p may also interact directly with the elongation form of RNA polymerase II *via* its conserved Set2 Rpb1 interacting (SRI) domain (Janevska *et al*. 2018). Meanwhile, it is reported that Set2p is most likely responsible for H3K36 methylation at euchromatic regions of the genome, while Ash1p methylates H3K36 at the subtelomeric region of all chromosomes (Janevska *et al*. 2018). In spite of showing the roles of H3K36me involved in vegetative growth, sporulation, SM biosynthesis and virulence in *F. fujikuroi*, this study also found that Ash1p deleted mutant exhibit an enhanced level of H3K27me3, an increased instability of subtelomeric regions and losses of the accessory chromosome XII, indicating that Ash1p may be involved in DNA repair process. Simultaneously, another study carried out in *N. crassa* also demonstrated that Ash1p-marked

chromatin can be further modified by methylation of H3K27, and Ash1p catalytic activity modulates the accumulation of H3K27me2/3 both positively and negatively (Bicocca *et al*. 2018). These findings provide new insights into the relationship between H3K36me, function of Ash1p and H3K27me establishment during chromatin structure change in filamentous fungi.

In eukaryotic cells, methylation of fourth lysine of H3 is normally associated with transcriptional activation (Santos-Rosa *et al*. 2002). Set1p is the catalytic component of Complex of Proteins Associated with Set1 (COMPASS), which mediates mono-, di- and tri-methylation of H3K4. It was first identified in *S. cerevisiae* (Nislow, Ray, and Pillus 1997). Set1p alone is not active as a lysine methyltransferase, only when within COMPASS can active the enzyme. Deletion of *S. cerevisiae* Set1p led to transcriptional silence and growth defects (Roguev 2001; Shilatifard 2012). In *F. graminearum*, H3K4me deposited by FgSet1p is required for the active transcription of genes involved in DON and AUR biosyntheses. In addition, FgSet1p plays an important role in responding to cell wall-damaging agents (Y. Liu *et al.* 2015). More recently, In *F. graminearum* and *F. fujikuroi*, a study showed that SM profiles in mutants lacking Ccl1p, a subunit of COMPASS, were strongly deviating from wild type ones (Studt, Janevska, *et al*. 2016). Similarly, knockout of the Set1p ortholog FvSet1p in *F. verticillioides* not only resulted to the inhibition of fungal growth and pathogenicity, but also to significant defects in FB1 biosynthesis and lower expression levels of FUM genes (Gu, Tahir, *et al*. 2017).

1.4.2.2 Histone acetylation

Histone acetylation occurs by the enzymatic addition of an acetyl group (COCH3) from acetyl-coA to the ε-amino group of lysine residues, producing coenzyme A as product. This process is catalyzed by various histone acetyltransferases (HATs). In contrast, the removal of acetyl groups from histone lysine residues is mediated by histone deacetylases (HDACs). The balance between the activities of HATs and HDACs decides the dynamic acetylation status of histones (Peserico and Simone 2011; Liu *et al*. 2017). Histone acetylation is tightly involved in the regulation of many cellular processes including chromatin dynamics and transcription, gene silencing, cell cycle progression, apoptosis, differentiation, DNA replication, DNA repair, nuclear import, and neuronal repression (Kuo and Allis 1998; Fan *et al*. 2015).

In fungi, histone acetylation has also been shown to greatly influence SM gene expression.

The HAT, general control protein 5 (Gcn5p), is a member of the highly conserved Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex which has been extensively studied in *S. cerevisiae* (Grant *et al*. 1997; Baker and Grant 2007). It is essential for the acetylation of several H3 lysines including H3K4, H3K9, H3K18 and H3K27 (Xue-Franzén et al. 2013). Lack of Gcn5 affected the transcription of 28 out of 47 putative SM gene clusters (M Rösler *et al*. 2016). Conversely, HDACs are also found to be involved in the regulation of secondary metabolism of *F. fujikuroi*. Targeted deletion was applied to remove three Zn^{2+}-dependent HDACs, including FfHDA1p, FfHDA2p and FfHDA3p. Results showed that both FfHDA1p and FfHDA2p are critical for SM biosynthesis in *F. fujikuroi*. FfHDA1p, which absence led to significant alterations in the acetylation state of SM gene clusters, participated in the regulation of at least four SMs, including bikaverin, fusarubins, fusaric acid and gibberellins (Studt *et al*. 2013). Furthermore, rice infection studies revealed that both FfHDA1p and FfHDA2p were required for GA-induced bakanae disease of rice. In *F. graminearum*, deletion of HDF1p resulted in 60 % reduction in HDAC activity and a significant inhibition in virulence and DON production (Y. Li *et al*. 2011a).

1.4.3 DNA methylation

DNA methylation was discovered as early as DNA was identified as the genetic material (Avery and McCARTY 1944). However, it was until 1980s that studies begun to demonstrate DNA methylation is involved in the gene regulation and cell differentiation (Holliday and Pugh 1975; Compere and Palmiter 1981). Nowadays, DNA methylation has been widely recognized as an epigenetic marker related with gene expression. It is catalyzed by a family of DNA methyltransferases (DNMTs) which transfer a methyl group from S-adenyl methionine (SAM) to the cytosine or adenine (Moore, Le, and Fan 2013). Cytosine is methylated on the carbon 5 of the pyrimidine ring (me5C), whereas adenine is methylated on the exocyclic NH_2 group at position 6 of the purine ring (6mA) (Iyer, Zhang, and Aravind 2016). Compared to adenine methylation, cytosine methylation is much well studied.

In mammals, DNA methylation is almost exclusively found in CpG dinucleotides, with the cytosines on both strands being usually methylated. However, non-CpG methylation can be detected in embryonic stem cells and hematopoietic progenitor cells (Haines, Rodenhiser, and Ainsworth 2001; Dodge *et al*. 2002; Lister *et al*. 2009; Kulis *et al*. 2015). In plants, DNA methylation occurs in all cytosine sequence contexts: CpG, CHG and CHH (H represents A, T

or C) (Zhang *et al.* 2006; Lister *et al*. 2008). In *Arabidopsis thaliana*, genome-wide DNA methylation is characterized by heavy methylation in heterochromatin, which is enriched with transposable elements (TEs) and other repetitive DNA sequences (Zhang *et al*. 2006; Henderson and Jacobsen 2007; H. Zhang, Lang, and Zhu 2018). Interspersed transposon-associated DNA methylation also exists in euchromatic chromosome arms (Zhang *et al*. 2006).

In fungi, modification of DNA occurs on both 5-methylcytosine (5mC) and 6mA bases (Seidl 2017; Mondo *et al*. 2017). In early-divergent fungi such as *Hesseltinella vesiculosa*, 6mA methylation marks are widespread in the region of active genes (Mondo *et al*. 2017). Conversely, 5mC methylation in eukaryotes is considered as a repressive epigenetic mark (Schübeler 2015). A few fungal species, including Dikarya (Ascomycota and Basidomycota), display increased prevalence of 5mC modifications; remarkable differences in methylation levels, however, have been reported among fungi. For example, *S. cerevisiae* and *Schizosaccharomyces pombe* lack 5mC, and *A. flavus* 5mC content is extremely limited (Liu et al. 2012). In *Armillaria bulbosa*, up to 4 % of total cytosines were identified as 5mC (Binz, D'Mello, and Horgen 1998; Capuano *et al*. 2014). In *Ascobolus immersus*, repeated genes are hyper-methylated (10-12% of the C residues) and silenced by a genome defence system named methylation induced premeiotically (MIP) which is dependent upon the gene *masc1* encoding a cytosine methyltransferase-like protein and takes place during sexual reproduction (Christophe Goyon, Nogueira, and Faugeron 1994; Malagnac *et al.* 1997; C Goyon 1996; Barra *et al.* 2000). In the genome of *N. crassa*, approximately 1.5 % of cytosines are methylated by the DNMT Dim2p, which is resulted from repeat-induced point mutation (RIP), a process related with MIP (Foss *et al*. 1995; Seymour *et al*. 2016). According to the results of mass spectrometry, in *F. graminearum, Fusarium ventricosum, F. fujikuroi* and *F. avenaceum*, levels of DNA methylation are less than 1% (N. Ponts, personal communication).

1.4.4 Summary

Chromatin remodelling, histone PTMs and DNA methylation act together as a network to control the dynamic changes of chromatin structure (Lasserre, Chung, and Vingron 2013). Up to now, important details of their functions and relationships remain unclear. Besides, as showed above, chromatin changes certainly play a key role on the production of SMs in filamentous fungi, but the greater bulk of the studies on epigenetic marks were performed in *N. crassa*. In my study, I focus on the function of histone variant H2A.Z **(See the Review below)** and the heterochromatic marks including HP1, Dim2p and Dim5p in *F. graminearum*.

Review Paper

Title:

H2A.Z function: a focus on fungi

Authors:

Z. Chen[1], N. Ponts[1,*]

Affiliations:

[1]UR1264-MycSA, INRA, Villenave d'Ornon, France

*Corresponding author: nadia.ponts@inra.fr

Word count: 6302

Abstract

Chromatin is a highly dynamic structure that closely relates with gene expression in eukaryotes. ATP-dependent chromatin remodeling, histone post-translational modification and DNA methylation are the main ways that mediate such plasticity. H2A.Z is a ubiquitous and highly conserved histone variant, which can be deposited or removed from nucleosomes by chromatin remodeling complex SWR1 or INO80, respectively, leading to altered chromatin state. In eukaryotes, H2A.Z has been found to be involved in a diverse range of biological processes, including genome stability, DNA repair and transcriptional regulation. Due to their formidable production of secondary metabolites, filamentous fungi play outstanding roles in pharmaceutical production, food safety and agriculture. During the last few years, chromatin structure changes was proven to be a key factor associated with secondary metabolism in fungi. However, studies on the function of H2A.Z are scarce. Here, we summarize current knowledge of H2A.Z functions with a focus on *F. graminearum* and *F. fujikuroi*, two pathogens known as 'plant killer'. We propose that H2A.Z is a potential target involved in the regulation of secondary metabolite biosynthesis by fungi.

Introduction

Fungi are a kingdom regrouping extremely diverse eukaryotes (“Stop Neglecting Fungi” 2017). Similar to plant cells, fungal cells include a cell wall outside of the cell membrane that surrounds the nucleus, mitochondria as well as an internal membrane system, including the endoplasmic reticulum and Golgi apparatus. However, unlike plant cells, fungal cells do not have chloroplasts or chlorophyll to do photosynthesis. Instead, they are heterotrophs, acquiring their food by absorbing dissolved molecules from the environment. It is estimated that there are 2.2 to 3.8 million fungal species on earth with a diversity of structures (Hawksworth and Lücking 2017). Among them, filamentous fungi, which are composed of a web of filaments called “hyphae”, are often overlooked. They are potent producers of heterogeneous secondary metabolites (SMs). Many of these metabolites are considered to be harmful, as in the case of mycotoxins which known as food spoilers, resulting in reduced yields and deteriorated grain quality such as aflatoxins (*e.g.,* aflatoxin B1; AFB1), fumonisins, zearalenone (ZEN), type B trichothecenes (*e.g.,* deoxynivalenol, or DON), type A trichothecenes (*e.g.,* T-2 toxin), and ochratoxin A (OTA) (Gruber-Dorninger, Jenkins, and Schatzmayr 2019; Alberti, Foster, and Bailey 2017; Hussein and Brasel 2001). Many of them are produced by *Fusarium* species, which are regarded as the most important fungal pathogens of plants and animals (Goswami and Kistler 2004). Nonetheless, SM effects can also be beneficial, as in the case of antibiotic compounds (Hamad 2010). The production of SMs in filamentous fungi are regulated by various factors, including nutrient source, pH, or light for example (Caracuel *et al*. 2003; Katoh *et al*. 2007; K. H. Wong, Hynes, and Davis 2008; Tisch and Schmoll 2010). More recently, chromatin structure changes were proposed to play a key role in the regulation of SM biosynthesis.

Inside the nucleus of fungal (and other eukaryotic) cells, genomic DNA is packaged with histones to form nucleosomes, which are the basic unit of chromatin. Each nucleosome consists of two copies of each of the four core canonical histones H2A, H2B, H3 and H4, with 145-147 base pairs (bp) of DNA wrapped around them (Richmond *et al*. 1984; Luger, Dechassa, and Tremethick 2012). These canonical histones are highly conserved across eukaryotic species and represent the major part of the total histone pool within an organism (Mariño-Ramírez *et al*. 2005). In most eukaryotes, each histone is encoded by multiple genes with high sequence similarity and deposited into chromatin only during the S phase of the cell cycle after DNA replication (Talbert and Henikoff 2014). In metazoans, histone mRNAs are devoid of introns and are not

polyadenylated. Instead, they have special stem-loop structures to trigger translation (Marzluff and Koreski 2017). In yeast, histone mRNAs are polyadenylated, polyA tail lengths varying during cell cycle (Beggs, James, and Bond 2012). They are expressed exclusively at the late G1 and S phase, *i.e.,* when DNA replication occurs (Kurat *et al*. 2014). Whilst histone genes are also largely devoid of introns, with few exceptions such as one H4 gene in *Yarrowia lipolytica*, Basidiomycota and Ascomycota have introns in their histone genes (Nishida and Yun 2011). At the protein level, N-terminal tails of histones are extensively marked by covalent post-translational modifications (or PTM) including methylation, phosphorylation, acetylation, or ubiquitylation, that impact gene expression by altering chromatin state (Marzluff and Koreski 2017). Additionally, non-allelic isoforms of canonical histones called histone variants exist in all eukaryotes. In contrast to canonical histones, most of them are encoded by a single gene containing one or more introns expressed throughout the cell cycle, and can thus be incorporated into nucleosomes during the whole cell cycles. To date, histone variants of all canonical histone have been recognized, except for H4 (Maze *et al*. 2014). In higher eukaryotes, H2A family encompasses the largest number of variants, including macroH2A, H2A.Bbd, H2A.X and H2A.Z, in which H2A.Z is considered as the most evolutionarily conserved one (van Daal et al. 1990; Thatcher and Gorovsky 1994). H2A.Z and H2A have typically ~60 % sequence similarity (see **Figure 1** for an example in yeast). It differs from H2A in the increased acidic patch and the carboxy-terminal α-helix included in the docking domain, which is a structure involved in the interaction of the H2A-H2B dimer with the $(H3\text{-}H4)_2$ tetramer (Luger *et al*. 1997; Suto *et al*. 2000).

H2A.Z has been identified by different names in various species, including hv1 in *Tetrahymena thermophila* (White *et al*. 1988), HTA8, HTA9, and HTA11 in *Arabidopsis thaliana* (March-Díaz and Reyes 2009), Htz1 in *Saccharomyces cerevisiae* (Jackson, Falciano, and Gorovsky 1996), or H2Av in *Drosophila melanogaster* (van Daal and Elgin 1992), for example. Within these different organisms, H2A.Z variants show high levels of sequence similarity. Conservation of H2A.Z throughout evolution indicates that it plays important roles in eukaryotic cells, that cannot be replaced by H2A. Actually, the absence of H2A.Z has been reported as lethal in many organisms such as mouse (Faast *et al*. 2001), *Drosophila* (van Daal and Elgin 1992), frogs and *Tetrahymena* (Liu, Li, and GorovskyMA 1996), but not in *S. cerevisiae* in which deletion of H2AZ stops nonetheless cell cycle progression (Adam *et al.* 2001; Jackson and Gorovsky 2000). Across eukaryotes, H2A.Z has been found to be involved in a diverse range of biological processes,

including genome stability (Downs *et al*. 2004; Krogan *et al*. 2004; Keogh *et al*. 2006), DNA repair (Kalocsay, Hiller, and Jentsch 2009; Xu *et al*. 2012; Gursoy-Yuzugullu, Ayrapetov, and Price 2015; Alatwi and Downs 2015) and transcriptional regulation (Marques *et al.* 2010; Deal and Henikoff 2011; Soboleva *et al.* 2014). In filamentous fungi, current knowledge on the function of H2A.Z is scarce. One study about the function of H2A.Z in filamentous fungi was carried out on *Neurospora crassa*, finding that H2A.Z regulates oxidative stress response (Dong *et al*. 2018). However, even though chromatin structure changes are of major importance in regulating the biosynthesis of several SMs, the role of H2A.Z has been overlooked. Owing to the outstanding role of filamentous fungi in pharmaceutical production, food safety, agriculture and the critical function of H2A.Z in various species, a better understanding on the role of chromatin dynamics mediated by H2A.Z in these fungi brooks no delay. In this review, we summarize current knowledge of H2A.Z functions with a focus on *Fusarium* spp. to lay the foundation for the further study of H2A.Z that will be carried out in these species.

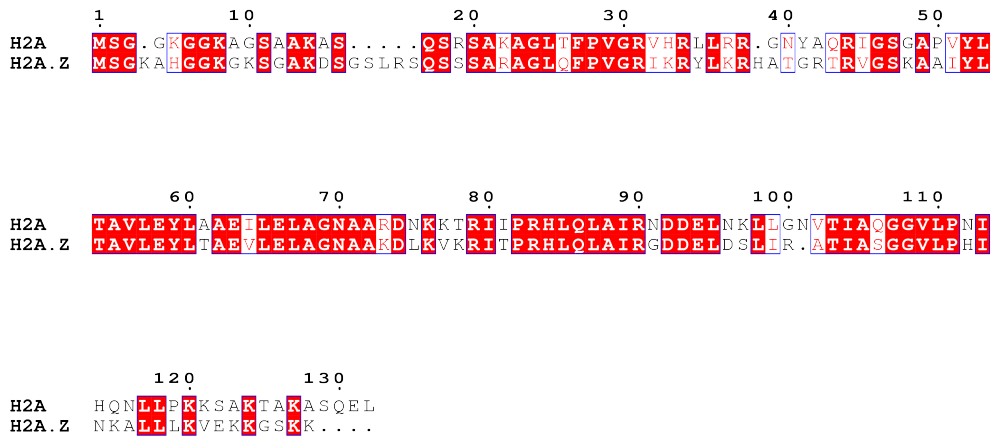


Figure 1. Sequence alignment of the whole sequences of *S. cerevisiae* H2A (gene ID: YBL003C) and H2A.Z (gene ID: YoL012) by ENDscript 3.0. Consensus threshold is 70%, if the similar score assigned to a column is greater than this value, residues are considered as highly similar and are colored in red and framed in blue. Below the threshold, residues are marked as weakly similar. In all cases, they are in white on a red background in case of strict identity.

H2A.Z in Fungi

Proteomes of 43 fungi were searched for domains matching the HMM profile for the C-terminal end of H2A (HMMER3 and PFAMv32 profile PF16211, (Bateman *et al*. 2000; Eddy 2009). The C-terminal end of H2A is known to play a crucial role in stabilizing nucleosome particles and recognition of the linker histone H1 (Vogler *et al*. 2010). The length and nature of the tail in both

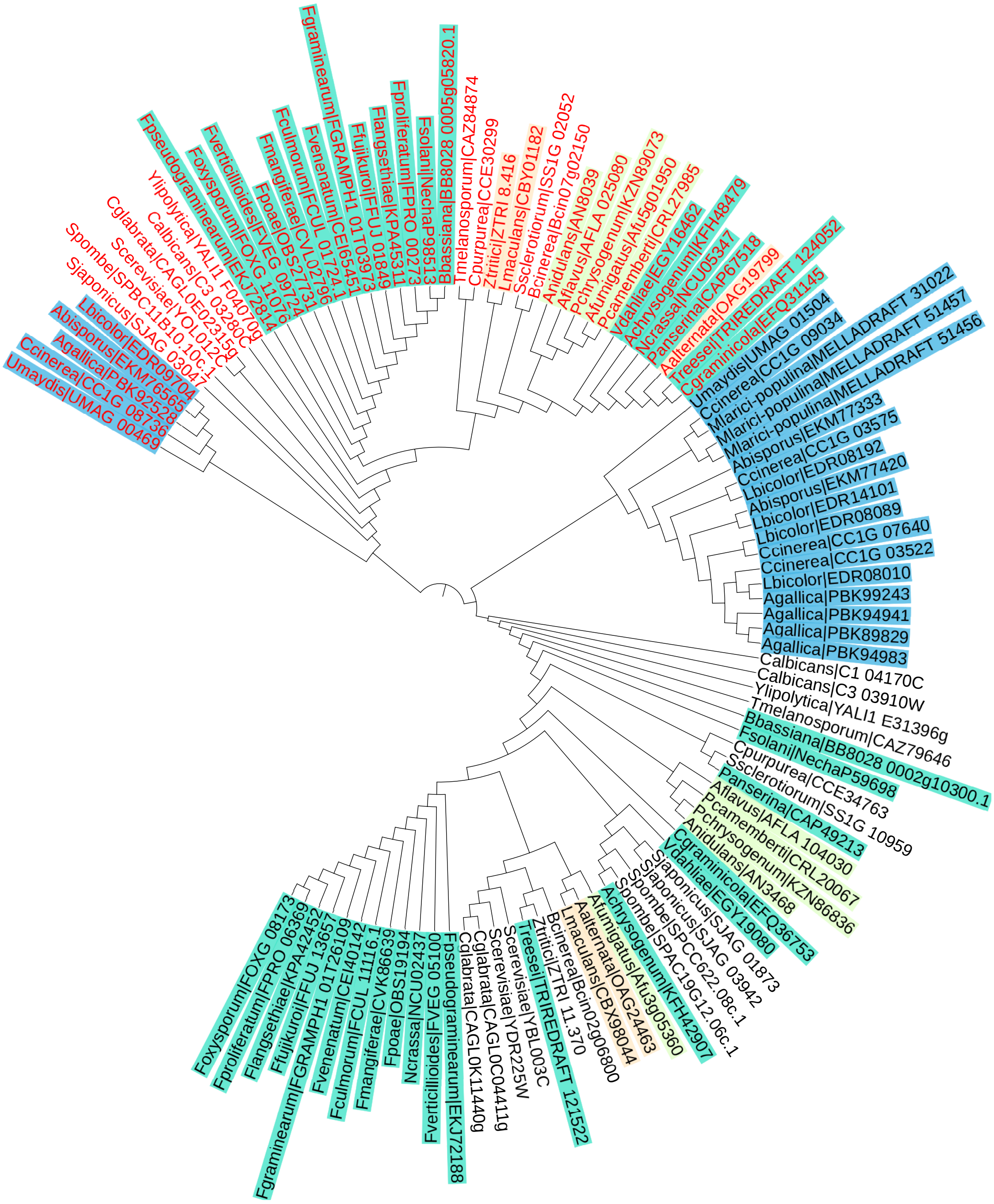


Figure 2 Phylogenetic tree based on H2A C-terminal domain homology. Domains were aligned with *hmmalign* program from HMMER3 suite using the PFAM HMM model PF (Bateman *et al*. 2000; Eddy 2009). Texts in red are H2A.Z, identified for their protein sequence homology with *S. cerevisiae* Htz1; texts in black are H2A, identified for their protein sequence homology with *S. cerevisiae* H2A. Texts with light green background are species belong to class Eurotiomycetes, dark green are species belong to class Sordariomycetes, blue are species belong division Basidiomycota, orange are species belong to class Dothideomycetes. Texts without background are other species.

H2A and H2A.Z have a conserved function in regulating association with nucleosomes (Wratting *et al*. 2012). Here, at least one H2A.Z variant is predicted in all fungi analyzed **(Supplemental Table S1)**. Examining partial sequence alignment of C-termini of the identified H2A/H2A.Z, high conservation of residues can be observed **(Supplemental Figure S1)**. H2A/H2A.Z putative identification was proposed from the examination of the derived phylogenetic tree **(Figure 2; PhyML 3.3)** (Guindon *et al*. 2010).

All analyzed fungi but one were found to possess one single histone H2A variant and one or more canonical H2A. Indeed, the Basidiomycota *Melampsora larici-populina*, the poplar leaf rust, hit on three proteins that all cluster with other H2A proteins. Regarding H2A, whilst all Pezizomycotina seem to possess one single H2A, yeasts and Basidiomycota have a varying number of them. Only the yeast *Yarrowia lipolytica* and the Basidiomycota *Ustilago maydis* are exceptions with one single H2A protein detected in their proteomes. Examining sequences in more details **(Supplemental Figure S1)**, among other differences, the presence of a glycine residue at position 8 of the domain seems to be a hallmark of H2A (*vs.* H2A.Z). Conversely, the exchange of an asparagine for an aspartic acid in position 4 as well as the exchange of proline 27 for leucin 26 are signatures of a H2A.Z domain.

Chromatin remodeling complexes mediate H2A.Z exchange for H2A

Functions of H2A.Z in cells are linked to the dynamics of its deposition and removal from nucleosomes. These processes are mediated by several ATP-dependent chromatin remodeling complexes, especially complexes belonging to the SNF2 superfamily (van Attikum, Fritsch, and Gasser 2007; Gerhold and Gasser 2014). Typically, SNF2 proteins contain two conserved motives, the DEXDc (DEAD-like helicases) and HelicC (helicase superfamily c-terminal domain) subdomains, which together constitute the ATPase domain and can use the energy derived from ATP hydrolysis to modify chromatin structure (Lusser and Kadonaga 2003; C. L. Smith and Peterson 2005). This superfamily of proteins consists of a large range of chromatin remodelers of varying functions, including histone dimer exchange (Ryan and Owen-Hughes 2011). **Figure 3** is a schematic representation of yeast subunits of chromatin remodeling complexes SWR1, INO80, and NuA4 that relate to exchange of H2A.Z, histone post-translational modification as well as DNA repair. Strikingly, several protein

subunits are shared between the three complexes, for example, Arp4p and Act1p are found in all of them, reflecting the close association between SWR1, INO80 and NuA4 complex.

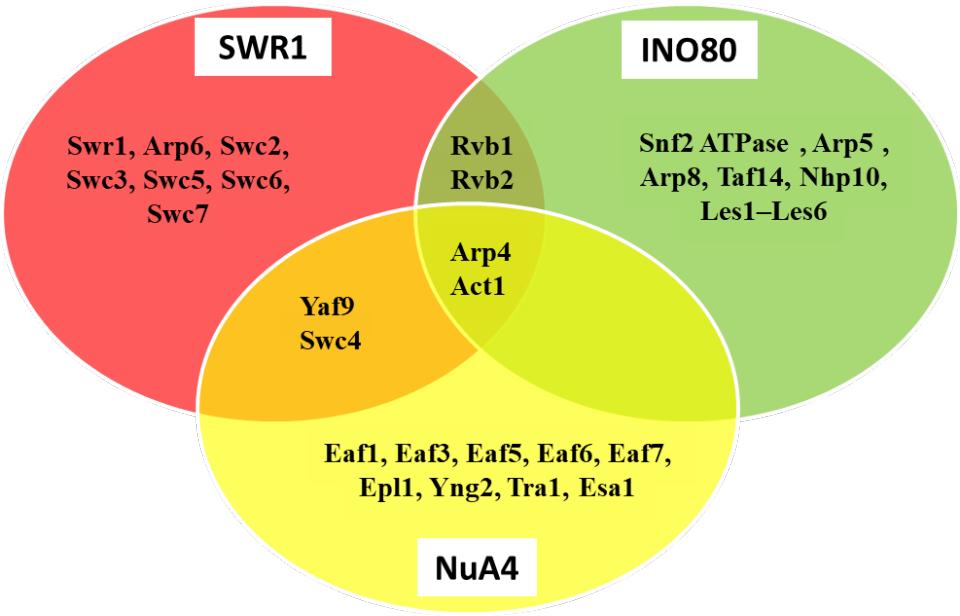


Figure 3 Proteins identified as subunits of SWR1, INO80 and NuA4 complexes in *S. cerevisiae.* Red: subunits of SWR1 complex; Green: subunits of INO80 complex; Yellow: subunits of NuA4 complex. Overlaps between complexes are common subunits.

The SWR-C/SWR1 complex

In *S. cerevisae*, the complex SWR1 belonging to SNF2 family is involved in the deposition of H2A.Z (Kobor *et al*. 2004). It can replace H2A-H2B dimers with H2A.Z-H2B ones. Yeast SWR1 complex includes 13 subunits. Among them, Act1p, Arp4p, Swc4p, Rvb1p, and Rvb2p are essential for cell viability, since the absence of any of the respective coding genes is lethal for the organism. In contrast, subunits Swr1p (catalytic subunit), Yaf9p, Arp6p, Swc2p, Swc3p, Swc5p, Swc6p, and Swc7p are not essential but are required for optimal function of the complex (Kobor *et al*. 2004; Krogan *et al*. 2003; Mizuguchi 2004). Complexes similar to the yeast SWR1 have been characterized in other organisms including mammalians and plants. In human, two complexes seem related to SWR1, namely SRCAP and Tip60/p400. In SRCAP complex, the SNF2 protein SRCAP (SWI2/SNF2-related CBP activator protein) is identified as the ortholog of Swr1p, contributing to the recruitment of H2A.Z variants on nucleosomes (Houjian Cai *et al*. 2006; Ruhl *et al*. 2006; J. Wong *et al*. 2007). The Tip60/p400 complex contains several chromatin-modifying enzymes, such as p400 ATPase and the Tip60 histone acetyltransferase, which also shows high sequence resemblance to Swr1p and SRCAP (Ikura

et al. 2000; Eissenberg, Wong, and Chrivia 2005). In *Drosophila*, the dTIP60 complex mediates the deposition of H2A.Z. The Domino protein, an ortholog of the yeast Swr1p, has been indicated as the catalytic subunit of the complex (Kusch *et al*. 2004).

Table 1 Genes encoding subunits of chromatin remodeling complex SWR1, INO80, NuA4 in yeast and their homologs in *F. graminearum* and *F. fujikuroi**

S. cerevisiae			***F.graminearum***	***F.fujikuroi***
Proteins	**Gene ID**	**Product description**	**Gene ID**	**Gene ID**
Swr1p	YDR334W	Chromatin-remodeling protein SWR1	FGRAMPH1_01G18675	FFUJ_05231
Arp6p	YLR085C	Actin-related protein	FGRAMPH1_01G09171	FFUJ_06545
Swc2p	YDR485C	Htz1p-binding component of the SWR1 complex	FGRAMPH1_01G06047	FFUJ_07909
Swc3p	YAL011W	Htz1p-binding component of the SWR1 complex	-	-
Swc5p	YBR231C	Htz1p-binding component of the SWR1 complex; protein abundance increases in response to DNA replication stress;	FGRAMPH1_01G24481	FFUJ_03149
Swc6p	YML041C	Htz1p-binding component of the SWR1 complex; required for vacuolar protein sorting	-	-
Swc7p	YLR385C	Component of the SWR1 complex that incorporates Htz1p into chromatin	-	-
Snf2p	YOR290C	Catalytic subunit of the SWI/SNF complex; involved in transcriptional regulation; contains DNA-stimulated ATPase activity	FGRAMPH1_01G24477	FFUJ_03151
Arp5p	YNL059C	promotes nucleosome shifts in the 3 prime direction	FGRAMPH1_01G20187	FFUJ_04662
Arp8p	YOR141C	has mRNA binding activity	FGRAMPH1_01G22879	FFUJ_03316
Taf14p	YPL129W	involved in RNA polymerase II transcription initiation and in chromatin modification; contains a YEATS domain	FGRAMPH1_01G14509	FFUJ_09554
Nhp10p	YDL002C	Non-essential INO80 complex subunit; preferentially binds DNA ends, protecting them from exonucleatic cleavage;	FGRAMPH1_01G23115	FFUJ_03111
Les1p	YFL013C	relocalizes to the cytosol in response to hypoxia	FGRAMPH1_01G24503	FFUJ_02630
Les2p	YNL215W	associates with the INO80 complex under low-salt conditions; protein abundance increases in response to DNA replication stress	FGRAMPH1_01G15023	FFUJ_09793
Les3p	YLR052W	Subunit of the INO80 complex	-	-
Les4p	YOR189W	target of the Mec1p/Tel1p DNA damage signaling pathway; proposed to link chromatin remodeling to replication checkpoint responses	-	-
Les5p	YER092W	Non-essential INO80 complex subunit; deletion affects telomere maintenance via recombination	-	-
Les6p	YEL044W	involved in regulation of chromosome segregation and maintenance of normal centromeric chromatin structure;	FGRAMPH1_01G20191	FFUJ_04660
Eaf1p	YDR359C	acts as a platform for assembly of NuA4 subunits into the native complex; required for initiation of pre-meiotic DNA replication,	FGRAMPH1_01G18101	FFUJ_08647
Eaf3p	YPR023C	Component of the Rpd3S histone deacetylase complex; nonessential component of the NuA4 acetyltransferase complex	FGRAMPH1_01G19347	FFUJ_13233
Eaf5p	YEL018W	Non-essential subunit of the NuA4 complex; Esa1p-associated factor	-	-

Eaf6p	YJR082C	Subunit of the NuA4; this complex acetylates histone H4 and NuA3 acetyltransferase complex that acetylates histone H3	-	-
Epl1p	YFL024C	conserved region at N-terminus is essential for interaction with the NPC (nucleosome core particle); required for autophagy	FGRAMPH1_01G24745	FFUJ_02514
Yng2p	YHR090C	positions Piccolo NuA4 for efficient acetylation of histone H4 or histone H2A;	-	-
Tra1p	YHR099W	Subunit of SAGA and NuA4 complexes; interacts with acidic activators (*e.g.*, Gal4p) which leads to transcription activation;	FGRAMPH1_01G19463	FFUJ_13284
Esa1p	YOR244W	Catalytic subunit of the NuA4; acetylates four conserved internal lysines of histone H4 N-terminal tail and can acetylate histone H2A; master regulator of cellular acetylation balance	FGRAMPH1_01G10071	FFUJ_06029
Arp4p	YJL081C	Nuclear actin-related protein involved in chromatin remodeling; component of chromatin-remodeling enzyme complexes	FGRAMPH1_01G02187	FFUJ_00687
Act1p	YFL039C	Actin; structural protein involved in cell polarization, endocytosis, and other cytoskeletal functions	FGRAMPH1_01G24551	FFUJ_02611
Rvb1p	YDR190C	ATP-dependent DNA helicase; conserved component of multiple complexes including the INO80 complex, the Swr1 complex, and the R2TP complex (Rvb1-Rvb2-Tah1-Pih1)	FGRAMPH1_01G17223	FFUJ_13385
Rvb2p	YPL235W	similar to Rvb1p	FGRAMPH1_01G19889	FFUJ_13493
Swc4p	YGR002C	incorporates Htz1p into chromatin; component of the NuA4 histone acetyltransferase complex	-	-
Yaf9p	YNL107W	may function to antagonize silencing near telomeres; interacts directly with Swc4p	FGRAMPH1_01G23049	FFUJ_03137

* Red: subunits of SWR1 complex; Green: subunits of INO80 complex; Yellow: subunits of NuA4 complex; Blue: subunits shared by these three complexes; Grey: subunits shared by SWR1 and NuA4 complex; Purple: subunits shared by INO80 and SWR1 complex.

The INO80 complex

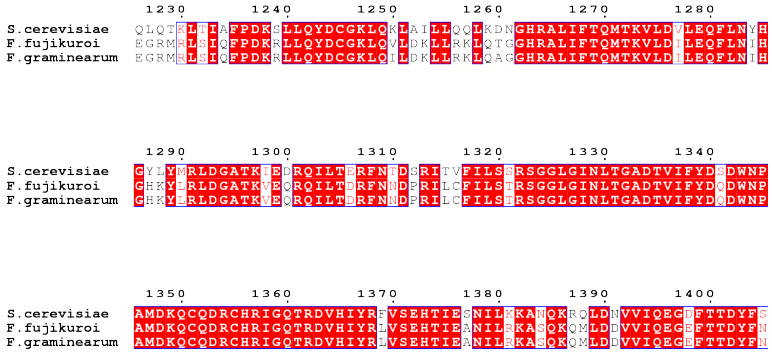
H2A.Z can be actively removed from nucleosomes by chromatin remodelers. In yeast and human, the SWR1-related Inositol requiring 80 (INO80) complex can remove H2A.Z from nucleosomes (Morrison and Shen 2009; Tosi *et al*. 2013; Alatwi and Downs 2015). INO80 complex is another subfamily of the SNF2 family that shares several subunits with SWR1 complex, and is usually related to DNA double-strand break (DSB) repair (Clapier and Cairns 2009; Chen *et al*. 2013). In humans, ANP32E is a histone chaperone reported to function by removing H2A.Z (Mao *et al*. 2014; Obri *et al*. 2014). Homolog of INO80 has also been identified in *Arabidopsis*, in contrast with its function in yeast and animals, it preferentially facilitates the enrichment of H2A.Z at the ends of repressor genes, at the main floral Flowering Locus C (FLC) and MADS Affecting Flowering 4/5 (MAF4/5) (Zhang *et al*. 2015).

Therefore, the roles of INO80 involving in H2A.Z exchange may be species or gene specific.

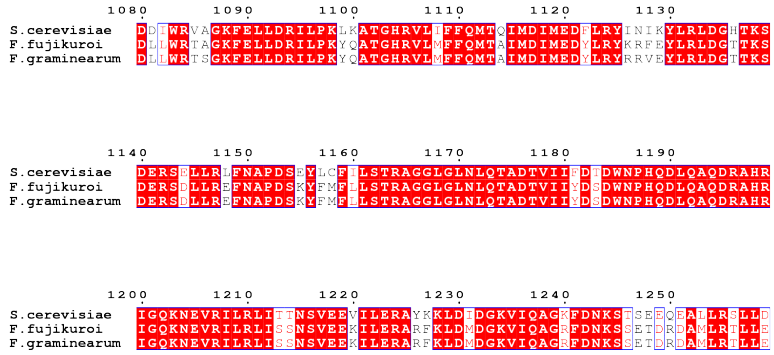
The NuA4 complex

The NuA4 histone acetyltransferase complex has a strong genetic and functional link with SWR1 complex. They share four subunits including Arp4p, Act1p, Yaf9p and Swc4p (Figure 3). Besides, the subunit Eaf1p has significant homology to Swr1p outside of the SWI/SNF-related ATPase domain (Auger *et al.* 2008; Altaf *et al.* 2010). In human and yeast, it was shown that the ability of SWR1 to replace canonical H2A from nucleosome by H2A.Z is greatly enhanced due to the prior acetylation of chromatin by NuA4, probably the subunit Eaf1p (Doyon and Côté 2004; J. Jin *et al*. 2005; Auger *et al.* 2008; Altaf *et al.* 2010). It was also observed that acetylation of histone H4 in yeast by Esa1p, the catalytic component of NuA4, is required for DNA double-strand break repair (Bird *et al*. 2002). Homologs of most subunits of *S. cerevisiae* SWR1, INO80, NuA4 complexes can be found in filamentous fungi, such as in the sordariomycetes *F. graminearum* and *F. fujikuroi* **(Table 1)**. Protein sequences of *S. cerevisiae* Swr1p (belongs to SWR1 complex), Snf2p (belongs to INO80 complex) and Esa1p (belongs to NuA4 complex) were aligned with their corresponding homologs in *F. graminearum* and *F. fujikuroi* **(Figure 4; Supplemental Figure S2)**. These proteins are identified as catalytic subunits of each complex. As shown in the results, highly conserved regions of the three protein can be found in both *F. graminearum* and *F. fujikuroi*. We believe that similar processes of H2A.Z exchange also occur in filamentous fungi. However, details of the process may differ from *S. cerevisiae*, because compositions of SWR1, INO80 and NuA4 complex are not totally equal between these species. For example, homolog of Yng2p, an essential subunit of NuA4 in *S. cerevisiae*, which is important for the efficient acetylation of histone H4 or histone H2A, is missing in *F. graminearum* and *F. fujikuroi*. Therefore, the already known functions and mechanisms of H2A.Z exchange in any other species including yeast cannot be copied directly to filamentous fungi. The potential role of H2A.Z in filamentous fungi needs further investigation.

A



B



C

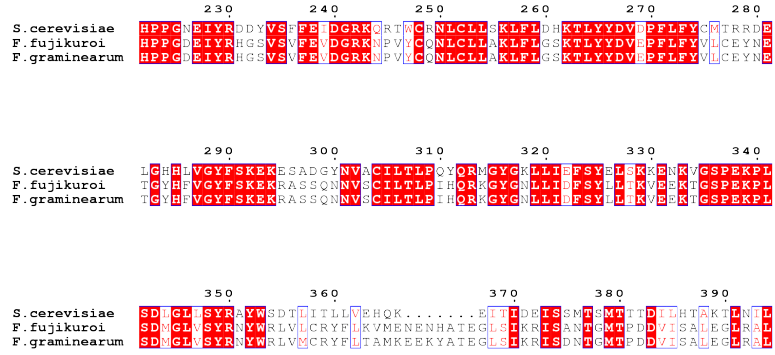


Figure 4. Partial conserved regions of subunits Swr1p **(A)**, Snf2p **(B)** and Esa1p **(C)** in *S. cerevisiae*, *F. fujikuroi* and *F. graminearum*. Sequence alignment was done by ENDscript 3.0. Consensus threshold is 70%, if the similar score assigned to a column is greater than this value, residues are considered as highly similar and are colored in red and framed in blue. Below the threshold, residues are marked as weakly similar. In all cases, they are in white on a red background in case of strict identify. Gene IDs are listed in **Table 1**. Alignments of the whole coding sequences of Swr1p, Snf2p and Esa1p in *S. cerevisiae*, *F. fujikuroi* and *F. graminearum* are presented in **Supplemental Figure S2.**

Post-translational modifications of H2A.Z

PTMs such as acetylation, phosphorylation, methylation, ubiquitylation and sumoylation have been abundantly described on N-terminal tails of canonical histones. They would influence the overall chromatin state by altering inter-nucleosomes interactions and finally regulate gene expression (Bannister and Kouzarides 2011; Audia and Campbell 2016). Similarly, PTMs also occur on the histone variant H2A.Z, leading to differences in the biochemical properties of H2A.Z-containing nucleosomes and marking functional regions of chromatin (Hu *et al*. 2011; Binda *et al.* 2013). N-terminal tail of H2A.Z is extensively acetylated on lysines K4, K7, K11 and K13, correlating with gene activation (Bruce *et al.* 2005; Boyne *et al.* 2006; G. Hu *et al.* 2013; Ku *et al.* 2012; Valdés-Mora *et al.* 2012). NuA4 is a histone acetyltransferase complex that shares several subunits with the SWR1 and INO80 complex **(Figure 3)**. Its primary acetylation target are lysines of histone H4. More recently, the function of NuA4 was shown to also involve the acetylation of H2A.Z (Smith *et al*. 1998; Allard *et al*. 1999; Keogh *et al*. 2006).

H2A.Z can also be mono-ubiquitinated at lysines K120, K121, and K125, mediated by the Polycomb Repressive Complex 1 (PRC1) (Sarcinella *et al*. 2007; Draker, Sarcinella, and Cheung 2011; Ku *et al*. 2012). This modification is generally considered as a silencing mark and appears to occur solely in mammals (Sarcinella *et al*. 2007). SUMO (Small Ubiquitin-like MOdifier) proteins are similar to ubiquitin and can also be covalently linked to lysines (Sevilla and Binda 2014). In *S. cerevisiae*, H2A.Z can be SUMOylated at lysines 126 and 133, and play an important role in DNA repair by relocating unrepaired chromosomal break to nuclear periphery (Kalocsay, Hiller, and Jentsch 2009).

H2A.Z further regulates gene expression through collaborating with other histone marks, particularly those on H3. For example, both in human and mouse embryonic stem (ES) cells, H2A.Z is co-localized with tri-methylation of K4 (H3K4me3) and K27 (H3K27me3) of histone H3 at both promoters and enhancers of genes involved in cell differentiation. In eukaryotic cells, H3K4me3 could create higher chromatin accessibility and is normally associated with transcriptional activation, while H3K27me3 is considered to mediate gene silencing (Santos-Rosa *et al.* 2002; Creyghton *et al.* 2008; Ku *et al.* 2012; G. Hu *et al.* 2013). In contrast, reduced H2A.Z deposition at the anthocyanin biosynthesis genes in *Arabidopsis* is accompanied by a

great increase in H3K4me3 but a decrease in H3K27me3, consistent with enhanced expression levels (Carter *et al*. 2018; Hanyang Cai *et al.* 2019). Moreover, it seems that H2A.Z can act as a functional substitute for H3K9me3 in chromatin for the recruitment of Heterochromatin Protein 1 (HP1) (Ryan and Tremethick 2018).

Recent studies indicated that SMs of many filamentous fungal are regulated by epigenetic modifications. Among them, histone methylation become the major currently known methods of modification. Even though deletion of HP1, which is linked with H3K9me3 and H2A.Z, did not show any cytological or morphological defect in both *N.crassa* and *F. graminearum*, several SM gene clusters were up or down regulated (K. M. Smith *et al.* 2011; Reyes-Dominguez *et al.* 2012). For example, in the HP1 deletion mutant of *F. graminearum*, expression of AUR genes required for the production of the pigment aurofusarin was greatly enhanced, while gene expression and metabolites involved in the deoxynivalenol (DON) pathway were inhibited. However, the absence of HP1 led to an increase of the heterochromatic mark H3K9me3 in both gene clusters (Reyes-Dominguez *et al.* 2012). In *F. verticillioides*, mutants lacking *Dim5* (or *Kmt1*), a lysine histone methyltransferase enzyme mediating H3K9me3 deposition, showed significant defects in fungal development and pathogenicity but increased tolerance to osmotic stress (Gu *et al*. 2017). Another two histone PTMs, H3K4me3 and H3K27me3, which have been proved to co-localize with H2A.Z, also play critical roles in the regulation of SMs production in filamentous fungi (Y. Liu *et al.* 2015; Connolly, Smith, and Freitag 2013). H3K4me3 is indeed required for the active transcription of genes involved in DON and aurofusarin biosynthesis in *F. graminearum* (Liu *et al*. 2015). In the same species, ChIP-sequencing showed that extensive segments, covering a third of the genome, were enriched with H3K27me3 (Connolly, Smith, and Freitag 2013). Removal of the mark by mutation of the methyltransferase subunit (KMT6) resulted in the activation of more than 1,500 genes, predominantly genes involved in the production or detoxification of SM or predicted to play a role in pathogenicity. Thus, H3K27me3 acts as a repressor of genes in *F. graminearum* (Connolly, Smith, and Freitag 2013). In *F. fujikuroi*, KMT6 appears to be essential. Knock down of KMT6 reduced H3K27me3 levels at the respective gene *loci* and induced four otherwise silent putative SM gene clusters accompanied by the accumulation of novel metabolites (Studt *et al*. 2016).

In summary, H2A.Z deposition or eviction on chromatin is associated with various PTMs such

as H3K4me3, H3K27me3 to control the accessibility of chromatin. In filamentous fungi, even though no research has been carried out to investigate whether H2A.Z functions in SM production more and more studies provide evidence that histone PTMs are key factors involved in these pathways. We hypothesize that H2A.Z also plays major roles in filamentous fungi to regulate the biosynthesis of SMs.

H2A.Z in controlling gene transcription

Roles of H2A.Z in controlling gene transcription have been extensively studied in human, *Drosophila melanogaster*, *A. thaliana* and budding yeast, and recently reviewed with a focus on its action in gene regulation (Giaimo *et al.* 2019). According to genome-wide localization maps of H2A.Z, nucleosomes containing H2A.Z are widespread in the organisms analyzed, but the distribution and density of H2A.Z vary between different chromosomes (Leach *et al*. 2000; Guillemette and Gaudreau 2006; Zhang, Roberts, and Cairns 2005). It is indicated that H2A.Z variant is preferentially enriched at the regions of transcriptional start sites (TSS) (Raisner *et al*. 2005; Zilberman *et al*. 2008; Mavrich *et al*. 2008), which suggested a relationship between H2A.Z and gene transcription. However, discordant arguments still exist about whether and how this histone variant is involved in transcriptional regulation.

RNA polymerase II (RNAPII) is a multiprotein complex found in eukaryotic cells which catalyzes the transcription of DNA to synthesize precursors of mRNA and most snRNA and microRNA (Kornberg 1999; Sims, Mandal, and Reinberg 2004). As a general transcription factor, TATA-binding protein (TBP) binds specifically to a DNA sequence called the TATA box in the promoter region of genes to initiate the recruitment of other factors required for RNA Pol II to begin transcription (Kornberg 2007). In yeast, RNAPII and TBP could not be efficiently recruited to *GAL1-10* promoter in cells lacking H2A.Z (Adam *et al*. 2001). Meanwhile, the elongation rate of RNAPII was slower in the absence of H2A.Z (Santisteban, Hang, and Smith 2011). Generally, the entry site of the first (+1) nucleosome is a larger barrier for RNAPII transiting for essentially all genes in *Drosophila*, while gene body nucleosomes are low barriers. Therefore, RNAPII would stall both at the entry site and near the dyad axis. Notably, H2A.Z enrichment at the +1 nucleosome correlates with decreased RNAPII stalling and depletion of H2A.Z from a nucleosome position results in a higher barrier to RNAPII,

suggesting that the high-energy barrier to RNAPII progression can be tuned by H2A.Z deposition. Meanwhile, H2A.Z incorporation at the +1 nucleosome regulates productive elongation by facilitating H2A.Z/H2B dimer loss without depletion of $(H3\text{-}H4)_2$ tetramers as H2A.Z levels anti-correlate with nucleosome turnover (Weber, Ramachandran, and Henikoff 2014; Subramanian, Fields, and Boyer 2015). Thus, H2A.Z deposition acts as an activator of gene transcription. Consistent with this idea, during the induced somatic cell reprogramming of mice, increased level of H2A.Z was detected in the high expressed genes involved in response to stress (hypoxia, oxygen levels, steroid hormone stimulus). Still in mice, Rispal *et al.* (2019) found that decrease of H2A.Z at promoters inhibits the binding of the CDX2 intestine-specific transcription factor. The authors proposed that genes containing H2A.Z can be easily marked as active signals for the recruitment of acetyltransferase enzyme complexes and supply access for the binding of transcription factors (Peserico and Simone 2011; Dong *et al*. 2016). However, a recent study in *N. crassa* revealed that H2A.Z is present at TSS of the oxidative stress resistant gene *catalase-3* and function as a negative regulator (Q. Dong *et al.* 2018). Under non-inductive condition, H2A.Z counteracts the positive effects of the transcription factor at cat-3 *locus*, CPC1, to achieve low level of cat-3 expression. Upon oxidative stress, H2A.Z is rapidly evicted from cat-3 locus to facilitate the recruitment of CPC1, contributing to robust and full cat-3 gene expression in response to external stimuli. Therefore, H2A.Z is here regarded as barrier for RNAPII transiting (Dong *et al*. 2018).

The deposition of H2A.Z also relates to DNA methylation, one of several epigenetic marks that cells use to control negatively gene transcription. In *A. thaliana*, it is reported that H2A.Z prevents genes from DNA methylation and reduce gene activity. Meanwhile, H2A.Z can also be excluded from the site of DNA methylation in the bodies of actively transcribed genes and in methylated transposons to affect gene silencing (Zilberman *et al*. 2008). Recently, however, further study demonstrated that there is only minor influence on the level of DNA methylation in a novel H2A.Z loss-function line of *A. thaliana* (Coleman-Derr and Zilberman 2012). In addition, the highest and lowest expressed genes have the least H2A.Z enrichment near TSS. Within gene bodies, H2A.Z deposition positively regulates gene responsiveness, but may negative correlates with gene constitutive expression. The authors suggested that a major function of gene body DNA methylation is to prevent the incorporation of H2A.Z within gene bodies, and thus stabilize the expression of constitutive genes, and on the other

hand H2A.Z deposition within unmethylated gene bodies could promote the expression of inducible genes, including genes response to developmental, biotic and abiotic stimuli (Coleman-Derr and Zilberman 2012). More recently, another study on *A. thaliana* also suggested that H2A.Z in gene bodies has a strong repressive effect on transcription, whereas in +1 nucleosomes, it is important for maintaining the activity of some genes (Sura *et al*. 2017). These findings are consistent with results in yeast indicating that H2A.Z incorporates in coding regions of genes that are involved in genotoxic stress responses (Sadeghi *et al*. 2011). In addition, Hartley and Madhani (2009) found in budding yeast that H2A.Z deposition is dispensable for nucleosome positioning, while the establishment of nucleosome-free region is necessary for H2A.Z deposition. finally, another study in yeast demonstrated that H2A.Z is selectively enriched at 5' regions of both active and inactive genes(Raisner *et al.* 2005). No correlation between the levels of H2A.Z deposition and either the transcription rate or RNA polymerase II occupancy can be observed (Raisner *et al*. 2005). Therefore, role of H2AZ in controlling gene transcription appears to be versatile and highly dependent on chromatin context.

Roles of H2A.Z on DNA repair

DNA double–strand breaks (DSB) occur as a result of genome damage, and cells have developed two major repair pathways: homologous recombination (HR) and the non-homologous end joining (NHEJ) (Hiom 2010; Grabarz *et al*. 2013). During DSB repair, a relaxed and open chromatin domain is required to facilitate the loading of repair factors (Kim *et al*. 2007; Sartori *et al*. 2007). Before the recruitment of 53BP1p and brca1p in the NHEJ pathway, components binding to the break site contain a variety of factors including MRX (MRN) complex and Ku proteins (Ku70 and Ku80) (Daley and Sung 2014). HR pathway is also initiated by the MRX complex in particular, then followed by the recruitment of other factors including such as RAD51 protein (Chatterjee and Walker 2017). In budding yeast, strains lacking the subunit of INO80 complex Ino80p, Arp5p, or Arp8p are hypersensitive to DNA damaging agents and to DSBs induced by the HO endonuclease (Attikum *et al.* 2004). ChIP experiments showed that Ino80p, Arp5p, and Arp8p are recruited to an HO-induced DSB, where a phosphorylated form of H2A accumulates, indicating that INO80-mediated

chromatin remodeling appears to facilitate processing of the DSBs repair (Attikum *et al.* 2004). Another study in yeast found H2A.Z is rapidly but transiently deposited at the break area and it is involved in DSB tethering. Meanwhile, H2A.Z SUMOylation is needed for DSB relocation (Kalocsay, Hiller, and Jentsch 2009). In human 293T cells, H2A.Z seems to be directly involved in the regulation of DSB repair through the HR and NHEJ pathway (Xu *et al.* 2012). It was observed rapidly and reversibly exchanged by p400 motor ATPase onto a unique DSB, promotes the acetylation and ubiquitination of histones, function together to shift the chromatin to an open domain at the site of DSB damage, therefore helps the loading of brca1 complex (Xu *et al.* 2012). H2A.Z exchange also restricts single-stranded DNA production by nucleases and is essential for loading of the Ku70/Ku80 DSB repair protein (Xu *et al*. 2012). Further analyses showed that, in mammalian cells, the deposition of H2A.Z on nucleosomes at DSBs is transient; it can be removed from chromatin flanking DNA damage by INO80 and histone chaperone Anp32e rapidly, which promotes DSB repair (Gursoy-Yuzugullu, Ayrapetov, and Price 2015; Alatwi and Downs 2015). They think it is because the rapid eviction of H2A.Z release H4 tail for acetylation, disrupting interactions between nucleosomes contribute to an open, flexible chromatin. By contrast, Taty-Taty *et al.* (2014) found the absence of H2A.Z impaired cell proliferation and viability but did not led to any DSB repair defects in the osteosarcoma U2OS cell lines and T-immortalized human fibroblasts. There was also no H2A.Z recruitment around DSBs that could be detected (Taty-Taty *et al.* 2014). Different ways to obtain H2A.Z depletion may help to explain the controversy. Xu *et al.* used shRNA to achieve a long-term and robust depletion, while Taty-Taty *et al.* only obtained a partial reduction in H2A.Z expression. Taken together, these studies revealed that H2A.Z plays a critical role in remodeling chromatin structure at DSBs. The underlying molecular mechanism might be that H2A.Z is actively accumulated on nucleosomes at DSBs. However, the H2A.Z-H2B dimer could be rapidly removed by INO80 complex and the histone chaperone ANE32E, resulting in loss of acidic patch and release of the H4 tail for acetylation by Tip60. Histone acetylation at DSBs can create a more open conformation of chromatin and further promotes ubiquitination, which is necessary for the loading of braca1.

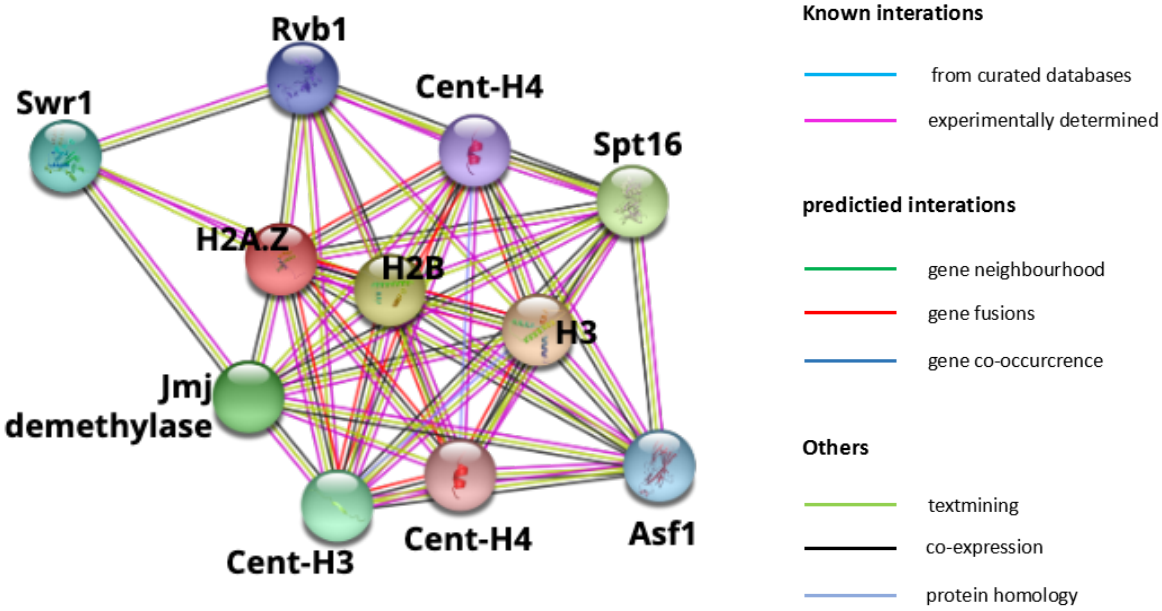
In filamentous fungi, many homologs of DNA repair factors of *S. cerevisiae* have been identified. For example, genetic and molecular analyses of *Neurospora* mutants revealed that

mei-3p (homolog of RAD51p), mus-51p and mus-52p (homologs of Ku70p and Ku80p) are involved in the HR and NHEJ pathway, respectively (Hatakeyama, Ishii, and Inoue 1995; Ninomiya *et al*. 2004; Ishibashi *et al*. 2006). Compared to yeast, HR frequencies in filamentous fungi are extremely low, meaning that DNA DSBs are predominantly repaired by NHEJ, which makes generation of gene knock-out mutants by HR time-consuming (Nielsen *et al*. 2006; Ishibashi *et al*. 2006; Nielsen, Nielsen, and Mortensen 2008). Therefore, studies have been extensively carried out to increase the efficiency of gene targeting by inhibiting NHEJ. Indeed, higher rate of gene targeting has been observed in KU-deficient mutants of *N. crassa*, *Aspergillus nidulans*, *A. fumigatus*, *A. sojae*, *A. oryzae*, and *Cryptococcus neoformans*. Taking into account the roles of H2A.Z in HR and NHEJ pathway in other organisms including yeast, maybe it is another potential target for the optimization of gene targeting in filamentous fungi.

H2A.Z affects nucleosome stability

Within the nucleosome, histone-histone or histone-DNA interactions play an important role in the stability of the entire particle. In some cases, H2A.Z stabilizes nucleosome structure (Park *et al.* 2004; Thambirajah *et al.* 2006; Jin and Felsenfeld 2007). When using fluorescence resonance energy transfer system to compare nucleosomes containing the histone variant H2A.Z with canonical nucleosomes containing replication-dependent major histones, found the dissociation of the (H2A.Z-H2B) dimer from the nucleosome occurs at higher salt concentrations, and over a broader salt range compared with the (H2A-H2B) dimer. A similar study revealed that H2A.Z could stabilize chromatin, but it is dependent on the acetylation of core histones (Thambirajah *et al.* 2006). Jin and Felsenfeld (2007) reported that all nucleosome core particles (NCPs) contain H3.3 reduced stability compared with H3 NCPs. in addition, NCPs that contain both H3.3 and H2A.Z are even less stable than NCPs containing H3.3 and H2A. Intriguingly, NCPs containing H3 and H2A.Z are at least as stable as H3/H2A NCPs. By contrast, some studies indicated that even though the crystal structure of NCP containing H2A.Z is similar to that of the nucleosome structure containing major histone proteins, histone H2A.Z variant may result in subtle destabilization of chromatin, which may be important for transcriptional activation (Suto *et al*. 2000; Abbott *et al*. 2001; Zhang,

Roberts, and Cairns 2005). The partners of H2A.Z within NCP may thus be key factors in determining nucleosome stability (Jin and Felsenfeld 2007). When H2A.Z is coupled with H3, it might form an unusually stable chromatin conformation. Otherwise, it may destabilize the nucleosomes. Such characteristics are consistent with the physiological roles of H2A.Z, especially its involvement in the transcriptional regulation and DNA repair.



Gene name	Annotation	Gene ID
H2A.Z	Histone Variant H2A.Z	FGRAMPH1_01G03973
H2B	Histone H2B	FGRAMPH1_01G26111
H3	Histone H3	FGRAMPH1_01G14931
Spt16	FACT complex subunit SPT16	FGRAMPH1_01G07139
Cent-H4	Histone H4 partial	FGRAMPH1_01G14929
Rvb1	RuvB-like helicase 1	FGRAMPH1_01G17223
Asf1	Histone chaperone ASF1	FGRAMPH1_01G00927
Swr1	Helicase SWR1	FGRAMPH1_01G18675
jmJ demethylase	JmjC domain-containing histone demethylase; targets tri- and dimethylated H3K36; predicted	FGRAMPH1_01G03799
Cent-H3	Histone H3 centromeric variant, putative	FGRAMPH1_01G06247
Cent-H4	Histone H4 partial	FGRAMPH1_01G18051

Figure 5 Predicted functional partners of H2A.Z in *F. graminearum*. FgH2A.Z (FGRAMPH1_01G03973P0) was used as query for The Simple Modular Architecture Research Tool (SMART;(Letunic, Doerks, and Bork 2015; Letunic and Bork 2018) to predict the corresponding interacting network of proteins pulling data from the Protein-Protein Interaction Networks data base STRING (Szklarczyk et al. 2019) using default settings. Each node represents all the proteins produced by a single, protein-coding gene locus. Edges represent protein-protein associations color coded by source of information (see legend in the figure).

In fungi, most of the studies of NCP focus on *S. cerevisiae*; the NCP of filamentous fungi has been much less characterized. As shown in **Figure 5**, in *F. graminearum*, H2A.Z interacts extensively with other proteins through both direct or indirect ways (Letunic, Doerks, and Bork 2015; Letunic and Bork 2018). For example, the relationship between H2A.Z and Swr1p has been experimentally determined, while it is predicted to co-occur with RuvB-like helicase1, histone H4 as well as an uncharacterized protein (Letunic, Doerks, and Bork 2015; Letunic and Bork 2018). There is also a co-expression with histone H2B and H3 **(Figure 5)**. In future studies on roles of H2A.Z in controlling nucleosome stability, taking into account the interaction of H2A.Z with other proteins may enlighten us more on the functions fulfilled.

Conclusions

Chromatin is a highly dynamic architecture mainly controlled by three ways, including ATP-dependent chromatin remodeling, histone variants and PTMs, and DNA methylation. H2A.Z is a highly conserved histone variant which can be deposited or removed from chromatin by the chromatin remodeling complex SWR1 or INO80, respectively. This process is accompanied by various histone PTMs as well as the conditions of DNA methylation, finally leading to the change of chromatin state. Normally, a more opened, flexible chromatin is necessary for many biological processes such as gene transcription and binding of DSB repair factors. According to the studies mentioned above, the status of chromatin in response to H2A.Z exchange varies between organisms, gene types as well as locations, meaning that the function of H2A.Z is very likely context-specific. It can act as a positive regulator under certain conditions, but might also be a negative regulator. Specific properties of H2A.Z-containing nucleosomes also linked with the incorporation with other histones or histone variants. For example, it was suggested that nucleosomes containing both H2A.Z and H3.3 histone variants are much less stable than nucleosomes of other composition (Jin and Felsenfeld, 2007).

In filamentous fungi, SMs biosynthesis highly associates with epigenetic marker such as H3K27me3, H3K4me3, and HP1. According to previous studies carried out in other species, some of these markers always co-localize with H2A.Z, suggesting the potential role of H2A.Z involved in filamentous fungi. Indeed, in *N. crassa*, it is reported that under normal and

oxidative stress conditions, H2A.Z antagonized the positive effect of the transcriptional activator CPC1 to regulate the expression of catalase-3, an oxidative resistant gene (Dong et al. 2018). Up to now, this is the only study that focuses on H2A.Z in filamentous fungi. As illustrated above, homologs of yeast Swr1p, Snf2p and Esa1p subunits, which are involved in the deposition/removal/acetylation of H2A.Z, can be identified in both *F. graminearum* and *F. fujikuroi*. We guess that H2A.Z also plays critical roles in central biological processes of *Fusarium* species, including the secondary metabolism.

Funding

Work was funded by ANR Grant ANR-18-CE91-0006 to N.P. Z.C. held a doctoral fellowship from the China Scholarship Council.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abbott, D. Wade, Vessela S. Ivanova, Xiaoying Wang, William M. Bonner, and Juan Ausió. 2001. "Characterization of the Stability and Folding of H2A.Z Chromatin Particles IMPLICATIONS FOR TRANSCRIPTIONAL ACTIVATION." *Journal of Biological Chemistry* 276 (45): 41945–49. https://doi.org/10.1074/jbc.M108217200.

Adam, M., F. Robert, M. Larochelle, and L. Gaudreau. 2001. "H2A.Z Is Required for Global Chromatin Integrity and for Recruitment of RNA Polymerase II under Specific Conditions." *Molecular and Cellular Biology* 21 (18): 6270–79.

Alatwi, H. E., and J. A. Downs. 2015. "Removal of H2A.Z by INO80 Promotes Homologous Recombination." *EMBO Reports* 16 (8): 986–94. https://doi.org/10.15252/embr.201540330.

Alberti, Fabrizio, Gary D. Foster, and Andy M. Bailey. 2017. "Natural Products from Filamentous Fungi and Production by Heterologous Expression." *Applied Microbiology and Biotechnology* 101 (2): 493–500. https://doi.org/10.1007/s00253-016-8034-2.

Allard, St|[eacute]|phane, Rhea T. Utley, Julie Savard, Astrid Clarke, Patrick Grant, Christopher J. Brandl, Lorraine Pillus, Jerry Workman, and Jacques C|[ocirc]|t|[eacute. 1999. "NuA4, an Essential Transcription Adaptor/Histone H4 Acetyltransferase Complex Containing Esa1p and the ATM-Related Cofactor Tra1p." *The*

EMBO Journal 18 (September): 5108–19. https://doi.org/10.1093/emboj/18.18.5108.

Altaf, Mohammed, Andréanne Auger, Julie Monnet-Saksouk, Joëlle Brodeur, Sandra Piquet, Myriam Cramet, Nathalie Bouchard, et al. 2010. "NuA4-Dependent Acetylation of Nucleosomal Histones H4 and H2A Directly Stimulates Incorporation of H2A.Z by the SWR1 Complex." *Journal of Biological Chemistry* 285 (21): 15966–77. https://doi.org/10.1074/jbc.M110.117069.

Attikum, Haico van, Olivier Fritsch, and Susan M Gasser. 2007. "Distinct Roles for SWR1 and INO80 Chromatin Remodeling Complexes at Chromosomal Double-Strand Breaks." *The EMBO Journal* 26 (18): 4113–25. https://doi.org/10.1038/sj.emboj.7601835.

Attikum, Haico van, Olivier Fritsch, Barbara Hohn, and Susan M. Gasser. 2004. "Recruitment of the INO80 Complex by H2A Phosphorylation Links ATP-Dependent Chromatin Remodeling with DNA Double-Strand Break Repair." *Cell* 119 (6): 777–88. https://doi.org/10.1016/j.cell.2004.11.033.

Audia, James E., and Robert M. Campbell. 2016. "Histone Modifications and Cancer." *Cold Spring Harbor Perspectives in Biology* 8 (4). https://doi.org/10.1101/cshperspect.a019521.

Auger, Andréanne, Luc Galarneau, Mohammed Altaf, Amine Nourani, Yannick Doyon, Rhea T. Utley, Dominique Cronier, Stéphane Allard, and Jacques Côté. 2008. "Eaf1 Is the Platform for NuA4 Molecular Assembly That Evolutionarily Links Chromatin Acetylation to ATP-Dependent Exchange of Histone H2A Variants." *Molecular and Cellular Biology* 28 (7): 2257–70. https://doi.org/10.1128/MCB.01755-07.

Bannister, Andrew J, and Tony Kouzarides. 2011. "Regulation of Chromatin by Histone Modifications." *Cell Research* 21 (3): 381–95. https://doi.org/10.1038/cr.2011.22.

Bateman, Alex, Ewan Birney, Richard Durbin, Sean R. Eddy, Kevin L. Howe, and Erik L. L. Sonnhammer. 2000. "The Pfam Protein Families Database." *Nucleic Acids Research* 28 (1): 263–66.

Beggs, Suzanne, Tharappel C. James, and Ursula Bond. 2012. "The PolyA Tail Length of Yeast Histone MRNAs Varies during the Cell Cycle and Is Influenced by Sen1p and Rrp6p." *Nucleic Acids Research* 40 (6): 2700–2711. https://doi.org/10.1093/nar/gkr1108.

Binda, Olivier, Ana Sevilla, Gary LeRoy, Ihor R. Lemischka, Benjamin A. Garcia, and Stéphane Richard. 2013. "SETD6 Monomethylates H2AZ on Lysine 7 and Is Required for the Maintenance of Embryonic Stem Cell Self-Renewal." *Epigenetics* 8 (2): 177–83. https://doi.org/10.4161/epi.23416.

Bird, Alexander W., David Y. Yu, Marilyn G. Pray-Grant, Qifeng Qiu, Kirsty E. Harmon, Paul C. Megee, Patrick A. Grant, M. Mitchell Smith, and Michael F. Christman. 2002. "Acetylation of Histone H4 by Esa1 Is Required for DNA Double-Strand Break Repair." *Nature* 419 (6905): 411–15. https://doi.org/10.1038/nature01035.

Boyne, Michael T., James J. Pesavento, Craig A. Mizzen, and Neil L. Kelleher. 2006. "Precise Characterization of Human Histones in the H2A Gene Family by Top Down Mass Spectrometry." *Journal of Proteome Research* 5 (2): 248–53. https://doi.org/10.1021/pr050269n.

Bruce, Kimberley, Fiona A. Myers, Evangelia Mantouvalou, Pascal Lefevre, Ian Greaves, Constanze Bonifer, David J. Tremethick, Alan W. Thorne, and Colyn Crane-Robinson. 2005. "The Replacement Histone H2A.Z in a Hyperacetylated Form Is a Feature of Active Genes in the Chicken." *Nucleic Acids Research* 33 (17): 5633–39. https://doi.org/10.1093/nar/gki874.

Cai, Hanyang, Man Zhang, Mengnan Chai, Qing He, Xinyu Huang, Lihua Zhao, and Yuan Qin. 2019. "Epigenetic Regulation of Anthocyanin Biosynthesis by an Antagonistic Interaction between H2A.Z and H3K4me3." *The New Phytologist* 221 (1): 295–308. https://doi.org/10.1111/nph.15306.

Cai, Houjian, Sarah Kauffman, Fred Naider, and Jeffrey M. Becker. 2006. "Genomewide Screen Reveals a Wide Regulatory Network for Di/Tripeptide Utilization in Saccharomyces Cerevisiae." *Genetics* 172 (3): 1459–76.

https://doi.org/10.1534/genetics.105.053041.

Caracuel, Zaira, M. Isabel G. Roncero, Eduardo A. Espeso, Clara I. González-Verdejo, Fe I. García-Maceira, and Antonio Di Pietro. 2003. “The PH Signalling Transcription Factor PacC Controls Virulence in the Plant Pathogen Fusarium Oxysporum.” *Molecular Microbiology* 48 (3): 765–79. https://doi.org/10.1046/j.1365-2958.2003.03465.x.

Carter, Benjamin, Brett Bishop, Kwok Ki Ho, Ru Huang, Wei Jia, Heng Zhang, Pete E. Pascuzzi, Roger B. Deal, and Joe Ogas. 2018. “The Chromatin Remodelers PKL and PIE1 Act in an Epigenetic Pathway That Determines H3K27me3 Homeostasis in Arabidopsis.” *The Plant Cell* 30 (6): 1337–52. https://doi.org/10.1105/tpc.17.00867.

Chatterjee, Nimrat, and Graham C. Walker. 2017. “Mechanisms of DNA Damage, Repair and Mutagenesis.” *Environmental and Molecular Mutagenesis* 58 (5): 235–63. https://doi.org/10.1002/em.22087.

Chen, Menzies, Katherine Licon, Rei Otsuka, Lorraine Pillus, and Trey Ideker. 2013. “Decoupling Epigenetic and Genetic Effects through Systematic Analysis of Gene Position.” *Cell Reports* 3 (1): 128–37. https://doi.org/10.1016/j.celrep.2012.12.003.

Clapier, Cedric R., and Bradley R. Cairns. 2009. “The Biology of Chromatin Remodeling Complexes.” *Annual Review of Biochemistry* 78: 273–304. https://doi.org/10.1146/annurev.biochem.77.062706.153223.

Coleman-Derr, Devin, and Daniel Zilberman. 2012. “Deposition of Histone Variant H2A.Z within Gene Bodies Regulates Responsive Genes.” *PLoS Genetics* 8 (10): e1002988. https://doi.org/10.1371/journal.pgen.1002988.

Connolly, Lanelle R., Kristina M. Smith, and Michael Freitag. 2013. “The Fusarium Graminearum Histone H3 K27 Methyltransferase KMT6 Regulates Development and Expression of Secondary Metabolite Gene Clusters.” Edited by Hiten D. Madhani. *PLoS Genetics* 9 (10): e1003916. https://doi.org/10.1371/journal.pgen.1003916.

Creyghton, Menno P., Styliani Markoulaki, Stuart S. Levine, Jacob Hanna, Michael A. Lodato, Ky Sha, Richard A. Young, Rudolf Jaenisch, and Laurie A. Boyer. 2008. “H2AZ Is Enriched at Polycomb Complex Target Genes in ES Cells and Is Necessary for Lineage Commitment.” *Cell* 135 (4): 649–61. https://doi.org/10.1016/j.cell.2008.09.056.

Daal, A. van, and S. C. Elgin. 1992. “A Histone Variant, H2AvD, Is Essential in Drosophila Melanogaster.” *Molecular Biology of the Cell* 3 (6): 593–602. https://doi.org/10.1091/mbc.3.6.593.

Daal, A. van, E. M. White, S. C. Elgin, and M. A. Gorovsky. 1990. “Conservation of Intron Position Indicates Separation of Major and Variant H2As Is an Early Event in the Evolution of Eukaryotes.” *Journal of Molecular Evolution* 30 (5): 449–55.

Daley, James M., and Patrick Sung. 2014. “53BP1, BRCA1, and the Choice between Recombination and End Joining at DNA Double-Strand Breaks.” *Molecular and Cellular Biology* 34 (8): 1380–88. https://doi.org/10.1128/MCB.01639-13.

Deal, Roger B., and Steven Henikoff. 2011. “The INTACT Method for Cell Type-Specific Gene Expression and Chromatin Profiling in Arabidopsis Thaliana.” *Nature Protocols* 6 (1): 56–68. https://doi.org/10.1038/nprot.2010.175.

Dong, Fulu, Zhenwei Song, Jiali Yu, Baole Zhang, BaoChun Jiang, Ying Shen, Youde Lu, Chunlei Song, Peiqing Cong, and Honglin Liu. 2016. “Dynamic Changes in Occupancy of Histone Variant H2A.Z during Induced Somatic Cell Reprogramming.” *Stem Cells International* 2016. https://doi.org/10.1155/2016/3162363.

Dong, Qing, Yajun Wang, Shaohua Qi, Kexin Gai, Qun He, and Ying Wang. 2018. “Histone Variant H2A.Z Antagonizes the Positive Effect of the Transcriptional Activator CPC1 to Regulate Catalase-3 Expression under Normal and Oxidative Stress Conditions.” *Free Radical Biology and Medicine* 121 (June): 136–48. https://doi.org/10.1016/j.freeradbiomed.2018.05.003.

Downs, Jessica A., Stéphane Allard, Olivier Jobin-Robitaille, Ali Javaheri, Andréanne Auger, Nathalie Bouchard, Stephen J. Kron, Stephen P. Jackson, and Jacques Côté. 2004. "Binding of Chromatin-Modifying Activities to Phosphorylated Histone H2A at DNA Damage Sites." *Molecular Cell* 16 (6): 979–90. https://doi.org/10.1016/j.molcel.2004.12.003.

Doyon, Yannick, and Jacques Côté. 2004. "The Highly Conserved and Multifunctional NuA4 HAT Complex." *Current Opinion in Genetics & Development* 14 (2): 147–54. https://doi.org/10.1016/j.gde.2004.02.009.

Draker, Ryan, Elizabeth Sarcinella, and Peter Cheung. 2011. "USP10 Deubiquitylates the Histone Variant H2A.Z and Both Are Required for Androgen Receptor-Mediated Gene Activation." *Nucleic Acids Research* 39 (9): 3529–42. https://doi.org/10.1093/nar/gkq1352.

Eddy, Sean R. 2009. "A New Generation of Homology Search Tools Based on Probabilistic Inference." *Genome Informatics. International Conference on Genome Informatics* 23 (1): 205–11. https://doi.org/10.1142/9781848165632_0019.

Eissenberg, Joel C., Madeline Wong, and John C. Chrivia. 2005. "Human SRCAP and Drosophila Melanogaster DOM Are Homologs That Function in the Notch Signaling Pathway." *Molecular and Cellular Biology* 25 (15): 6559–69. https://doi.org/10.1128/MCB.25.15.6559-6569.2005.

Faast, R., V. Thonglairoam, T. C. Schulz, J. Beall, J. R. Wells, H. Taylor, K. Matthaei, P. D. Rathjen, D. J. Tremethick, and I. Lyons. 2001. "Histone Variant H2A.Z Is Required for Early Mammalian Development." *Current Biology: CB* 11 (15): 1183–87.

Gerhold, Christian B., and Susan M. Gasser. 2014. "INO80 and SWR Complexes: Relating Structure to Function in Chromatin Remodeling." *Trends in Cell Biology* 24 (11): 619–31. https://doi.org/10.1016/j.tcb.2014.06.004.

Giaimo, Benedetto Daniele, Francesca Ferrante, Andreas Herchenröther, Sandra B. Hake, and Tilman Borggrefe. 2019. "The Histone Variant H2A.Z in Gene Regulation." *Epigenetics & Chromatin* 12 (1): 37. https://doi.org/10.1186/s13072-019-0274-9.

Goswami, Rubella S., and H. Corby Kistler. 2004. "Heading for Disaster: Fusarium Graminearum on Cereal Crops." *Molecular Plant Pathology* 5 (6): 515–25. https://doi.org/10.1111/j.1364-3703.2004.00252.x.

Grabarz, Anastazja, Josée Guirouilh-Barbat, Aurélia Barascu, Gaëlle Pennarun, Diane Genet, Emilie Rass, Susanne M. Germann, Pascale Bertrand, Ian D. Hickson, and Bernard S. Lopez. 2013. "A Role for BLM in Double-Strand Break Repair Pathway Choice: Prevention of CtIP/Mre11-Mediated Alternative Nonhomologous End-Joining." *Cell Reports* 5 (1): 21–28. https://doi.org/10.1016/j.celrep.2013.08.034.

Gruber-Dorninger, Christiane, Timothy Jenkins, and Gerd Schatzmayr. 2019. "Global Mycotoxin Occurrence in Feed: A Ten-Year Survey." *Toxins* 11 (7): 375. https://doi.org/10.3390/toxins11070375.

Gu, Qin, Tiantian Ji, Xiao Sun, Hai Huang, Hao Zhang, Xi Lu, Liming Wu, Rong Huo, Huijun Wu, and Xuewen Gao. 2017. "Histone H3 Lysine 9 Methyltransferase FvDim5 Regulates Fungal Development, Pathogenicity and Osmotic Stress Responses in Fusarium Verticillioides." *FEMS Microbiology Letters* 364 (19). https://doi.org/10.1093/femsle/fnx184.

Guillemette, Benoît, and Luc Gaudreau. 2006. "H2A.Z: A Histone Variant That Decorates Gene Promoters." *Médecine Sciences : M/S* 22 (December): 941–46. https://doi.org/10.1051/medsci/20062211941.

Guindon, Stéphane, Jean-François Dufayard, Vincent Lefort, Maria Anisimova, Wim Hordijk, and Olivier Gascuel. 2010. "New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0." *Systematic Biology* 59 (3): 307–21. https://doi.org/10.1093/sysbio/syq010.

Gursoy-Yuzugullu, Ozge, Marina K. Ayrapetov, and Brendan D. Price. 2015. "Histone Chaperone Anp32e Removes H2A.Z from DNA Double-Strand Breaks and Promotes Nucleosome Reorganization and DNA

Repair." *Proceedings of the National Academy of Sciences* 112 (24): 7507–12. https://doi.org/10.1073/pnas.1504868112.

Hamad, Bashar. 2010. "The Antibiotics Market." *Nature Reviews. Drug Discovery* 9 (9): 675–76. https://doi.org/10.1038/nrd3267.

Hartley, Paul D., and Hiten D. Madhani. 2009. "Mechanisms That Specify Promoter Nucleosome Location and Identity." *Cell* 137 (3): 445–58. https://doi.org/10.1016/j.cell.2009.02.043.

Hatakeyama, S., C. Ishii, and H. Inoue. 1995. "Identification and Expression of the Neurospora Crassa Mei-3 Gene Which Encodes a Protein Homologous to Rad51 of Saccharomyces Cerevisiae." *Molecular & General Genetics: MGG* 249 (4): 439–46. https://doi.org/10.1007/bf00287106.

Hawksworth, David, and Robert Lücking. 2017. "Fungal Diversity Revisited: 2.2 to 3.8 Million Species." *Microbiology Spectrum* 5 (July). https://doi.org/10.1128/microbiolspec.FUNK-0052-2016.

Hiom, Kevin. 2010. "Coping with DNA Double Strand Breaks." *DNA Repair* 9 (12): 1256–63. https://doi.org/10.1016/j.dnarep.2010.09.018.

Hu, Gangqing, Kairong Cui, Daniel Northrup, Chengyu Liu, Chaochen Wang, Qingsong Tang, Kai Ge, David Levens, Colyn Crane-Robinson, and Keji Zhao. 2013. "H2A.Z Facilitates Access of Active and Repressive Complexes to Chromatin in Embryonic Stem Cell Self-Renewal and Differentiation." *Cell Stem Cell* 12 (2): 180–92. https://doi.org/10.1016/j.stem.2012.11.003.

Hu, Yongfeng, Yuan Shen, Natalia Conde e Silva, and Dao-Xiu Zhou. 2011. "The Role of Histone Methylation and H2A.Z Occupancy during Rapid Activation of Ethylene Responsive Genes." *PLOS ONE* 6 (11): e28224. https://doi.org/10.1371/journal.pone.0028224.

Hussein, H. S., and J. M. Brasel. 2001. "Toxicity, Metabolism, and Impact of Mycotoxins on Humans and Animals." *Toxicology* 167 (2): 101–34.

Ikura, T., V. V. Ogryzko, M. Grigoriev, R. Groisman, J. Wang, M. Horikoshi, R. Scully, J. Qin, and Y. Nakatani. 2000. "Involvement of the TIP60 Histone Acetylase Complex in DNA Repair and Apoptosis." *Cell* 102 (4): 463–73. https://doi.org/10.1016/s0092-8674(00)00051-9.

Ishibashi, Kazuma, Keiichiro Suzuki, Yoshinori Ando, Chihiro Takakura, and Hirokazu Inoue. 2006. "Nonhomologous Chromosomal Integration of Foreign DNA Is Completely Dependent on MUS-53 (Human Lig4 Homolog) in Neurospora." *Proceedings of the National Academy of Sciences of the United States of America* 103 (40): 14871–76. https://doi.org/10.1073/pnas.0604477103.

Jackson, J. D., V. T. Falciano, and M. A. Gorovsky. 1996. "A Likely Histone H2A.F/Z Variant in Saccharomyces Cerevisiae." *Trends in Biochemical Sciences* 21 (12): 466–67.

Jackson, J. D., and M. A. Gorovsky. 2000. "Histone H2A.Z Has a Conserved Function That Is Distinct from That of the Major H2A Sequence Variants." *Nucleic Acids Research* 28 (19): 3811–16.

Jin, Chunyuan, and Gary Felsenfeld. 2007. "Nucleosome Stability Mediated by Histone Variants H3.3 and H2A.Z." *Genes & Development* 21 (12): 1519–29. https://doi.org/10.1101/gad.1547707.

Jin, Jingji, Yong Cai, Tingting Yao, Aaron J. Gottschalk, Laurence Florens, Selene K. Swanson, José L. Gutiérrez, et al. 2005. "A Mammalian Chromatin Remodeling Complex with Similarities to the Yeast INO80 Complex." *The Journal of Biological Chemistry* 280 (50): 41207–12. https://doi.org/10.1074/jbc.M509128200.

Kalocsay, Marian, Natalie Jasmin Hiller, and Stefan Jentsch. 2009. "Chromosome-Wide Rad51 Spreading and SUMO-H2A.Z-Dependent Chromosome Fixation in Response to a Persistent DNA Double-Strand Break." *Molecular Cell* 33 (3): 335–43. https://doi.org/10.1016/j.molcel.2009.01.016.

Katoh, H., K. Ohtani, H. Yamamoto, and K. Akimitsu. 2007. “Overexpression of a Gene Encoding a Catabolite Repression Element in Alternaria Citri Causes Severe Symptoms of Black Rot in Citrus Fruit.” *Phytopathology* 97 (5): 557–63. https://doi.org/10.1094/PHYTO-97-5-0557.

Keogh, Michael-Christopher, Thomas A. Mennella, Chika Sawa, Sharon Berthelet, Nevan J. Krogan, Adam Wolek, Vladimir Podolny, et al. 2006. “The Saccharomyces Cerevisiae Histone H2A Variant Htz1 Is Acetylated by NuA4.” *Genes & Development* 20 (6): 660–65. https://doi.org/10.1101/gad.1388106.

Kim, Jong-Soo, Jason T. Heale, Weihua Zeng, Xiangduo Kong, Tatiana B. Krasieva, Alexander R. Ball, and Kyoko Yokomori. 2007. “In Situ Analysis of DNA Damage Response and Repair Using Laser Microirradiation.” *Methods in Cell Biology* 82: 377–407. https://doi.org/10.1016/S0091-679X(06)82013-3.

Kobor, Michael S., Shivkumar Venkatasubrahmanyam, Marc D. Meneghini, Jennifer W. Gin, Jennifer L. Jennings, Andrew J. Link, Hiten D. Madhani, and Jasper Rine. 2004. “A Protein Complex Containing the Conserved Swi2/Snf2-Related ATPase Swr1p Deposits Histone Variant H2A.Z into Euchromatin.” *PLoS Biology* 2 (5): E131. https://doi.org/10.1371/journal.pbio.0020131.

Kornberg, Roger D. 1999. “Eukaryotic Transcriptional Control.” *Trends in Cell Biology* 9 (12): M46–49. https://doi.org/10.1016/S0962-8924(99)01679-7.

———. 2007. “The Molecular Basis of Eukaryotic Transcription.” *Proceedings of the National Academy of Sciences of the United States of America* 104 (32): 12955–61. https://doi.org/10.1073/pnas.0704138104.

Krogan, Nevan J., Michael-Christopher Keogh, Nira Datta, Chika Sawa, Owen W. Ryan, Huiming Ding, Robin A. Haw, et al. 2003. “A Snf2 Family ATPase Complex Required for Recruitment of the Histone H2A Variant Htz1.” *Molecular Cell* 12 (6): 1565–76.

Krogan, Nevan J., Wen-Tao Peng, Gerard Cagney, Mark D. Robinson, Robin Haw, Gouqing Zhong, Xinghua Guo, et al. 2004. “High-Definition Macromolecular Composition of Yeast RNA-Processing Complexes.” *Molecular Cell* 13 (2): 225–39.

Ku, Manching, Jacob D. Jaffe, Richard P. Koche, Esther Rheinbay, Mitsuhiro Endoh, Haruhiko Koseki, Steven A. Carr, and Bradley E. Bernstein. 2012. “H2A.Z Landscapes and Dual Modifications in Pluripotent and Multipotent Stem Cells Underlie Complex Genome Regulatory Functions.” *Genome Biology* 13 (10): R85. https://doi.org/10.1186/gb-2012-13-10-r85.

Kurat, Christoph F., Judith Recht, Ernest Radovani, Tanja Durbic, Brenda Andrews, and Jeffrey Fillingham. 2014. “Regulation of Histone Gene Transcription in Yeast.” *Cellular and Molecular Life Sciences: CMLS* 71 (4): 599–613. https://doi.org/10.1007/s00018-013-1443-9.

Kusch, Thomas, Laurence Florens, W. Hayes Macdonald, Selene K. Swanson, Robert L. Glaser, John R. Yates, Susan M. Abmayr, Michael P. Washburn, and Jerry L. Workman. 2004. “Acetylation by Tip60 Is Required for Selective Histone Variant Exchange at DNA Lesions.” *Science (New York, N.Y.)* 306 (5704): 2084–87. https://doi.org/10.1126/science.1103455.

Leach, T. J., M. Mazzeo, H. L. Chotkowski, J. P. Madigan, M. G. Wotring, and R. L. Glaser. 2000. “Histone H2A.Z Is Widely but Nonrandomly Distributed in Chromosomes of Drosophila Melanogaster.” *The Journal of Biological Chemistry* 275 (30): 23267–72. https://doi.org/10.1074/jbc.M910206199.

Letunic, Ivica, and Peer Bork. 2018. “20 Years of the SMART Protein Domain Annotation Resource.” *Nucleic Acids Research* 46 (D1): D493–96. https://doi.org/10.1093/nar/gkx922.

Letunic, Ivica, Tobias Doerks, and Peer Bork. 2015. “SMART: Recent Updates, New Developments and Status in 2015.” *Nucleic Acids Research* 43 (Database issue): D257-260. https://doi.org/10.1093/nar/gku949.

Liu, X., B. Li, and null GorovskyMA. 1996. “Essential and Nonessential Histone H2A Variants in Tetrahymena

Thermophila." *Molecular and Cellular Biology* 16 (8): 4305–11.

Liu, Ye, Na Liu, Yanni Yin, Yun Chen, Jinhua Jiang, and Zhonghua Ma. 2015. "Histone H3K4 Methylation Regulates Hyphal Growth, Secondary Metabolism and Multiple Stress Responses in Fusarium Graminearum." *Environmental Microbiology* 17 (11): 4615–30. https://doi.org/10.1111/1462-2920.12993.

Luger, Karolin, Mekonnen L. Dechassa, and David J. Tremethick. 2012. "New Insights into Nucleosome and Chromatin Structure: An Ordered State or a Disordered Affair?" *Nature Reviews Molecular Cell Biology* 13 (June): 436.

Luger, Karolin, Armin W. Mäder, Robin K. Richmond, David F. Sargent, and Timothy J. Richmond. 1997. "Crystal Structure of the Nucleosome Core Particle at 2.8 Å Resolution." *Nature* 389 (6648): 251–60. https://doi.org/10.1038/38444.

Lusser, Alexandra, and James T. Kadonaga. 2003. "Chromatin Remodeling by ATP-Dependent Molecular Machines." *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 25 (12): 1192–1200. https://doi.org/10.1002/bies.10359.

Mao, Peng, Rithy Meas, Kathleen M. Dorgan, and Michael J. Smerdon. 2014. "UV Damage-Induced RNA Polymerase II Stalling Stimulates H2B Deubiquitylation." *Proceedings of the National Academy of Sciences of the United States of America* 111 (35): 12811–16. https://doi.org/10.1073/pnas.1403901111.

March-Díaz, Rosana, and Jose C. Reyes. 2009. "The Beauty of Being a Variant: H2A.Z and the SWR1 Complex in Plants." *Molecular Plant* 2 (4): 565–77. https://doi.org/10.1093/mp/ssp019.

Mariño-Ramírez, Leonardo, Maricel G Kann, Benjamin A Shoemaker, and David Landsman. 2005. "Histone Structure and Nucleosome Stability." *Expert Review of Proteomics* 2 (5): 719–29. https://doi.org/10.1586/14789450.2.5.719.

Marques, João Trindade, Kevin Kim, Pei-Hsuan Wu, Trevis M. Alleyne, Nadereh Jafari, and Richard W. Carthew. 2010. "Loqs and R2D2 Act Sequentially in the SiRNA Pathway in Drosophila." *Nature Structural & Molecular Biology* 17 (1): 24–30. https://doi.org/10.1038/nsmb.1735.

Marzluff, William F., and Kaitlin P. Koreski. 2017. "Birth and Death of Histone MRNAs." *Trends in Genetics: TIG* 33 (10): 745–59. https://doi.org/10.1016/j.tig.2017.07.014.

Mavrich, Travis N., Cizhong Jiang, Ilya P. Ioshikhes, Xiaoyong Li, Bryan J. Venters, Sara J. Zanton, Lynn P. Tomsho, et al. 2008. "Nucleosome Organization in the *Drosophila* Genome." *Nature* 453 (7193): 358–62. https://doi.org/10.1038/nature06929.

Maze, Ian, Kyung-Min Noh, Alexey A. Soshnev, and C. David Allis. 2014. "Every Amino Acid Matters: Essential Contributions of Histone Variants to Mammalian Development and Disease." *Nature Reviews. Genetics* 15 (4): 259–71. https://doi.org/10.1038/nrg3673.

Mizuguchi, G. 2004. "ATP-Driven Exchange of Histone H2AZ Variant Catalyzed by SWR1 Chromatin Remodeling Complex." *Science* 303 (5656): 343–48. https://doi.org/10.1126/science.1090701.

Morrison, Ashby J., and Xuetong Shen. 2009. "Chromatin Remodelling beyond Transcription: The INO80 and SWR1 Complexes." *Nature Reviews. Molecular Cell Biology* 10 (6): 373–84. https://doi.org/10.1038/nrm2693.

Nielsen, Jakob B., Michael L. Nielsen, and Uffe H. Mortensen. 2008. "Transient Disruption of Non-Homologous End-Joining Facilitates Targeted Genome Manipulations in the Filamentous Fungus Aspergillus Nidulans." *Fungal Genetics and Biology: FG & B* 45 (3): 165–70. https://doi.org/10.1016/j.fgb.2007.07.003.

Nielsen, Michael L., Line Albertsen, Gaëlle Lettier, Jakob B. Nielsen, and Uffe H. Mortensen. 2006. "Efficient PCR-Based Gene Targeting with a Recyclable Marker for Aspergillus Nidulans." *Fungal Genetics and Biology:*

FG & B 43 (1): 54–64. https://doi.org/10.1016/j.fgb.2005.09.005.

Ninomiya, Yuuko, Keiichiro Suzuki, Chizu Ishii, and Hirokazu Inoue. 2004. "Highly Efficient Gene Replacements in Neurospora Strains Deficient for Nonhomologous End-Joining." *Proceedings of the National Academy of Sciences of the United States of America* 101 (33): 12248–53. https://doi.org/10.1073/pnas.0402780101.

Nishida, Hiromi, and Choong-Soo Yun. 2011. "Extraction of Tentative Mobile Introns in Fungal Histone Genes." *Mobile Genetic Elements* 1 (1): 78–79. https://doi.org/10.4161/mge.1.1.15431.

Obri, Arnaud, Khalid Ouararhni, Christophe Papin, Marie-Laure Diebold, Kiran Padmanabhan, Martin Marek, Isabelle Stoll, et al. 2014. "ANP32E Is a Histone Chaperone That Removes H2A.Z from Chromatin." *Nature* 505 (7485): 648–53. https://doi.org/10.1038/nature12922.

Park, Young-Jun, Pamela N. Dyer, David J. Tremethick, and Karolin Luger. 2004. "A New Fluorescence Resonance Energy Transfer Approach Demonstrates That the Histone Variant H2AZ Stabilizes the Histone Octamer within the Nucleosome." *The Journal of Biological Chemistry* 279 (23): 24274–82. https://doi.org/10.1074/jbc.M313152200.

Peserico, Alessia, and Cristiano Simone. 2011. "Physical and Functional HAT/HDAC Interplay Regulates Protein Acetylation Balance." In *Journal of Biomedicine & Biotechnology*. https://doi.org/10.1155/2011/371832.

Raisner, Ryan M., Paul D. Hartley, Marc D. Meneghini, Marie Z. Bao, Chih Long Liu, Stuart L. Schreiber, Oliver J. Rando, and Hiten D. Madhani. 2005. "Histone Variant H2A.Z Marks the 5′ Ends of Both Active and Inactive Genes in Euchromatin." *Cell* 123 (2): 233–48. https://doi.org/10.1016/j.cell.2005.10.002.

Reyes-Dominguez, Yazmid, Stefan Boedi, Michael Sulyok, Gerlinde Wiesenberger, Norbert Stoppacher, Rudolf Krska, and Joseph Strauss. 2012. "Heterochromatin Influences the Secondary Metabolite Profile in the Plant Pathogen Fusarium Graminearum." *Fungal Genetics and Biology* 49 (1–3): 39–47. https://doi.org/10.1016/j.fgb.2011.11.002.

Richmond, T. J., J. T. Finch, B. Rushton, D. Rhodes, and A. Klug. 1984. "Structure of the Nucleosome Core Particle at 7 Å Resolution." *Nature* 311 (October): 532.

Rispal, Jérémie, Lucie Baron, Jean-François Beaulieu, Martine Chevillard-Briet, Didier Trouche, and Fabrice Escaffit. 2019. "The H2A.Z Histone Variant Integrates Wnt Signaling in Intestinal Epithelial Homeostasis." *Nature Communications* 10 (1): 1–14. https://doi.org/10.1038/s41467-019-09899-z.

Ruhl, Donald D., Jingji Jin, Yong Cai, Selene Swanson, Laurence Florens, Michael P. Washburn, Ronald C. Conaway, Joan Weliky Conaway, and John C. Chrivia. 2006. "Purification of a Human SRCAP Complex That Remodels Chromatin by Incorporating the Histone Variant H2A.Z into Nucleosomes." *Biochemistry* 45 (17): 5671–77. https://doi.org/10.1021/bi060043d.

Ryan, Daniel P., and Tom Owen-Hughes. 2011. "Snf2-Family Proteins: Chromatin Remodellers for Any Occasion." *Current Opinion in Chemical Biology* 15 (5): 649–56. https://doi.org/10.1016/j.cbpa.2011.07.022.

Ryan, Daniel P., and David J. Tremethick. 2018. "The Interplay between H2A.Z and H3K9 Methylation in Regulating HP1α Binding to Linker Histone-Containing Chromatin." *Nucleic Acids Research* 46 (18): 9353–66. https://doi.org/10.1093/nar/gky632.

Sadeghi, Laia, Carolina Bonilla, Annelie Strålfors, Karl Ekwall, and J. Peter Svensson. 2011. "Podbat: A Novel Genomic Tool Reveals Swr1-Independent H2A.Z Incorporation at Gene Coding Sequences through Epigenetic Meta-Analysis." *PLoS Computational Biology* 7 (8): e1002163. https://doi.org/10.1371/journal.pcbi.1002163.

Santisteban, Maria Soledad, Mingda Hang, and M. Mitchell Smith. 2011. "Histone Variant H2A.Z and RNA Polymerase II Transcription Elongation." *Molecular and Cellular Biology* 31 (9): 1848–60.

https://doi.org/10.1128/MCB.01346-10.

Santos-Rosa, Helena, Robert Schneider, Andrew J. Bannister, Julia Sherriff, Bradley E. Bernstein, N. C. Tolga Emre, Stuart L. Schreiber, Jane Mellor, and Tony Kouzarides. 2002. “Active Genes Are Tri-Methylated at K4 of Histone H3.” *Nature* 419 (6905): 407–11. https://doi.org/10.1038/nature01080.

Sarcinella, Elizabeth, Philip C. Zuzarte, Priscilla N. I. Lau, Ryan Draker, and Peter Cheung. 2007. “Monoubiquitylation of H2A.Z Distinguishes Its Association with Euchromatin or Facultative Heterochromatin.” *Molecular and Cellular Biology* 27 (18): 6457–68. https://doi.org/10.1128/MCB.00241-07.

Sartori, Alessandro A., Claudia Lukas, Julia Coates, Martin Mistrik, Shuang Fu, Jiri Bartek, Richard Baer, Jiri Lukas, and Stephen P. Jackson. 2007. “Human CtIP Promotes DNA End Resection.” *Nature* 450 (7169): 509–14. https://doi.org/10.1038/nature06337.

Sevilla, Ana, and Olivier Binda. 2014. “Post-Translational Modifications of the Histone Variant H2az.” *Stem Cell Research* 12 (1): 289–95. https://doi.org/10.1016/j.scr.2013.11.004.

Sims, Robert J, Subhrangsu S Mandal, and Danny Reinberg. 2004. “Recent Highlights of RNA-Polymerase-II-Mediated Transcription.” *Current Opinion in Cell Biology* 16 (3): 263–71. https://doi.org/10.1016/j.ceb.2004.04.004.

Smith, Corey L., and Craig L. Peterson. 2005. “ATP-Dependent Chromatin Remodeling.” *Current Topics in Developmental Biology* 65: 115–48. https://doi.org/10.1016/S0070-2153(04)65004-6.

Smith, Edwin R., Arri Eisen, Weigang Gu, Martin Sattah, Antonio Pannuti, Jianxin Zhou, Richard G. Cook, John C. Lucchesi, and C. David Allis. 1998. “ESA1 Is a Histone Acetyltransferase That Is Essential for Growth in Yeast.” *Proceedings of the National Academy of Sciences of the United States of America* 95 (7): 3561–65.

Smith, Kristina M., Pallavi A. Phatale, Christopher M. Sullivan, Kyle R. Pomraning, and Michael Freitag. 2011. “Heterochromatin Is Required for Normal Distribution of Neurospora Crassa CenH3 ▿.” *Molecular and Cellular Biology* 31 (12): 2528–42. https://doi.org/10.1128/MCB.01285-10.

Soboleva, Tatiana A., Maxim Nekrasov, Daniel P. Ryan, and David J. Tremethick. 2014. “Histone Variants at the Transcription Start-Site.” *Trends in Genetics* 30 (5): 199–209. https://doi.org/10.1016/j.tig.2014.03.002.

“Stop Neglecting Fungi.” 2017. *Nature Microbiology* 2 (8): 17120. https://doi.org/10.1038/nmicrobiol.2017.120.

Studt, Lena, Sarah M. Rösler, Immo Burkhardt, Birgit Arndt, Michael Freitag, Hans-Ulrich Humpf, Jeroen S. Dickschat, and Bettina Tudzynski. 2016. “Knock-down of the Methyltransferase Kmt6 Relieves H3K27me3 and Results in Induction of Cryptic and Otherwise Silent Secondary Metabolite Gene Clusters in Fusarium Fujikuroi.” *Environmental Microbiology* 18 (11): 4037–54. https://doi.org/10.1111/1462-2920.13427.

Subramanian, Vidya, Paul A. Fields, and Laurie A. Boyer. 2015. “H2A.Z: A Molecular Rheostat for Transcriptional Control.” *F1000prime Reports* 7: 01. https://doi.org/10.12703/P7-01.

Sura, Weronika, Michał Kabza, Wojciech M. Karlowski, Tomasz Bieluszewski, Marta Kus-Slowinska, Łukasz Pawełoszek, Jan Sadowski, and Piotr A. Ziolkowski. 2017. “Dual Role of the Histone Variant H2A.Z in Transcriptional Regulation of Stress-Response Genes.” *The Plant Cell* 29 (4): 791–807. https://doi.org/10.1105/tpc.16.00573.

Suto, R. K., M. J. Clarkson, D. J. Tremethick, and K. Luger. 2000. “Crystal Structure of a Nucleosome Core Particle Containing the Variant Histone H2A.Z.” *Nature Structural Biology* 7 (12): 1121–24. https://doi.org/10.1038/81971.

Szklarczyk, Damian, Annika L. Gable, David Lyon, Alexander Junge, Stefan Wyder, Jaime Huerta-Cepas, Milan Simonovic, et al. 2019. “STRING V11: Protein-Protein Association Networks with Increased Coverage,

Supporting Functional Discovery in Genome-Wide Experimental Datasets." *Nucleic Acids Research* 47 (D1): D607–13. https://doi.org/10.1093/nar/gky1131.

Talbert, Paul B., and Steven Henikoff. 2014. "Environmental Responses Mediated by Histone Variants." *Trends in Cell Biology* 24 (11): 642–50. https://doi.org/10.1016/j.tcb.2014.07.006.

Taty-Taty, Gemael-Cedrick, Celine Courilleau, Muriel Quaranta, alexandre carayon, catherine chailleux, Francois Aymard, Didier Trouche, and Yvan Canitrot. 2014. "H2A.Z Depletion Impairs Proliferation and Viability but Not DNA Double-Strand Breaks Repair in Human Immortalized and Tumoral Cell Lines." *Cell Cycle* 13 (3): 399–407. https://doi.org/10.4161/cc.27143.

Thambirajah, Anita A., Deanna Dryhurst, Toyotaka Ishibashi, Andra Li, Allison H. Maffey, and Juan Ausió. 2006a. "H2A.Z Stabilizes Chromatin in a Way That Is Dependent on Core Histone Acetylation." *Journal of Biological Chemistry* 281 (29): 20036–44. https://doi.org/10.1074/jbc.M601975200.

———. 2006b. "H2A.Z Stabilizes Chromatin in a Way That Is Dependent on Core Histone Acetylation." *Journal of Biological Chemistry* 281 (29): 20036–44. https://doi.org/10.1074/jbc.M601975200.

Thatcher, T. H., and M. A. Gorovsky. 1994. "Phylogenetic Analysis of the Core Histones H2A, H2B, H3, and H4." *Nucleic Acids Research* 22 (2): 174–79.

Tisch, Doris, and Monika Schmoll. 2010. "Light Regulation of Metabolic Pathways in Fungi." *Applied Microbiology and Biotechnology* 85 (5): 1259–77. https://doi.org/10.1007/s00253-009-2320-1.

Tosi, Alessandro, Caroline Haas, Franz Herzog, Andrea Gilmozzi, Otto Berninghausen, Charlotte Ungewickell, Christian B. Gerhold, et al. 2013. "Structure and Subunit Topology of the INO80 Chromatin Remodeler and Its Nucleosome Complex." *Cell* 154 (6): 1207–19. https://doi.org/10.1016/j.cell.2013.08.016.

Valdés-Mora, Fátima, Jenny Z. Song, Aaron L. Statham, Dario Strbenac, Mark D. Robinson, Shalima S. Nair, Kate I. Patterson, David J. Tremethick, Clare Stirzaker, and Susan J. Clark. 2012. "Acetylation of H2A.Z Is a Key Epigenetic Modification Associated with Gene Deregulation and Epigenetic Remodeling in Cancer." *Genome Research* 22 (2): 307–21. https://doi.org/10.1101/gr.118919.110.

Vogler, Christine, Claudia Huber, Tanja Waldmann, Ramona Ettig, Lora Braun, Annalisa Izzo, Sylvain Daujat, et al. 2010. "Histone H2A C-Terminus Regulates Chromatin Dynamics, Remodeling, and Histone H1 Binding." *PLoS Genetics* 6 (12). https://doi.org/10.1371/journal.pgen.1001234.

Weber, Christopher M., Srinivas Ramachandran, and Steven Henikoff. 2014. "Nucleosomes Are Context-Specific, H2A.Z-Modulated Barriers to RNA Polymerase." *Molecular Cell* 53 (5): 819–30. https://doi.org/10.1016/j.molcel.2014.02.014.

White, J. H., J. F. DiMartino, R. W. Anderson, K. Lusnak, D. Hilbert, and S. Fogel. 1988. "A DNA Sequence Conferring High Postmeiotic Segregation Frequency to Heterozygous Deletions in Saccharomyces Cerevisiae Is Related to Sequences Associated with Eucaryotic Recombination Hotspots." *Molecular and Cellular Biology* 8 (3): 1253–58.

Wong, Jonathan, Yuko Nakajima, Stefan Westermann, Ching Shang, Jung-Seog Kang, Crystal Goodner, Pantea Houshmand, et al. 2007. "A Protein Interaction Map of the Mitotic Spindle." *Molecular Biology of the Cell* 18 (10): 3800–3809. https://doi.org/10.1091/mbc.E07-06-0536.

Wong, Koon Ho, Michael J. Hynes, and Meryl A. Davis. 2008. "Recent Advances in Nitrogen Regulation: A Comparison between Saccharomyces Cerevisiae and Filamentous Fungi." *Eukaryotic Cell* 7 (6): 917–25. https://doi.org/10.1128/EC.00076-08.

Wratting, Daniel, Angela Thistlethwaite, Michael Harris, Leo A. H. Zeef, and Catherine B. Millar. 2012. "A Conserved Function for the H2A.Z C Terminus." *Journal of Biological Chemistry* 287 (23): 19148–57.

https://doi.org/10.1074/jbc.M111.317990.

Xu, Ye, Marina K. Ayrapetov, Chang Xu, Ozge Gursoy-Yuzugullu, Yiduo Hu, and Brendan D. Price. 2012. "Histone H2A.Z Controls a Critical Chromatin Remodeling Step Required for DNA Double-Strand Break Repair." *Molecular Cell* 48 (5): 723–33. https://doi.org/10.1016/j.molcel.2012.09.026.

Zhang, Chi, Lin Cao, Liang Rong, Zengxuan An, Wangbin Zhou, Jinbiao Ma, Wen-Hui Shen, Yan Zhu, and Aiwu Dong. 2015. "The Chromatin-Remodeling Factor AtINO80 Plays Crucial Roles in Genome Stability Maintenance and in Plant Development." *The Plant Journal* 82 (4): 655–68. https://doi.org/10.1111/tpj.12840.

Zhang, Haiying, Douglas N. Roberts, and Bradley R. Cairns. 2005. "Genome-Wide Dynamics of Htz1, a Histone H2A Variant That Poises Repressed/Basal Promoters for Activation through Histone Loss." *Cell* 123 (2): 219–31. https://doi.org/10.1016/j.cell.2005.08.036.

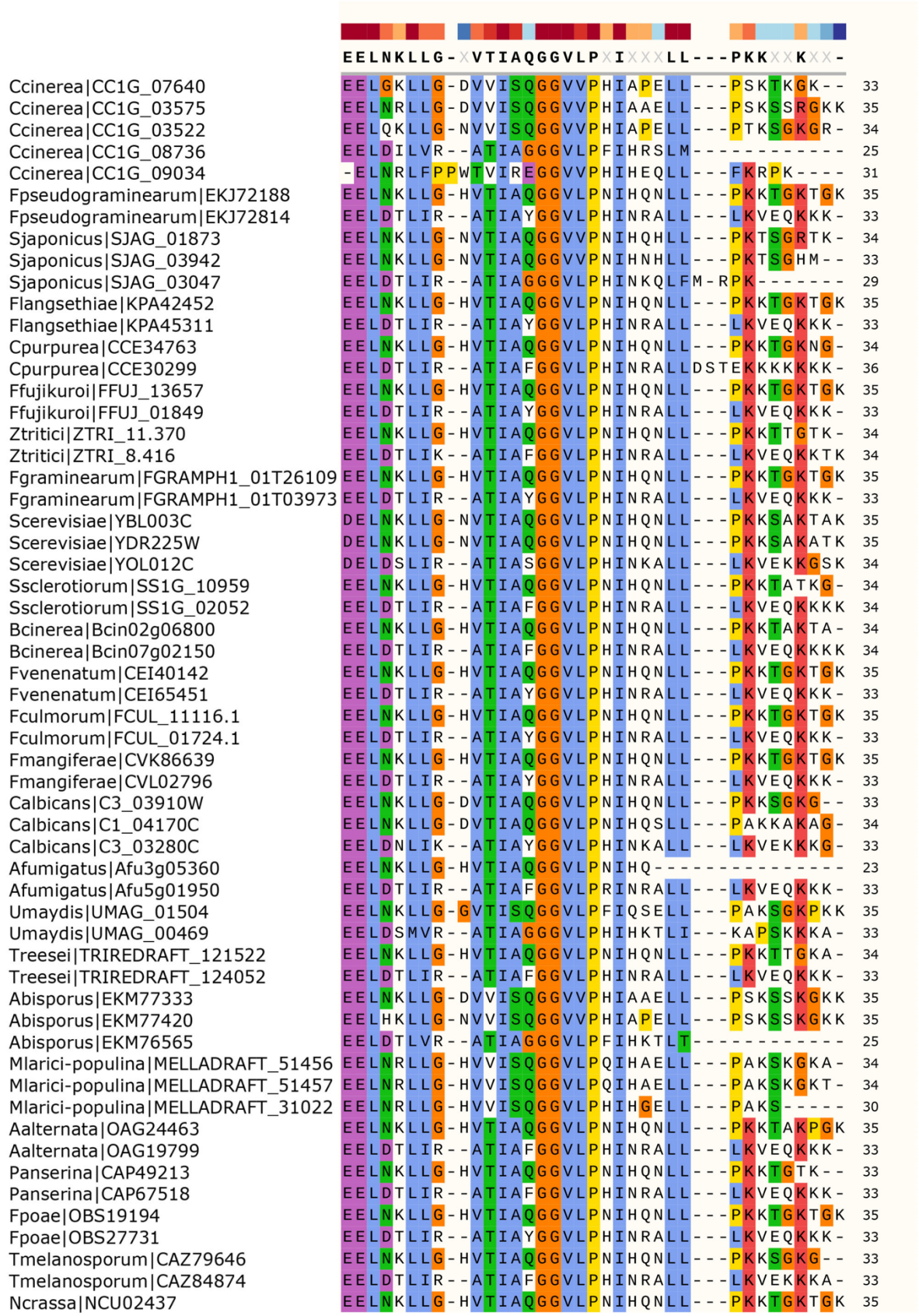
Zilberman, Daniel, Devin Coleman-Derr, Tracy Ballinger, and Steven Henikoff. 2008. "Histone H2A.Z and DNA Methylation Are Mutually Antagonistic Chromatin Marks." *Nature* 456 (7218): 125–29. https://doi.org/10.1038/nature07324.

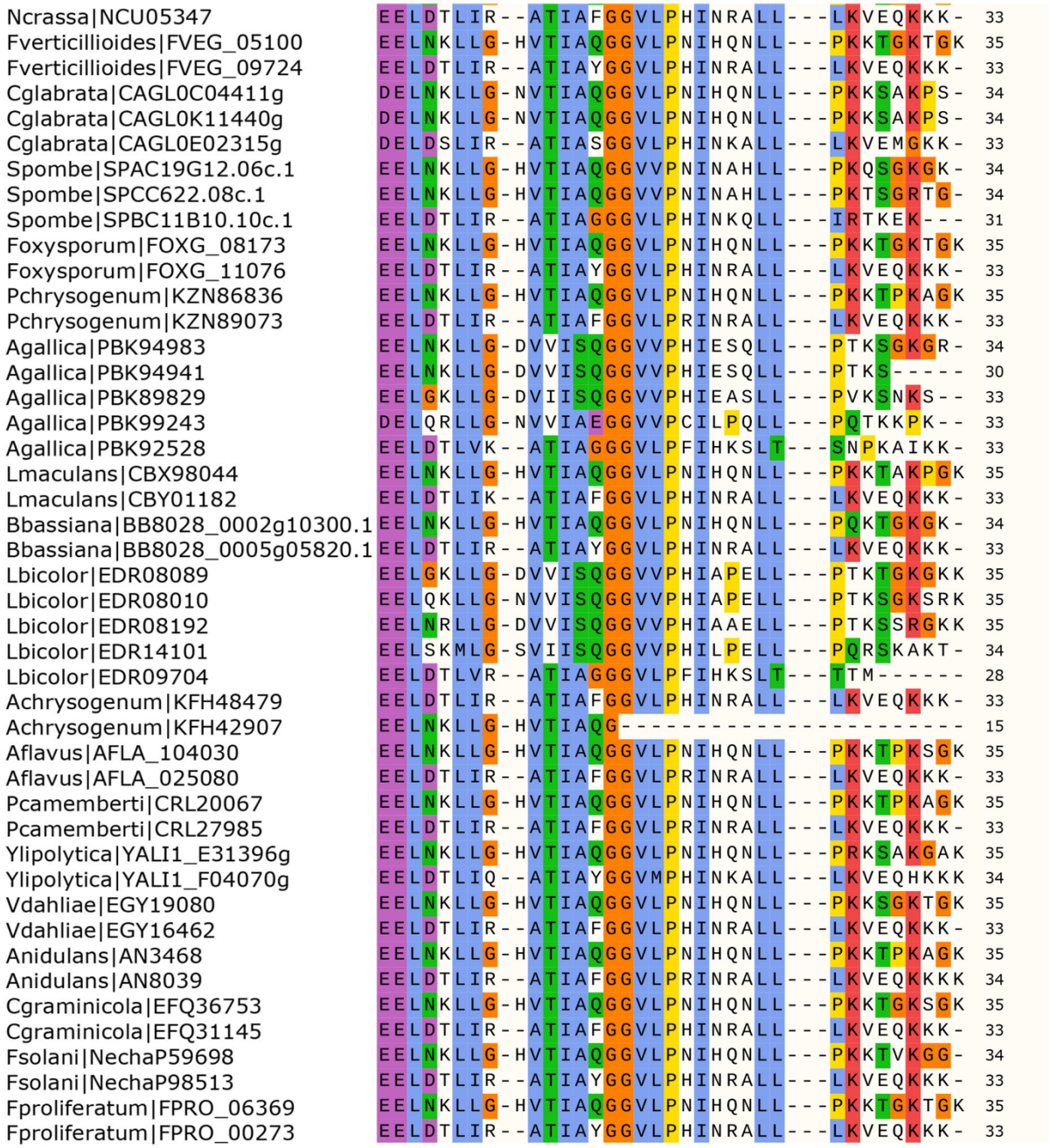
Supplemental data:

Supplemental Table S1. Protein count per species, found searching for the HMM profile for H2A C-terminus proteome-wide*

Division	Subdivision	Class	Species	Protein count
Ascomycota	Pezizomycotina	Dothideomycetes	*A.alternata*	2
			L.maculans	2
			Z.tritici	2
		Eurotiomycetes	*A.flavus*	2
			A.fumigatus	2
			A.nidulans	2
			P.camemberti	2
			P.chrysogenum	2
		Leotiomycetes	*B.cinerea*	2
			S.sclerotiorum	2
		Pezizomycetes	*T.melanosporum*	2
		Sordariomycetes	*A.chrysogenum*	2
			B.bassiana	2
			C.graminicola	2
			C.purpurea	2
			F.culmorum	2
			F.fujikuroi	2
			F.graminearum	2
			F.langsethiae	2
			F.mangiferae	2
			F.oxysporum	2
			F.poae	2
			F.proliferatum	2
			F.pseudograminearum	2
			F.solani	2
			F.venenatum	2
			F.verticillioides	2
			N.crassa	2
			P.anserina	2
			T.reesei	2
			V.dahliae	2
	Saccharomycotina		*C.albicans*	3
			C.glabrata	3
			S.cerevisiae	3
			Y.lipolytica	2
	Taphrinomycotina		*S.japonicus*	3
			S.pombe	3
Basidiomycota	Agaricomycotina	Agaricomycetes	*A.bisporus*	3
			A.gallica	5
			C.cinerea	5
			L.bicolor	5
	Pucciniomycotina		*M.larici-populina*	3
	Ustilaginomycotina		*U.maydis*	2

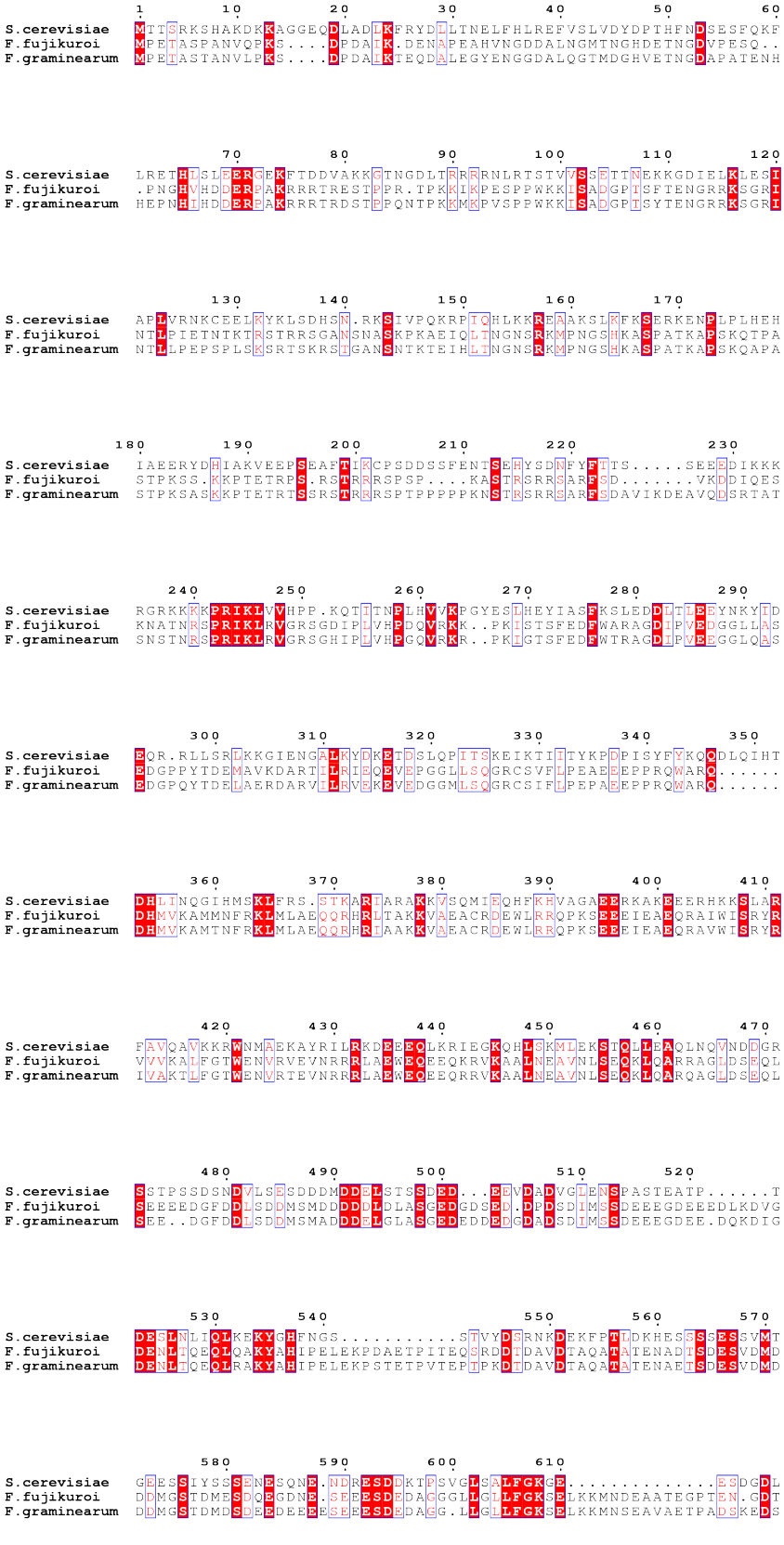
*HMMER3 and PFAM version 32 were used; proteomes were downloaded from FungiDBv45 and EnsemblFungi45; hits are predicted

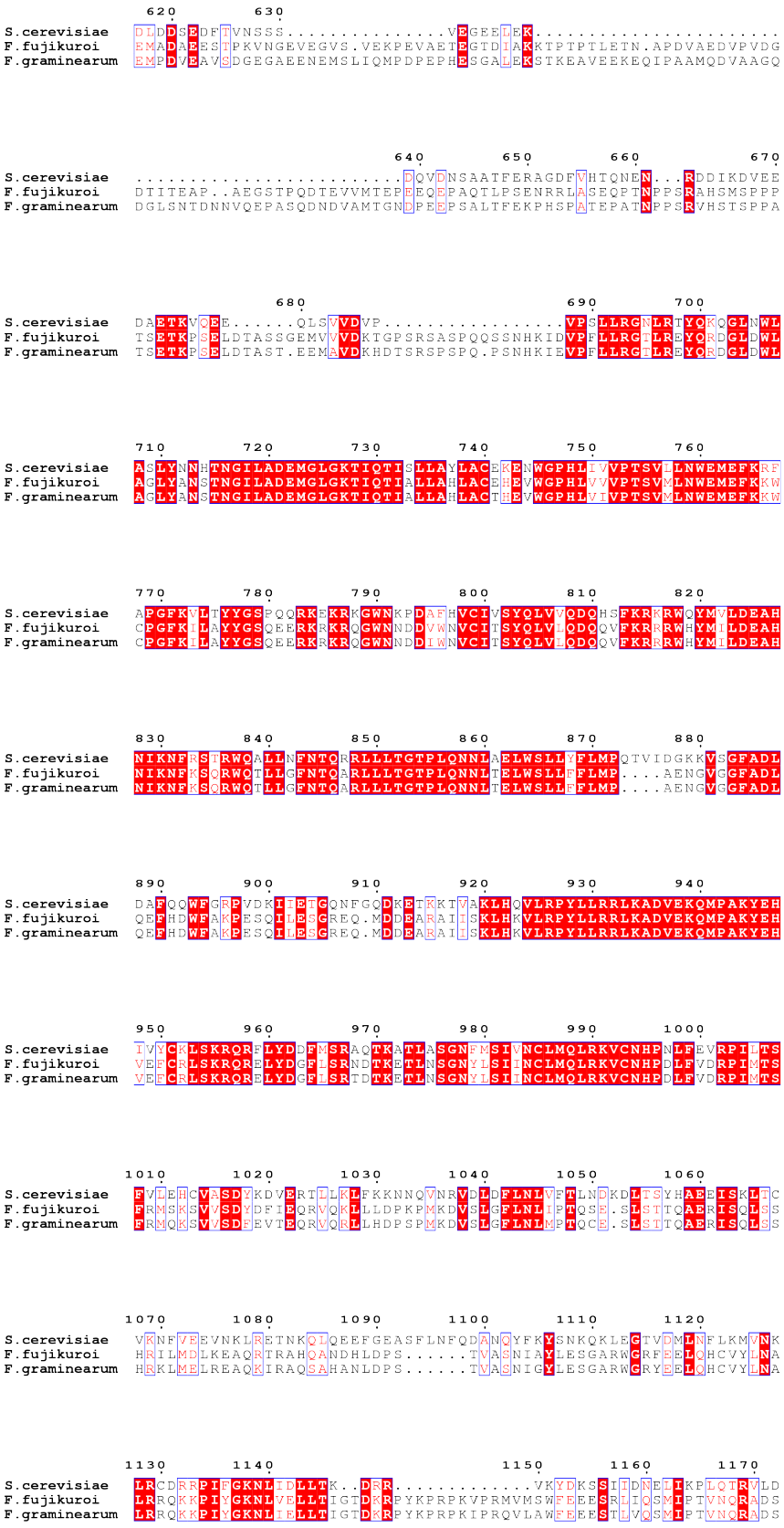

EELNKLLG-XVTIAQGGVLPXIXXXLL---PKKXXKXX-
Ccinerea|CC1G_07640 EELGKLLG-DVVISQGGVVPHIAPELL---PSKTKGK-- 33
Ccinerea|CC1G_03575 EELNRLLG-DVVISQGGVVPHIAAELL---PSKSSRGKK 35
Ccinerea|CC1G_03522 EELQKLLG-NVVISQGGVVPHIAPELL---PTKSGKGR- 34
Ccinerea|CC1G_08736 EELDILVR--ATIAGGGVLPFIHRSLM---------- 25
Ccinerea|CC1G_09034 -ELNRLFPPWTVIREGGVVPHIHEQLL---FKRPK---- 31
Fpseudograminearum|EKJ72188 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Fpseudograminearum|EKJ72814 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Sjaponicus|SJAG_01873 EELNKLLG-NVTIAQGGVVPNIHQHLL---PKTSGRTK- 34
Sjaponicus|SJAG_03942 EELNKLLG-NVTIAQGGVVPNIHNHLL---PKTSGHM-- 33
Sjaponicus|SJAG_03047 EELDTLIR--ATIAGGGVLPHINKQLFM-RPK------- 29
Flangsethiae|KPA42452 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Flangsethiae|KPA45311 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Cpurpurea|CCE34763 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKNG- 34
Cpurpurea|CCE30299 EELDTLIR--ATIAFGGVLPHINRALLDSTEKKKKKKK- 36
Ffujikuroi|FFUJ_13657 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Ffujikuroi|FFUJ_01849 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Ztritici|ZTRI_11.370 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTTGTK- 34
Ztritici|ZTRI_8.416 EELDTLIK--ATIAFGGVLPHINRALL---LKVEQKKTK 34
Fgraminearum|FGRAMPH1_01T26109 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Fgraminearum|FGRAMPH1_01T03973 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Scerevisiae|YBL003C DELNKLLG-NVTIAQGGVLPNIHQNLL---PKKSAKTAK 35
Scerevisiae|YDR225W DELNKLLG-NVTIAQGGVLPNIHQNLL---PKKSAKATK 35
Scerevisiae|YOL012C DELDSLIR--ATIASGGVLPHINKALL---LKVEKKGSK 34
Ssclerotiorum|SS1G_10959 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTATKG- 34
Ssclerotiorum|SS1G_02052 EELDTLIR--ATIAFGGVLPHINRALL---LKVEQKKKK 34
Bcinerea|Bcin02g06800 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTAKTA- 34
Bcinerea|Bcin07g02150 EELDTLIR--ATIAFGGVLPHINRALL---LKVEQKKKK 34
Fvenenatum|CEI40142 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Fvenenatum|CEI65451 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Fculmorum|FCUL_11116.1 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Fculmorum|FCUL_01724.1 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Fmangiferae|CVK86639 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Fmangiferae|CVL02796 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Calbicans|C3_03910W EELNKLLG-DVTIAQGGVLPNIHQNLL---PKKSGKG-- 33
Calbicans|C1_04170C EELNKLLG-DVTIAQGGVLPNIHQSLL---PAKKAKAG- 34
Calbicans|C3_03280C EELDNLIK--ATIAYGGVLPHINKALL---LKVEKKKG- 33
Afumigatus|Afu3g05360 EELNKLLG-HVTIAQGGVLPNIHQ---------- 23
Afumigatus|Afu5g01950 EELDTLIR--ATIAFGGVLPRINRALL---LKVEQKKK- 33
Umaydis|UMAG_01504 EELNKLLG-GVTISQGGVLPFIQSELL---PAKSGKPKK 35
Umaydis|UMAG_00469 EELDSMVR--ATIAGGGVLPHIHKTLI---KAPSKKKA- 33
Treesei|TRIREDRAFT_121522 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTTGKA- 34
Treesei|TRIREDRAFT_124052 EELDTLIR--ATIAFGGVLPHINRALL---LKVEQKKK- 33
Abisporus|EKM77333 EELNKLLG-DVVISQGGVVPHIAAELL---PSKSSKGKK 35
Abisporus|EKM77420 EELHKLLG-NVVISQGGVVPHIAPELL---PSKSSKGKK 35
Abisporus|EKM76565 EELDTLVR--ATIAGGGVLPFIHKTLT---------- 25
Mlarici-populina|MELLADRAFT_51456 EELNRLLG-HVVISQGGVLPQIHAELL---PAKSKGKA- 34
Mlarici-populina|MELLADRAFT_51457 EELNRLLG-HVVISQGGVLPQIHAELL---PAKSKGKT- 34
Mlarici-populina|MELLADRAFT_31022 EELNRLLG-HVVISQGGVLPHIHGELL---PAKS----- 30
Aalternata|OAG24463 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTAKPGK 35
Aalternata|OAG19799 EELDTLIR--ATIAFGGVLPHINRALL---LKVEQKKK- 33
Panserina|CAP49213 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGTK-- 33
Panserina|CAP67518 EELDTLIR--ATIAFGGVLPHINRALL---LKVEQKKK- 33
Fpoae|OBS19194 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35
Fpoae|OBS27731 EELDTLIR--ATIAYGGVLPHINRALL---LKVEQKKK- 33
Tmelanosporum|CAZ79646 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKSGKG-- 33
Tmelanosporum|CAZ84874 EELDTLIR--ATIAFGGVLPHINRALL---LKVEKKKA- 33
Ncrassa|NCU02437 EELNKLLG-HVTIAQGGVLPNIHQNLL---PKKTGKTGK 35

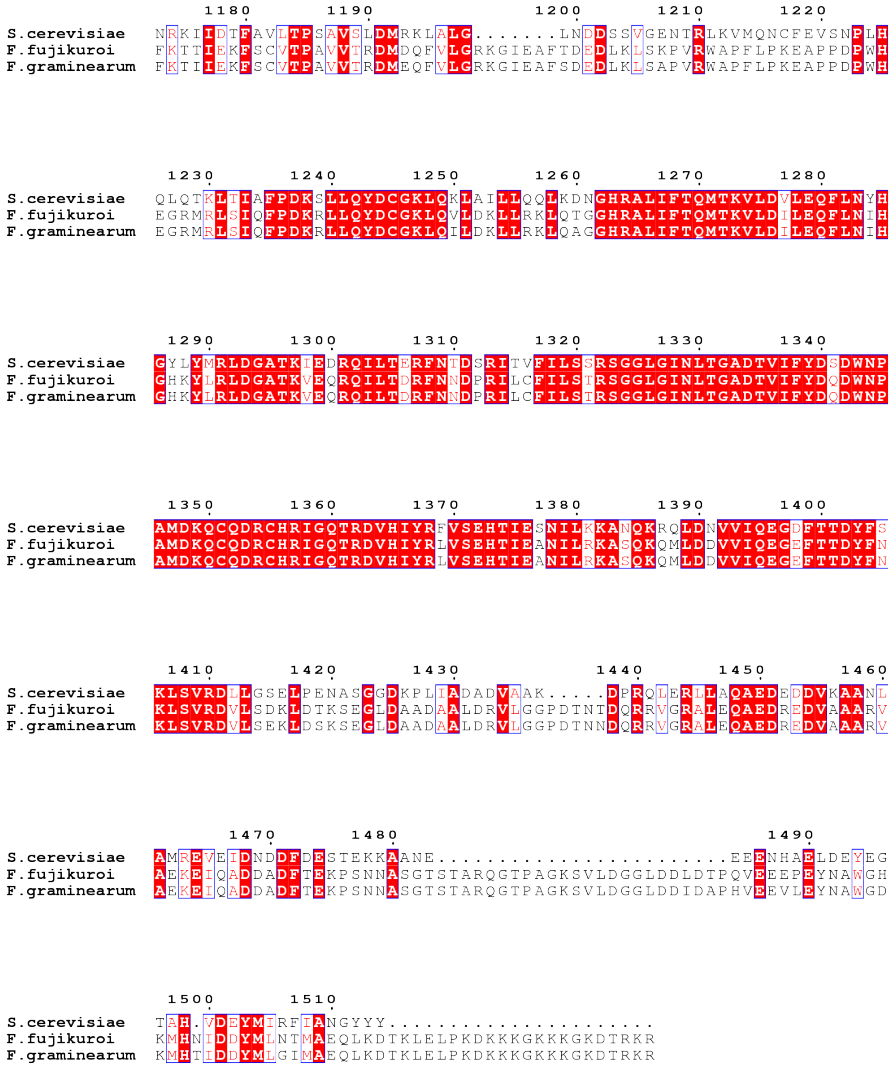


Supplemental Figure S1. Alignment of identified H2A and H2A.Z fungal sequences. Alignments were performed with hmmalign from HMMER3 software suite and displayed with SnapGene viewer.

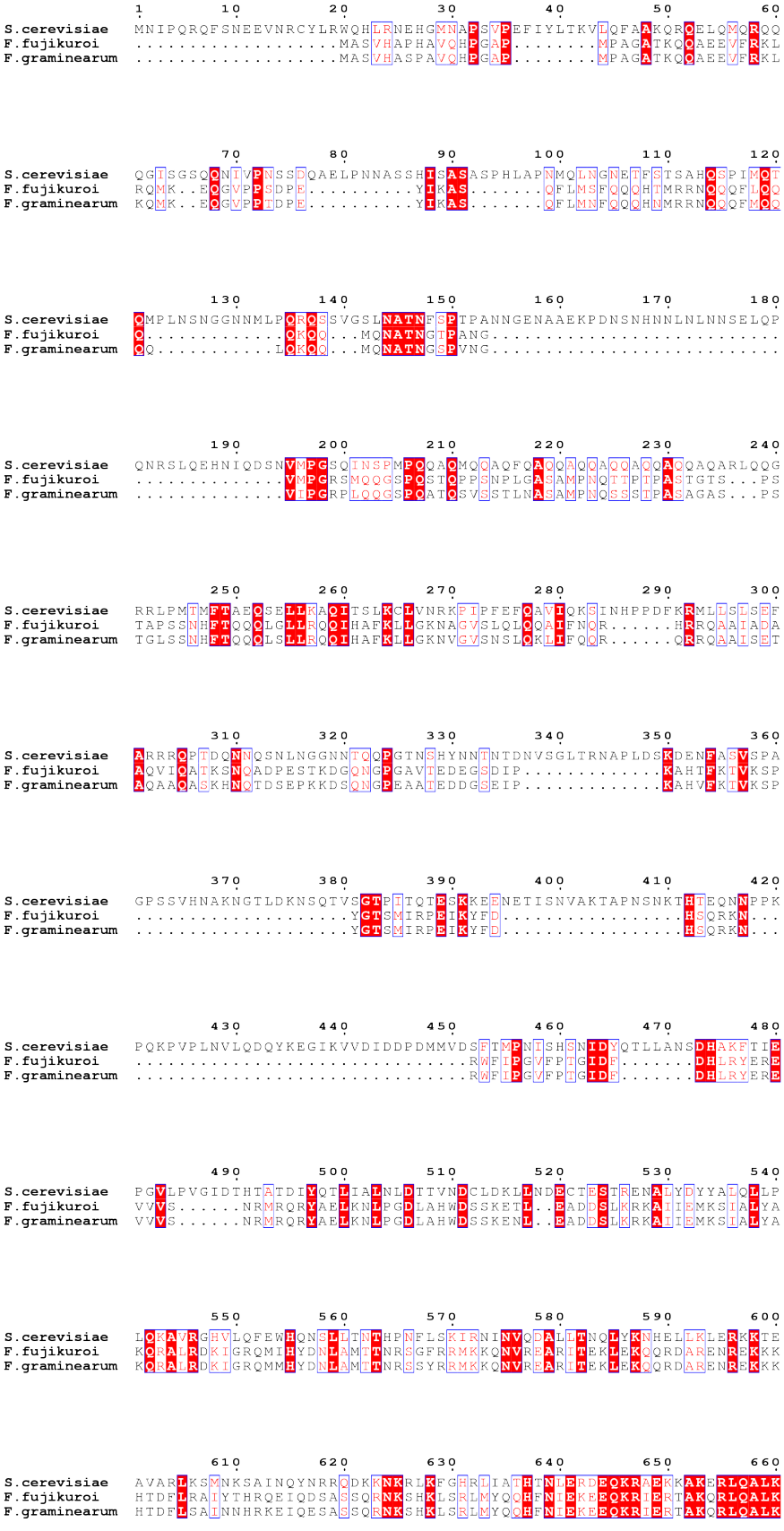
A

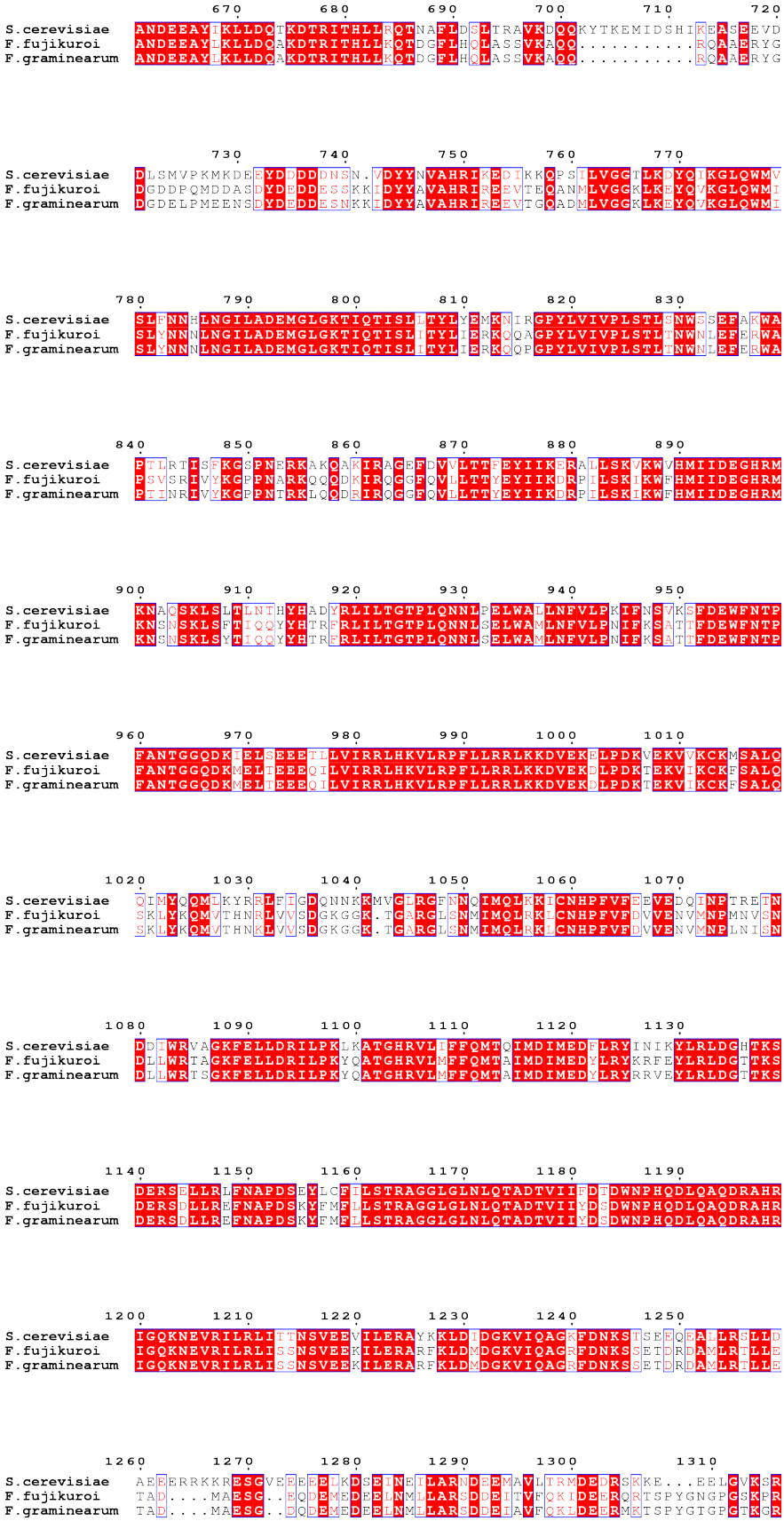


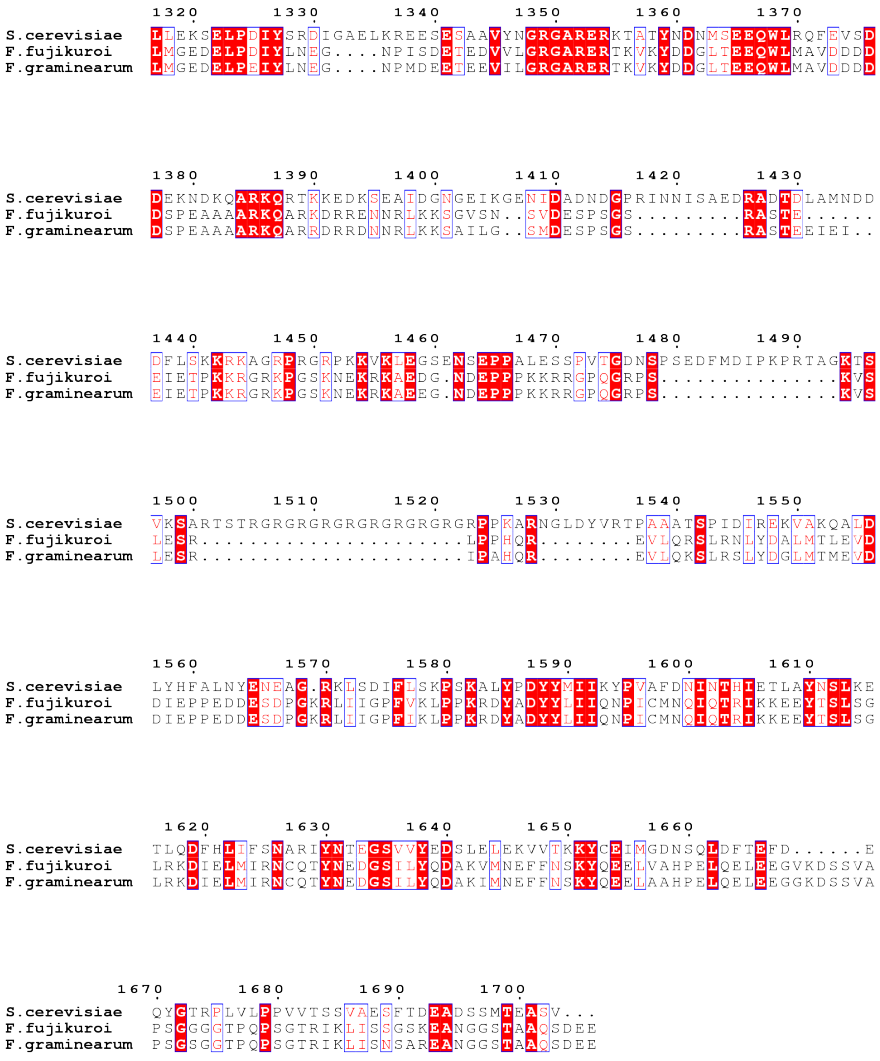

S.cerevisiae
F.fujikuroi
F.graminearum


1180 1190 1200 1210 1220
S.cerevisiae NRKIIDTFAVLTPSAVSILDMRKLALG.....LNDDSSVGENTRLKVMQNCFEVSNPLH
F.fujikuroi FKTTIEKFSCVTPAVVTRDMDQFVLGRKGIEAFTDEDLKLSKPVRWAPFLPKEAPPDPWH
F.graminearum FKTIIEKFSCVTPAVVTRDMEQFVLGRKGIEAFSDEDLKLSAPVRWAPFLPKEAPPDPWH
1230 1240 1250 1260 1270 1280
S.cerevisiae QLQTKLTIAFPDKSLLQYDCGKLQKLAILLQQLKDNGHRALIFTQMTKVLDVLEQFLNYH
F.fujikuroi EGRMRLSIQFPDKRLLQYDCGKLQVLDKLLRKLQTGGHRALIFTQMTKVLDILEQFLNIH
F.graminearum EGRMRLSIQFPDKRLLQYDCGKLQILDKLLRKLQAGGHRALIFTQMTKVLDILEQFLNIH
1290 1300 1310 1320 1330 1340
S.cerevisiae GYLYMRLDGATKIEDRQILTERFNTDSRITVFILSSRSGGLGINLTGADTVIFYDSDWNP
F.fujikuroi GHKYLRLDGATKVEQRQILTDRFNNDPRILCFILSTRSGGLGINLTGADTVIFYDQDWNP
F.graminearum GHKYLRLDGATKVEQRQILTDRFNNDPRILCFILSTRSGGLGINLTGADTVIFYDQDWNP
1350 1360 1370 1380 1390 1400
S.cerevisiae AMDKQCQDRCHRIGQTRDVHIYRFVSEHTIESNILKKANQKRQLDNVVIQEGDFTTDYFS
F.fujikuroi AMDKQCQDRCHRIGQTRDVHIYRLVSEHTIEANILRKASQKQMLDDVVIQEGEFTTDYFN
F.graminearum AMDKQCQDRCHRIGQTRDVHIYRLVSEHTIEANILRKASQKQMLDDVVIQEGEFTTDYFN
1410 1420 1430 1440 1450 1460
S.cerevisiae KLSVRDLLGSELPENASCGDKPLIADADVAAK.....DPRQLERLLAQAEDEDDVKAANL
F.fujikuroi KLSVRDVLSDKLDTKSEGLDAADAALDRVLGGPDTNTDQQRRVGRALEQAEDREDVAAARV
F.graminearum KLSVRDVLSEKLDSKSEGLDAADAALDRVLGGPDTNNDQQRRVGRALEQAEDREDVAAARV
1470 1480 1490
S.cerevisiae AMREVEIDNDDFDESTEKKAANE.....EEENHAELDEYEG
F.fujikuroi AEKEIQADDADFTEKPSNNASGTSTARQGTPAGKSVLDGGLDDLDTPQVEEPEYNAWGH
F.graminearum AEKEIQADDADFTEKPSNNASGTSTARQGTPAGKSVLDGGLDDIDAPHVEEVLEYNAWGD
1500 1510
S.cerevisiae TAH.VDEYMTRFIANGYYY.....
F.fujikuroi KMHNIDDYMLNTMAEQLKDTKLELPKDKKKGKKKGKDTRKR
F.graminearum KMHTIDDYMLGIMAEQLKDTKLELPKDKKKGKKKGKDTRKR

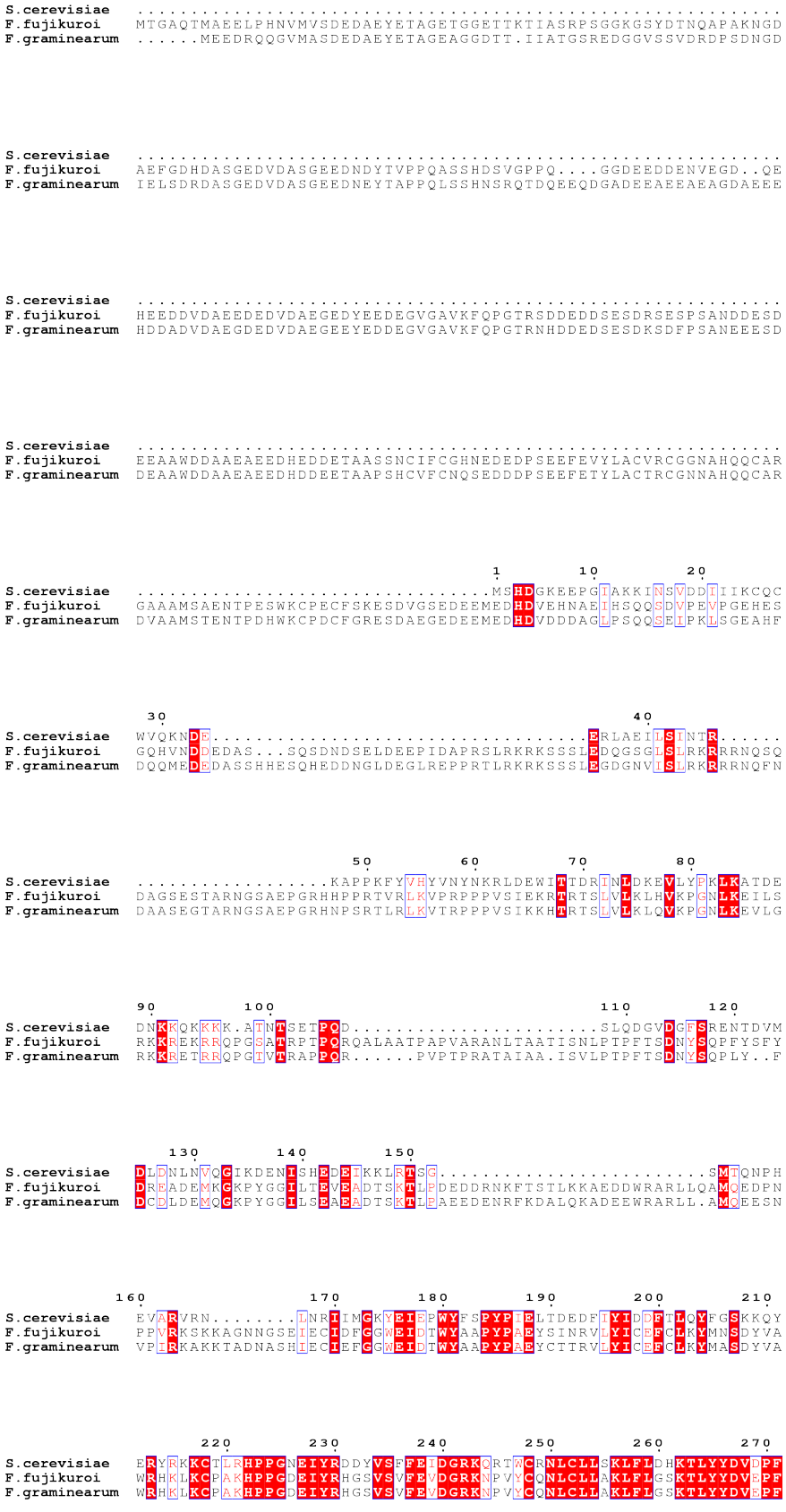
B




670 680 690 700 710 720
S.cerevisiae ANDEEAYIKLLDQIKDTRITHLLRQTNAFLDSLTRAVKDQQKYTKEMIDSHIKEASEEVD
F.fujikuroi ANDEEAYLKLLDQAKDTRITHLLKQTDGFLHQLASSVKAQQ.....RQAAERYG
F.graminearum ANDEEAYLKLLDQAKDTRITHLLKQTDGFLHQLASSVKAQQ.....RQAAERYG
730 740 750 760 770
S.cerevisiae DLSMVPKMKDEEYDDDDDNSN.VDYYNVAHRIKEDIKKQPSILVGGTLKEYQIKGLQWMV
F.fujikuroi DGDDPQMDDASDYDEDDESSKKIDYYAVAHRIREEVTEQANMLVGGKLKEYQVKGLQWMI
F.graminearum DGDELPMEENSDYDEDDESNKKIDYYAVAHRIREEVTGQADMLVGGKLKEYQVKGLQWMI
780 790 800 810 820 830
S.cerevisiae SLFNNHLNGILADEMGLGKTIQTISLITYLYEMKNIRGPYLVIVPLSTLSNWSSEFAKWA
F.fujikuroi SLYNNNLNGILADEMGLGKTIQTISLITYLIERKQQAGPYLVIVPLSTLTNWNLEFERWA
F.graminearum SLYNNNLNGILADEMGLGKTIQTISLITYLIERKQQPGPYLVIVPLSTLTNWNLEFERWA
840 850 860 870 880 890
S.cerevisiae PTLRTISFKGSPNERKAKQAKIRAGEFDVVLTTFEYIIKERAILSKVKWYHMIIDEGHRM
F.fujikuroi PSVSRIVYKGPPNARKQQQDKIRQGGFQVLLTTYEYIIKDRPILSKIKWFHMIIDEGHRM
F.graminearum PTINRIVYKGPPNTRKLQQDRIRQGGFQVLLTTYEYIIKDRPILSKIKWFHMIIDEGHRM
900 910 920 930 940 950
S.cerevisiae KNAQSKLSLTLNTHYHADYRLILTGTPLQNNLPELWALLNFVLPKIFNSVKSFDEWFNTP
F.fujikuroi KNSNSKLSFTIQQYYHTRFRLILTGTPLQNNLSELWAMLNFVLPNIFKSATTFDEWFNTP
F.graminearum KNSNSKLSYTIQQYYHTRFRLILTGTPLQNNLSELWAMLNFVLPNIFKSATTFDEWFNTP
960 970 980 990 1000 1010
S.cerevisiae FANTGGQDKIELSEEETLLVIRRLHKVLRPFLLRRLKKDVEKELPDKVEKVVKCKMSALQ
F.fujikuroi FANTGGQDKMELTEEEQILVIRRLHKVLRPFLLRRLKKDVEKDLPDKTEKVIKCKFSALQ
F.graminearum FANTGGQDKMELTEEEQILVIRRLHKVLRPFLLRRLKKDVEKDLPDKTEKVIKCKFSALQ
1020 1030 1040 1050 1060 1070
S.cerevisiae QIMYQQMLKYRRLFIGDQNNKKMVGLRGFNNQIMQLKKICNHPFVFEEVEDQINPTRETN
F.fujikuroi SKLYKQMVTHNRLVVSDGKGGK.TGARGLSNMIMQLRKLCNHPFVFDVVENVMNPMNVSN
F.graminearum SKLYKQMVTHNKLVVSDGKGGK.TGARGLSNMIMQLRKLCNHPFVFDVVENVMNPLNISN
1080 1090 1100 1110 1120 1130
S.cerevisiae DDTWRVAGKFELLDRILPKLKATGHRVLIFFQMTQIMDIMEDFLRYINIKYLRLDGHTKS
F.fujikuroi DDLWRTAGKFELLDRILPKYQATGHRVLMFFQMTAIMDIMEDVLRYKRFEYLRLDGTTKS
F.graminearum DDLWRTSGKFELLDRILPKYQATGHRVLMFFQMTAIMDIMEDVLRYRRVEYLRLDGTTKS
1140 1150 1160 1170 1180 1190
S.cerevisiae DERSELLRLFNAPDSEYLCFLLSTRAGGLGLNLQTADTVIIFDTDWNPHQDLQAQDRAHR
F.fujikuroi DERSDLLREFNAPDSKYFMFLLSTRAGGLGLNLQTADTVIIYDSDWNPHQDLQAQDRAHR
F.graminearum DERSDLLREFNAPDSKYFMFLLSTRAGGLGLNLQTADTVIIYDSDWNPHQDLQAQDRAHR
1200 1210 1220 1230 1240 1250
S.cerevisiae IGQKNEVRILRLITTNSVEEKILERAYKKLDIDGKVIQAGKFDNKSTSEEQEALLRSLLD
F.fujikuroi IGQKNEVRILRLISSNSVEEKILERARFKLDMDGKVIQAGRFDNKSSETDRDAMLRFLLE
F.graminearum IGQKNEVRILRLISSNSVEEKILERARFKLDMDGKVIQAGRFDNKSSETDRDAMLRFLLE
1260 1270 1280 1290 1300 1310
S.cerevisiae AEEERRKKRESGVEEEELKDSEINEILARNDEEMAVLTRMDEDRSKKE...EELGVKSR
F.fujikuroi TAD....MAESG..EQDEMEDEELNMILARSDDEIAVFQKLDERRQRTSPYGNGPGSKPR
F.graminearum TAD....MAESG..DQDEMEDEELNMILARSDDEIAVFQKLDERRMKTSPYGTGPGTKGR


1320 1330 1340 1350 1360 1370
S.cerevisiae LIEKSELPDIYSRDIGAELKREESESGAAVYNGRGARERKTATYNDNMSEEQWLRQFEVSD
F.fujikuroi LMGEDELPDIYLNEG....NPISDETEDVVLGRGARERTKVKYDDGLTEEQWLMAVDDDD
F.graminearum LMGEDELPEIYLNEG....NPMDEETEEVILGRGARERTKVKYDDGLTEEQWLMAVDDDD
1380 1390 1400 1410 1420 1430
S.cerevisiae DEKNDKQARKQRTKKEDKSEAIDGNGEIKGENIDADNDGPRINNISAEDRADTDLAMNDD
F.fujikuroi DSPEAAAARKQARKDRRENNRLKKSGVSN..SVDESPSGS.........RASTE......
F.graminearum DSPEAAAARKQARRDRRDNNRLKKSAILG..SMDESPSGS.........RASTEEIEI..
1440 1450 1460 1470 1480 1490
S.cerevisiae DFLSKKRKAGRPRGRPKKVKLEGSENSEPPALESSPVTGDNSPSEDFMDIPKPRTAGKTS
F.fujikuroi EIETPKKRGRKPGSKNEKRKAEDG.NDEPPPKKRRGPQGRPS...............KVS
F.graminearum EIETPKKRGRKPGSKNEKRKAEEG.NDEPPPKKRRGPQGRPS...............KVS
1500 1510 1520 1530 1540 1550
S.cerevisiae VKSARTSTRGRGRGRGRGRGRGRGRPPKARNGLDYVRTPAAATSPIDIREKVAKQALD
F.fujikuroi LESR.....................LPPHQR........EVLQRSLRNLYDALMTLEVD
F.graminearum LESR.....................IPAHQR........EVLQKSLRSLYDGLMTMEVD
1560 1570 1580 1590 1600 1610
S.cerevisiae LYHFALNYENEAG.RKLSDIFLSKPSKALYPDYYMIIKYPVAFDNINTHIETLAYNSLKE
F.fujikuroi DIEPPEDDESDPGKRLIIGPFVKLPPKRDYADYYLIIQNPICMNQIQTRIKKEEYTSLSG
F.graminearum DIEPPEDDESDPGKRLIIGPFIKLPPKRDYADYYLIIQNPICMNQIQTRIKKEEYTSLSG
1620 1630 1640 1650 1660
S.cerevisiae TLQDFHLIFSNARIYNTPGSVVYEDSLELEKVVTKKYCEIMGDNSQLDFTEFD.....E
F.fujikuroi LRKDIELMIRNCQTYNEDGSILYQDAKVMNEFFNSKYQEELVAHPELQELEEGVKDSSVA
F.graminearum LRKDIELMIRNCQTYNEDGSILYQDAKIMNEFFNSKYQEELAAHPELQELEEGGKDSSVA
1670 1680 1690 1700
S.cerevisiae QYGTRPLVIPPVVTSSVAESFTDEADSSMTSASV...
F.fujikuroi PSGGGTPQPSGTRIKLISSGSKEANGGSTAAQSDEE
F.graminearum PSGSGGTPQPSGTRIKLISNSAREANGGSTAAQSDEE

C



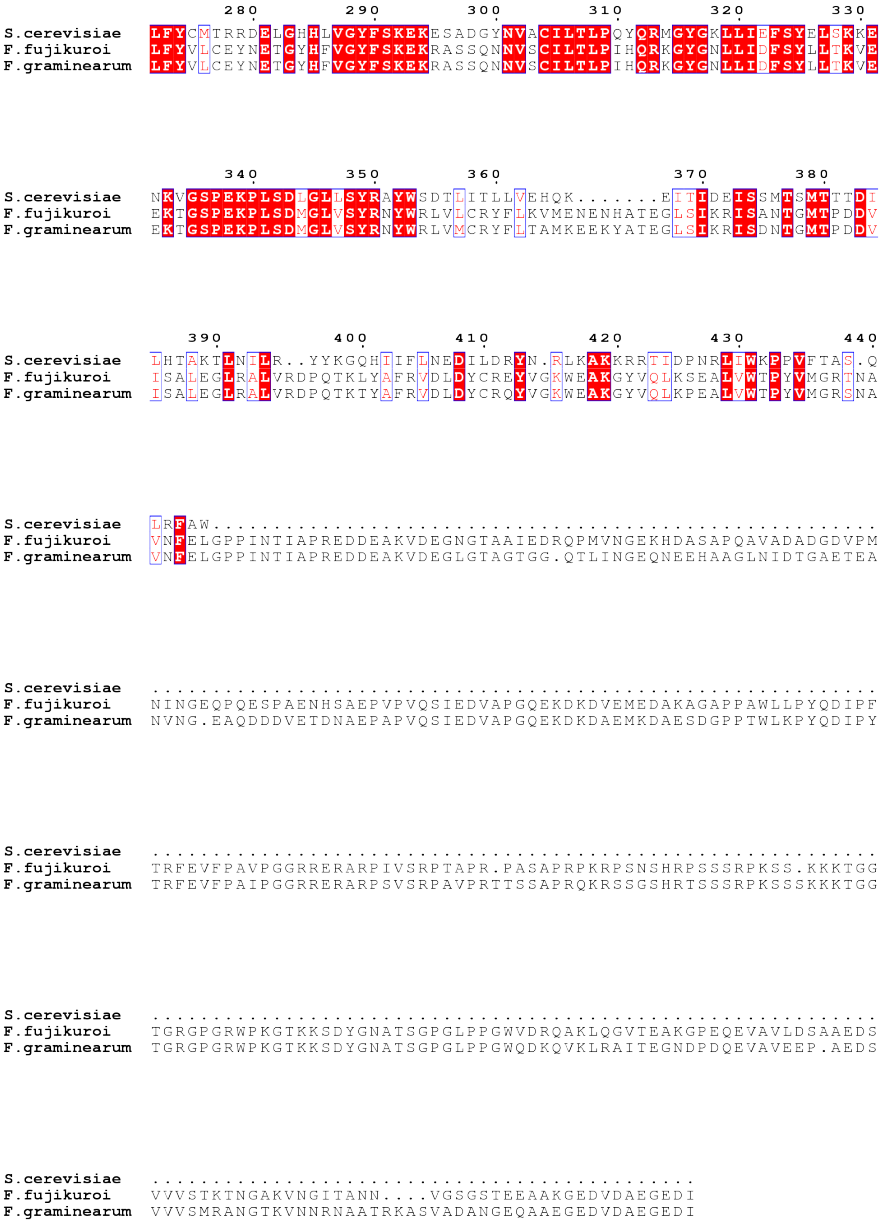


Figure S2. Alignments of the whole coding sequences of protein Swr1p **(A)**, Snf2p **(B)** and Esa1p **(C)** in *S. cerevisiae*, *F. fujikuroi* and *F. graminearum*. Sequence alignment was done by ENDscript 3.0. Consensus threshold is 70%, if the similar score assigned to a column is greater than this value, residues are considered as highly similar and are colored in red and framed in blue. Below the threshold, residues are marked as weakly similar. In all cases, they are in white on a red background in case of strict identify. Gene IDs are listed in **Table 1.**

Summary

Contamination of food with mycotoxins is a significant risk to human and animal health. Reports from European Food Safety Authority (EFSA) indicate that nearly half of the food derived from cereals and unprocessed grains collected between 2007 and 2012 in 21 European countries were contaminated with TCTB, and especially DON (EFSA, 2013). These mycotoxins are produced by fungal phytopathogens on growing kernels, before harvest. On account of the global climate change, this situation may become increasingly serious (Medina *et al*. 2017). Therefore, preventing the production of mycotoxins is a task which brooks no delay. In Europe, DON is predominantly produced by *F. graminearum*. It is the primary causal agent of FHB in barley and wheat, which is considered as one of the most devastating plant disease (Windels 2000; Gilbert and Haber 2013). Even though the sequential steps of the TCTB biosynthetic pathway are fairly well described, the molecular events involved in regulating this pathway are complex and remain, so far, widely misunderstood.

Recent studies highlighted PTMs of canonical histones as well as their variants as critical players in the regulation of mycotoxin and other SMs biosyntheses in filamentous fungi, by altering chromatin structure. In *F. graminearium*, it was shown that the HDAC, HDF1p could be involved in the activation of DON production (Y. Li *et al*. 2011b). Similarly, H3K4me3 deposited by FgSet1p is required for the active transcription of genes involved in DON and AUR biosynthesis (Liu *et al.* 2015). In contrast, H3K27me3 histone mark represses 14% of its genome, including genes involved in secondary metabolic pathways (Connolly, Smith, and Freitag 2013). In *N. crassa*, the binding site created by H3K9me3 is necessary for the recruitment of HP1 and furthermore, Dim2p, which are involved in the establishment and maintenance of heterochromatin (Lomberk, Wallrath, and Urrutia 2006). It forms also histone deacetylation complexes that work in parallel with DNA methylation to assemble silent chromatin. In *F. graminearum*, deletion of HP1 impedes the expression of genes involved in the DON pathway but promotes the production of AUR (Smith *et al*. 2011; Reyes-Dominguez *et al*. 2012).

H2A.Z, a highly evolutionarily conserved histone variant which is ubiquitous in eukaryotes, can exchange dynamically with the canonical histone H2A in nucleosomes by the chromatin

remodeling complex. It is shown to be involved in a diverse range of biological processes, including genome stability, DNA repair, transcriptional regulation and telomere silencing (Coleman-Derr and Zilberman 2012). However, the underlying mechanisms of these functions remain unclear. In some species, the function of H2A.Z appears to be essential (Sevilla and Binda 2014). In *Arabidopsis*, human and mouse ES cells, H2A.Z was reported to be closely associated with the histone markers such as H3K9me3, H3K4me3 and H3K27me3 (Santos-Rosa *et al*. 2002; Creyghton *et al*. 2008; Ku *et al*. 2012; Hu *et al*. 2013). Up to now, the only one study targeted directly on the function of H2A.Z in filamentous fungi was carried out on *N. crassa*, and identifying a role in oxidative stress response (Dong *et al*. 2018).

We hypothesize that in *F. graminearum*, H2A.Z may be involved in important biological processes including those invloved in the production of SMs. Thus, the goal of my project is to identify the major changes in chromatin structure in *F. graminearum* involved in pathogenicity and SM production, with a focus on the function of histone variant H2A.Z. There are three objectives: **(1)** to investigate whether H2A.Z is essential in *F. graminearum*; **(2)** to identify roles played by H2A.Z in development, pathogenicity and SM production in *F. graminearum*; **(3)** to decipher potential relationships between H2A.Z and the heterochromatic markers including HP1, H3K9me3 and DNA methylation in controlling development, pathogenicity and SM production in *F. graminearum.*

Chapter 2

Results Part 1: Main Project

Synopsis

Mainly produced by the filamentous fungus *F. graminearum* in Europe, the mycotoxin DON is toxic to human and animals upon ingestion. Recent studies indicated that chromatin structure changes play a critical role in the regulation of mycotoxin biosynthesis in filamentous fungi. H2A.Z is a highly conserved histone variant in eukaryotes which can replace canonical histone H2A on nucleosomes, altering the structure of chromatin. Indeed, it has been reported to be involved in a diverse range of central biological processes, including genome stability, DNA repair, transcriptional regulation and telomere silencing (Coleman-Derr and Zilberman 2012). However, the underlying mechanisms of these functions remain unclear. Up to now, the only one study targeted directly on the function of H2A.Z in filamentous fungi was carried out on *N. crassa*, and identifyied a role in oxidative stress response (Dong et al. 2018). Here, we hypothesized that H2A.Z may be involved in important biological processes of *F. graminearum* including those involved in the production of secondary metabolism. Therefore, in this study, we aim at characterizing the roles played by the histone variant H2A.Z in controlling development, metabolism and virulence in *F. graminearum*.

Using split-marker approach, a reverse genetics approach which has been proved to work efficiently in the creation of mutants in *Fusarium*, we tried to delete H2A.Z in four *F. graminearum* strains with different genomic backgrounds and characteristics including I156, I349, I605 and I812. However, compared with previous projects that involved knocking out other genes in *F. graminearum* with same method in our lab, deletion of H2A.Z was much tougher and time-consuming. After one year of repeated attempts, only six mutants lacking H2A.Z derived from I156 (N = 4), I349 (N = 1), and I812 (N = 1). All efforts on I605 consistently failed.

All mutants exhibit deficiency in sporulation, germination, radial growth and DON production; however, intensities in the observed effects depend on the considered genetic background. Same method was used to add back wild-type H2A.Z in the I349ΔH2A.Z background to see whether it can rescue the deficiency. Surprisingly, the transformation failed again and again. Ultimately, we obtained two H2A.Z complemented mutants, but

phenotyping revealed that there were very little differences between ΔH2A.Z and H2A.Z add-back mutants **(these results are described in the research paper next section)**.

Whilst PCR and Southern blot are usually and routinely used in the lab to confirm the correctness of the engineered KO mutant (and these techniques were used to primarily very all mutants before phenotyping), this unusual and suspicious phenomenon led us to send all the mutants for whole-genome sequencing. Results revealed that, although H2A.Z has been totally removed from the genome, compensatory mutations occur at other sites in each mutant regardless of the genetic background, particularly in genes involved in chromatin remodeling **(Table 1 in Research Paper)**. For example, several compensatory mutations happen in genes encoding proteins related with histone PTMs. Mutations are also detected on histone H3 and *Swr1*, which mediates the deposition of H2A.Z on nucleosomes. Strikingly, exactly one extra mutation on *Swr1* is observed for both add-back mutants (collected from two independent post-transformation selection plates), the same one for both mutants, questioning the level of randomness of compensation or, in other words, indicating reduced degrees of freedom in the selection of suitable possible options for effective compensation **(see discussion in Research Paper)**. Considering our results as a whole, we draw the conclusion that H2A.Z is essential in *F. graminearum*. It is the occurrence of compensatory mutations that rescued part of the lethality caused by H2A.Z deletion. Meanwhile, the mutations are more likely to happen on genes involved in the same functional module of H2A.Z. We hypothesize that profound reorganizations of gene networks allow such plasticity, with certain consequences in terms of evolution and adaptation. Such hypothesis might be tested by transcriptomic analyses.

Additional results

In order to see whether H2A.Z overexpressed (OE) mutant exhibits reverse phenotype compared to ΔH2A.Z mutants, pGPD, a strong commonly used constitutive yeast promoter was fused to H2A.Z to replace the native promoter at the *locus* **(Figure 13)**. After validation by PCR, southern blot as well as whole genome sequencing, we obtained three H2A.Z.

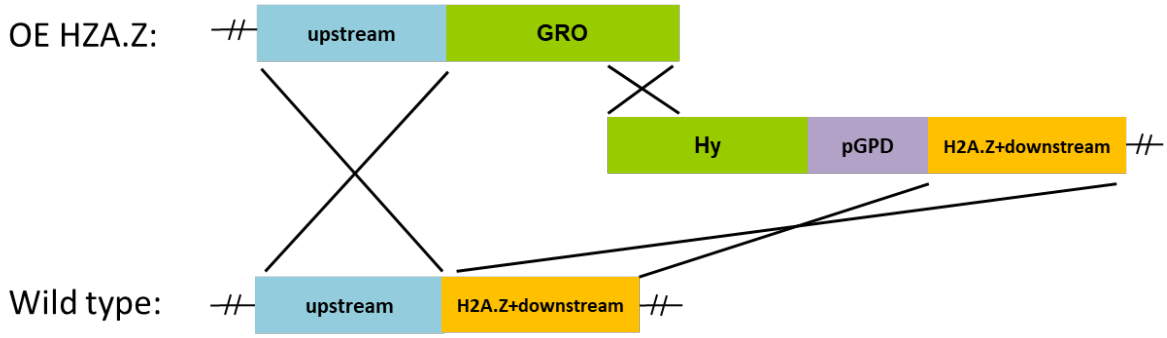


Figure 13. Schematic representation of the split-marker strategy used to overexpress H2A.Z (see Materials and Methods in the research paper). A strong promoter pGPD amplified from the plasmid PAN52-leGFP was inserted to overexpress H2A.Z. The hygromycin B resistance gene was used as selectable marker.

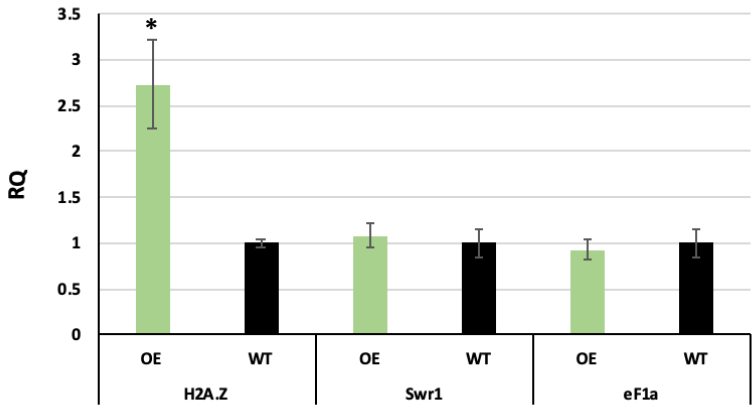


Figure 14. Relative quantification (RQ) of gene expression (*H2A.Z, Swr1, eF1a*) in OE mutant. The I349 wild type strain is used as the reference sample (RQ=1). P-value<0.05 *

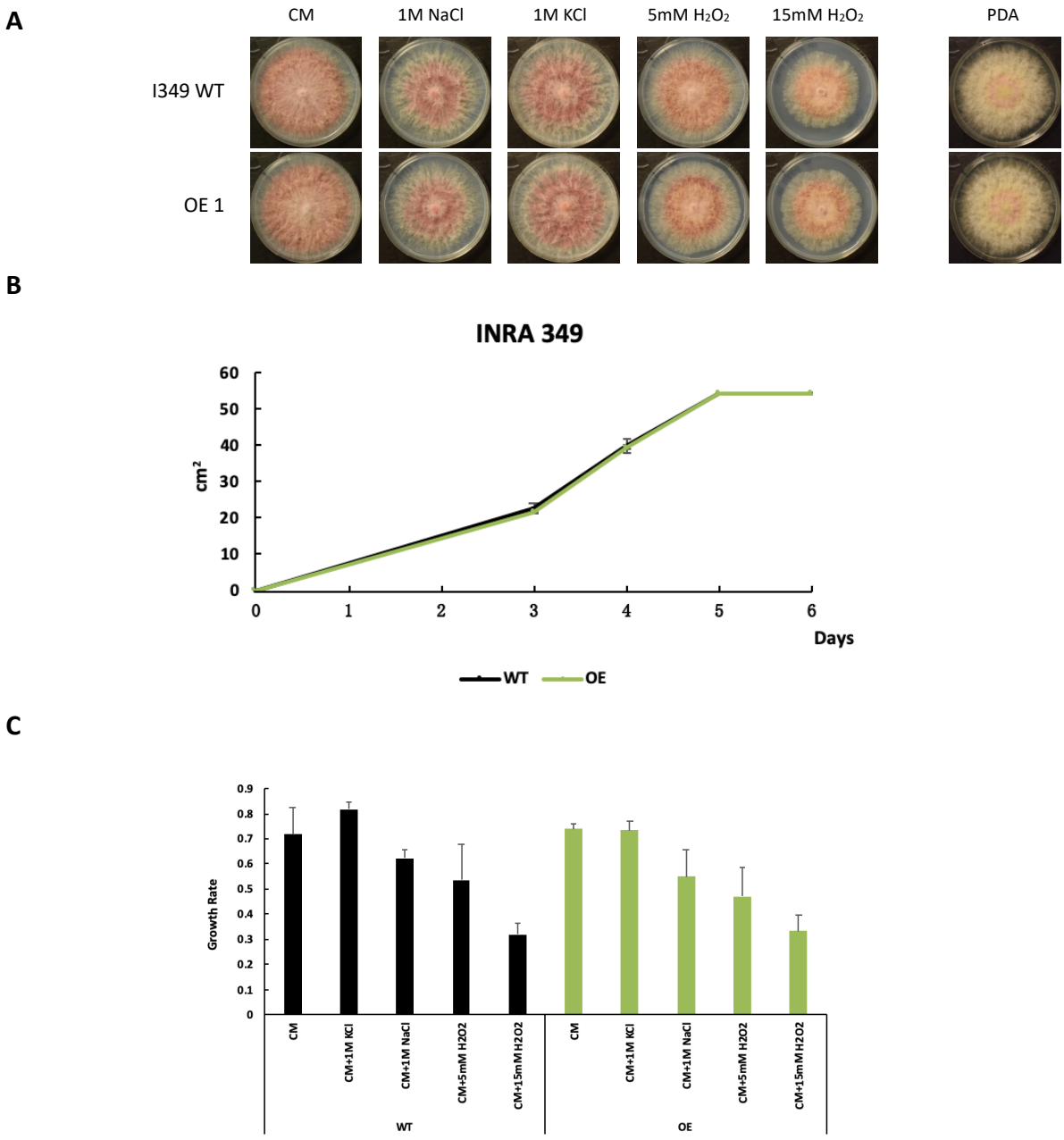


Figure 15. Radial growth of INRA 349 wild-type strain and H2A.Z overexpressed mutant under standard condition (CM), osmotic stress (CM+1M NaCl/KCl) and oxidative stress (CM+5mM/15mM H_2O_2). (A) A 3mm diameter plug of each sample were incubated on each petri dish for up to 5 days at 25°C in the dark. (B) Mycelium growth curve under standard condition during 6 days. (C) The growth rate of the wild type and mutants under standard condition and stresses between 3 and 4 days of inoculation.

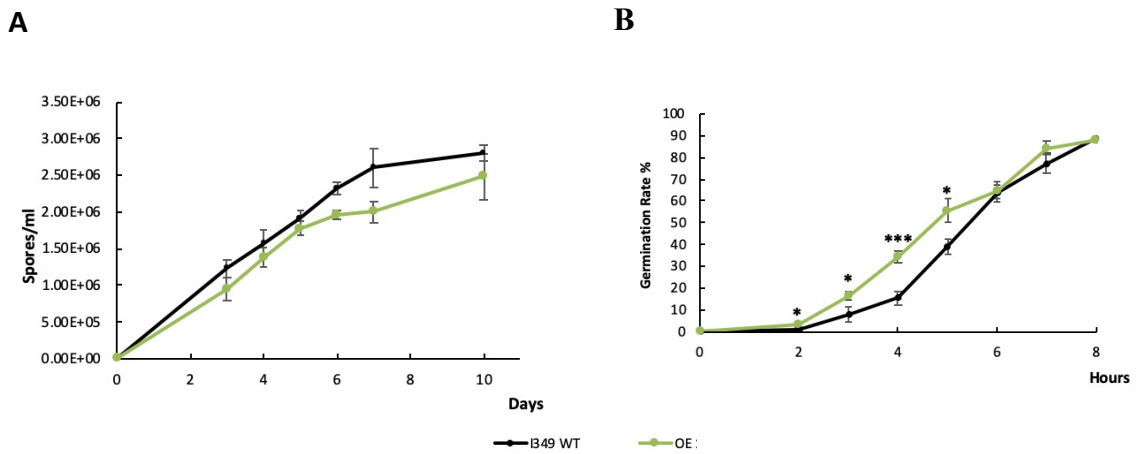


Figure 16. Comparison of sporulation and germination rate between INRA 349 wild-type strain and H2A.Z overexpressed mutant. (A) For each sample, a 3mm implant was incubated in 10 mL of CMC liquid medium for up to 10 days at 25°C and 180rpm in the dark. Spores were counted by Thoma under microscope every day. Bars denote standard errors from three repeated experiments. No significant difference can be observed; (B) 1,15E+06 5 days old spores were incubated in 10ml MS sucrose at 25°C. Germination rates were counted under microscope every hour. P-value<0.05 *, p-value<0.01 **, p-value<0.001 *** (student's t-test).

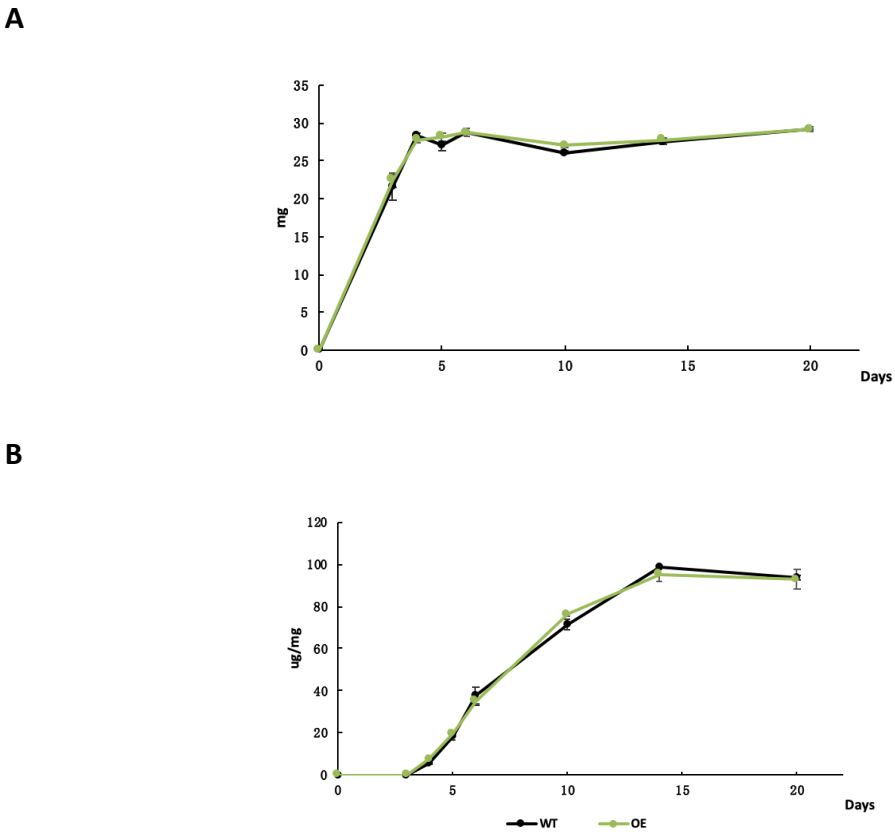


Figure 17. Comparison of biomass (A) and DON production/ biomass (B) between INRA 349 wild-type strains and H2A.Z OE mutant. 8E+04 spores were inoculated in MS glucose liquid medium for up to 20 days at 25°C in the dark. TCTB were extracted and measured by ethyl acetate and HPLC-DAD at the age of 3, 4, 5, 6, 10, 14, 20days respectively.

OE mutants in the background of I349 wild type, in which H2A.Z is nearly three times overexpressed **(Figure 14)**. We measured radial growth under standard and stress conditions **(Figure 15)**, sporulation and germination rate **(Figure 16)**, DON and 15-ADON production **(Figure 17)** in OE mutants and compared our results to those obtained in wild-type **(see Materials and Methods in Research paper)**. No significant difference in phenotype can be observed with the exception of a faster germination rate in OE mutants that fades with time and becomes similar to wild-type.

We did qPCR to measure the expression level of several other genes. Results show that the expression of *Swr1*, which is involved in the deposition of H2A.Z, in OE:H2A.Z is similar to the one measured in wild-type **(Figure 14)**. This might explain why overexpression of H2A.Z almost has no impact on the phenotype of *F. graminearum*. Another possibility is that H2A.Z expression level in our mutant does not reach a threshold sufficient to alter the phenotype. In future experiments, the OE mutant will undergo RNA-seq to check the expression of other genes. A stronger promoter may also be used to create new OE mutants with higher H2A.Z expression level.

Research Paper

Effect of H2A.Z deletion is rescued by compensatory mutations in *Fusarium graminearum*

Zhenhui Chen[1], Enric Zehraoui[1], Anna K. Atanasoff-Kardjalieff[2], Joseph Strauss[2], Lena Studt[2], Nadia Ponts[1,*].

[1]INRA UR1264-MycSA, Villenave d'Ornon, France. [2]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna (BOKU), Tulln, Austria

* To whom correspondence should be addressed. **Email: nadia.ponts@inra.fr**

Classification

Microbiology

Keywords

Suppression; mycotoxin; chromatin

Author Contributions

N.P. designed research; Z.C., E.Z., and A.K.A.K. performed research; Z.C., J.S., L.S. and N.P. analyzed data; Z.C. and N.P. wrote the paper.

This PDF file includes:

Main Text
Figures 1 to 6
Tables 1
Supplemental Figures S1 to S4
Supplemental Tables S1 to S2

Abstract

Fusarium head blight is a destructive disease of grains resulting in reduced yields and contamination of grains with mycotoxins worldwide; *Fusarium graminearum* is its major causal agent. Chromatin structure changes have been shown to play key roles in regulating mycotoxin biosynthesis in filamentous fungi. Using a split-marker approach in three *F. graminearum* strains INRA156, INRA349 and INRA812, we knocked out the gene encoding H2A.Z, a ubiquitous histone variant reported to be involved in a diverse range of biological processes in yeast, plants and animals but has been rarely studied in filamentous fungi. All Δ*H2A.Z* mutants exhibit defects in development including radial growth, sporulation, germination and sexual reproduction, but with varying degrees of severity between them. Besides, heterogeneity of osmotic and oxidative stress response as well as mycotoxin production was observed in Δ*H2A.Z* strains. Adding-back wild-type *H2A.Z* in INRA349-Δ*H2A.Z* could not rescue the phenotypes. Whole genome sequencing revealed that, although *H2A.Z* has been totally removed from the genome and the deletion cassette is inserted at *H2A.Z locus* only, mutations occur at other *loci* in each mutant regardless of the considered genetic background. Our results underline the genetic plasticity of *F. graminearum* facing detrimental gene perturbation. These findings suggest that intergenic suppressions rescue deleterious phenotypes in Δ*H2A.Z* strains, and that *H2A.Z* is essential in *F. graminearum*.

Significance Statement

The functions of histone variant H2A.Z has been widely studied in yeast and higher eukaryotes. However, there is a significant lack of knowledge of the role of H2A.Z and its regulation in filamentous fungi. Here we demonstrate for the first time that complete removal of *H2A.Z* in the genome of *F. graminearum* triggers genetic suppression. The suppressors detected open new possibilities to investigate in more depth the network linking *H2A.Z* and other genes in *F. graminearum,* which can be presented as an experimental bridge between yeasts and higher organisms, and used to decipher unresolved questions concerning H2A.Z.

Introduction

Fusarium.graminearum, a homothallic filamentous fungus, is the major causal agent responsible for the devastating disease Fusarium head blight (FHB) of wheat, barley and other small grain cereal crops worldwide (1, 4). In wheat, FHB affects the head and leads to the wilt of kernels, reducing yield at harvest and causing billions of dollars losses (5, 6). As an additional serious concern, the presence of *F. graminearum* in kernels results in the contamination of cereals with mycotoxins, especially the extremely stable type B trichothecenes (TCTB) including deoxynivalenol (DON) and its acetylated C-15 derivatives (15-ADON). The presence of these mycotoxins on cereals, persistent in derived food and feed products, threatens the health of humans and animals (7–9). Most of the genes involved in the TCTB biosynthetic pathway have been identified, and are referred to as *Tri* genes (10, 11). Expression levels of these genes are regulated by many environmental factors such as temperature (12), nutritional sources (13–15), or oxidative stress (16, 17). A growing body of evidence indicates that chromatin structure changes play a critical role in the regulation of mycotoxin biosynthesis in filamentous fungi.

Like other eukaryotic cells, genomic DNA of fungal cells is packaged together with histones to form nucleosomes, which are the basic units of chromatin. Each nucleosome consists of two copies of each of the four core canonical histones H2A, H2B, H3 and H4, with 145-147 base pairs (bp) of DNA wrapped around them (18, 19). These canonical histones are highly conserved across eukaryotic species and represent the major part of histones within an organism. The N-terminal tails of histones are extensively marked by covalent post-translational modifications (PTMs) including methylation, phosphorylation, or acetylation for example. The combinatorial positioning of these histone marks impact gene expression by altering chromatin state according to a not yet deciphered “Histone Code” (20). Additionally, non-allelic isoforms of canonical histones called histone variants also exist in all eukaryotes (21, 22). The H2A family encompasses the largest number of variants (23); H2A.Z is considered as the most evolutionarily conserved one (24, 25). Generally, H2A.Z and H2A have 60 % amino acid sequence similarity. It differs from H2A in the increased acidic patch and the carboxy-terminal α-helix included in the docking domain, which is a structure involved in the interaction of the H2A-H2B dimer with the (H3-H4)2 tetramer (26, 27). H2A.Z has been identified in various species, including *Arabidopsis thaliana* (28), *Saccharomyces cerevisiae* (29), *Drosophila melanogaster* (30), and human (31). It has been found involving in a diverse range of biological processes, including genome stability (32–34), DNA repair (35–38) and transcriptional regulation (39–41). The absence of H2A.Z may be lethal in many organisms such as mouse (42), Drosophila (30), frogs (43) and Tetrahymena (44), but not in *S. cerevisiae* (45, 46). The dynamic process of H2A.Z deposition/removal from nucleosomes is mediated by ATP-dependent chromatin remodeling complexes, especially complexes belonging to the SNF2 superfamily (47, 48). SNF2 proteins contain two conserved motifs the DEXDc and HelicC subdomains, which together constitute the ATPase domain and can

use the energy derived from ATP hydrolysis to modify chromatin structure (49, 50). In *S. cerevisae*, the complex SWR1 is involved in the recruitment of H2A.Z. It can replace the H2A-H2B by H2A.Z-H2B dimers. Yeast SWR1 complex includes 13 subunits including Swr1p (catalytic subunit), Act1p, Arp4p, Swc4p, and Rvb1p (51–53). By contrast, the removal of H2A.Z from nucleosomes is mediated by the SWR1-related Inositol requiring 80 (INO80) complex (38, 54, 55). INO80 shares several subunits with SWR1 complex and its functions are usually associated with DNA double-strand break repair (56, 57). Homologs for H2A.Z and most subunits of yeast SWR1 and INO80 complex can be found in *F. graminearum*, meaning that similar biological processes may also occur.

Various studies illustrated that functions of H2A.Z in cells are based on its collaboration with other histone marks, particularly those that decorate histone H3 tails. For example, both in human and mouse ES cells, H2A.Z is enriched at enhancers and promoters marked by H3K4me3, a mark that activates gene expression (58, 59). Moreover, it seems that H2A.Z can act as a functional substitute for H3K9me3 in chromatin, recruiting Heterochromatin Protein 1 (HP1) when H3K0me3 levels are low for example (60). Still in mouse ES cells, H2A.Z strongly co-localizes with H3K27me3 and Polycomb Repressive Complexes (PRCs) 1 and 2 near the TSS of genes involved in cell differentiation, and thus helps to keep these genes silenced (61). In filamentous fungi, major roles of chromatin structure changes in regulating the biosynthesis of mycotoxins and other secondary metabolites have been evidenced. For example, in *F. graminearum*, it was shown that the histone deacetylase HDF1 could be involved in the activation of DON production (62). Similarly, H3K4me3 deposited by FgSet1 is required for the active transcription of genes involved in the biosynthesis of both DON and the pigment aurofusarin (63). By contrast, the histone mark H3K27me3 represses 14 % of *F. graminearum*'s genome, including genes involved in secondary metabolism (2). However, regarding the function of H2A.Z in fungi, so far only one study to our best knowledge was carried out on *Neurospora crassa* studying specifically the function of H2A.Z and identifying a role in oxidative stress response (64).

Here, we hypothesized that H2A.Z plays important roles in controlling development and metabolism in *F. graminearum*. Using a reverse genetics approach on three different strains, we evidence that, although H2A.Z has been totally removed from the genome, compensatory mutations occur at other sites in each mutant in all genetic backgrounds. We found that deletion of *H2A.Z* produces a spectrum of highly variable phenotypic consequences, including in mutants originating from the same wild-type (WT) strain. Compensatory mutations occurred in each one of the generated mutants, revealing an unsuspected genome plasticity. As a whole, our results reveal a flexible network of genes that, by interacting with H2A.Z within the same functional network, can re-wire itself to rescue deleterious phenotypes.

Results

Deletion of *H2A.Z* provokes heterogenous developmental defects

To elucidate the function of H2A.Z in *F. graminearum*, we generated six deletion mutants in three different strains (INRA349, INRA156, and INRA812; see the materials and methods section) by targeted replacement of the *H2A.Z* gene with a hygromycin resistance cassette (HygR) as a selectable marker (Fig.S1). We confirmed by PCR, Southern blot, and whole genome resequencing that our target *locus* was indeed replaced by HygR, and that the latter did not insert elsewhere in the genome (data not shown). We started phenotyping by investigating colony morphology of all wild-type (WT) and Δ*H2A.Z* mutant strains following incubation on two different solid media, complete medium (CM) and potato dextrose agar (PDA). Results were consistent for both used media (Fig.1A). Compared with their respective WT strains, all Δ*H2A.Z* mutants displayed a growth deficiency, although with varying degrees of severity. Growth of INRA156Δ*H2A.Z*#3 and INRA349Δ*H2A.Z*, were dramatically decreased ($p < 0.001$; Fig.1B and 1C), and formed colonies with visibly shorter and more condensed hyphae. Inhibition was less intense for INRA156Δ*H2A.Z*#1, #2, and #4 and INRA812Δ*H2A.Z* although still significant (Fig.1B and 1D). In addition, on the macroscopic scale, INRA156Δ*H2A.Z*#2 displayed a less pigmented aspect than WT and derived mutants (Fig.1A). Since fungal growth is polarized and extend at hyphal tips, we tested the possible different abilities of mycelium in active *vs.* not active growing state to resume growth. Three plugs were sampled from the center, middle and edge of pre-cultured plates and transferred to new Petri dishes to resume growth (Fig.2A). Results are shown in Fig.2B. All sub-cultures resumed growth similarly with the exception of INRA156Δ*H2A.Z*#1 and, to a lesser extent, INRA349Δ*H2A.Z* for which the outmost sampled plugs started to grow earlier than the others.

To investigate whether or not phenotype heterogeneity could be observed for other developmental traits, we explored the ability of all WT and mutants to produce asexual spores (macroconidia). Kinetics of conidia production in a spore-inducing medium was followed for up to 10 days for all WT and Δ*H2A.Z* strains. Deletion of *H2A.Z* impeded sporulation in all INRA349, INRA156, and INRA812 mutants Δ*H2A.Z vs.* WT (Fig.3A, 3B, and 3C). Moreover, the kinetics of germination was monitored for INRA349 with its mutant Δ*H2A.Z*. For WT strain, germination was nearly complete (90 %) after eight hours (Fig.3D), an observation consistent with a previously published assay that used the same strain (65). However, in the same time span, less than 20 % of the spores of INRA349Δ*H2A.Z* had germinated.

Finally, we considered the hypothesis of *H2A.Z* deletion affecting sexual reproduction. *F. graminearum* is homothallic, meaning it possesses both compatible alleles in its genome, and can thus undergo sexual reproduction without having to find a compatible partner. After induction of sexual reproduction, perithecia form and sexual spores (ascospores) are ejected in the air for dissemination. Here, after induction on carrot agar, INRA156Δ*H2A.Z*#1 and Δ*H2A.Z*#3 seem to

have completely lost the ability to differentiate into perithecia (Fig.4). Fewer but healthy perithecia, which could discharge ascospores themselves able to germinate and grow, were produced by INRA156Δ*H2A.Z*#2 and Δ*H2A.Z*#4, although there was a 4-day delay for INRA156Δ*H2A.Z*#4. Finally, INRA349 both WT and mutant could never produce perithecia, in our conditions (data not shown).

Heterogeneity of stress responses and DON/15-ADON production in ΔH2A.Z strains

We pursued our exploration and investigated the ability to respond to osmotic/ionic stress and oxidative stress, which are common and major stresses for the fungus (66). We thus cultured our WT and mutant strains on CM agar plates supplemented with NaCl 1M, KCl 1M, H_2O_2 5 mM, or H_2O_2 15 mM, or not supplemented. Results are shown Fig.5 and Fig.S2. As a general observation, considering growth rate, all three WT strains showed little sensitivity to KCl but increased with NaCl. Similarly, H_2O_2 had important measurable effects only when applied at 15 mM rather than 5 mM (Fig.5, FigS2A, and Table S1). Nonetheless, both NaCl and KCl caused striking discoloration of the mycelium for all WT strains (Fig.S2B). H_2O_2 also caused some levels of discolorations with disparities between strains. INRA349 is indeed the only strain less colored with H_2O_2 5 mM, but also the one coping the best with H_2O_2 15 mM (Fig.5B, Fig.S2B, and Table S1). Regarding Δ*H2A.Z* strains, all showed similar or slightly decreased sensitivity to H_2O_2 (for INRA156Δ*H2A.Z*#1 and INRA349Δ*H2A.Z* with H_2O_2 15 mM) when compared to their respective WT, except for INRA156Δ*H2A.Z*#4 that showed reduced growth rate with H_2O_2 5 mM (Fig.5A and Table S1). In contrast, INRA349Δ*H2A.Z* and INRA156Δ*H2A.Z*#3 showed high sensitivity to both NaCl and KCl, other mutants being similar to their respective WT. Meanwhile, similar to WT, a reduction of pigment accumulation could be observed in all mutant strains in response to stress caused by both NaCl or KCl (Fig.S2B). Surprisingly, whilst INRA156 and INRA812 WT strains showed varying levels of discoloration with H_2O_2 15 mM, the derived mutants resembled their not-treated counterparts (Fig.S2B).

The ability to produce mycotoxins was finally investigated in all our mutants and compared to their respective WT. The strains used in this study produce low to high amounts of DON and its derivative 15-ADON, which are virulence factors that help the colonization and spread of fungus within wheat spikes (67). We monitored the kinetics of DON+15-ADON production of WT and mutants for all strains (Fig.6). For all WT strains, toxin production could be detected after three days of culture post-inoculation (dpi) in our conditions. WT strain INRA349 produced high levels of toxins (more than 100 µg/mg of dry biomass), whereas WT INRA156 and INRA812 produced only low amounts of toxins (less than 3 µg/mg of dry biomass). In all Δ*H2A.Z* strains but INRA812Δ*H2A.Z* for which toxins could also be detected after 3 dpi, a 24-hour lag was observed and toxins could only be detected at 4 dpi. In all mutants but INRA156Δ*H2A.Z*#1 and INRA156Δ*H2A.Z*#2, toxins stopped accumulating quickly after induction and levels remained

extremely low throughout. Surprisingly, in INRA156Δ*H2A.Z*#1 and INRA156Δ*H2A.Z*#2, toxin production was stimulated and toxins accumulated at levels higher than WT.

Intergenic suppressors rescue deleterious phenotypes in Δ*H2A.Z* and Δ*H2A.Z*::*H2A.Z* strains

The heterogeneity of phenotypes presented above was puzzling. Moreover, our numerous attempts to add back *H2A.Z* led to the obtention of a single mutant INRA349ΔH2A.Z::H2A.Z that had successfully re-integrated *H2A.Z* at its *locus* (Fig.S3) but could not fully rescue any of the deficiencies caused by *H2A.Z* deletion (Fig.S4). All WT, Δ*H2A.Z*, and Δ*H2A.Z::H2AZ* strains were thus sequenced by Whole Genome Sequencing (WGS) and all subsequent genome sequences scrutinized. As expected, the sequencing confirmed that H2A.Z coding sequence was absent from all Δ*H2A.Z* strains and successfully added back in INRA349Δ*H2A.Z::H2A.Z*. More surprisingly, we discovered in each Δ*H2A.Z* strain one or more mutation elsewhere in the genome that we hypothesized to be compensatory mutations (Table 1), considered to have beneficial effects on fitness when a deleterious mutation is present (68). Moreover, INRA349Δ*H2A.Z::H2A.Z* had one extra mutation compared to INRA349Δ*H2A.Z*, its parental strain. As a whole, compensatory mutations were detected in eight distinct genes, all involving single nucleotide variations that could be deletion, insertion, or replacement of a nucleotide by another. Strikingly, all concerned genes are either currently annotated as “hypothetical protein” in the reference functional database FungiDB (v45; (69, 70) or they encode proteins involved in chromatin remodeling. In three instances, the same gene is affected in two Δ*H2A.Z* mutants from two different parental strains, albeit not with the exact same mutation. The first case regards INRA349Δ*H2A.Z* and INRA156Δ*H2A.Z*#4 that each carry a version of a frameshift mutation in an essential subunit of the histone deacetylase Rpd3S complex (FGRAMPH1_01G23597; reviewed in (71)). Two other mutations occurred in genes encoding proteins involved in methylation and demethylation of histones, namely FGRAMPH1_01G18925 encoding the Jarid1 H3K4 demethylase (in INRA156Δ*H2A.Z*#1) and FGRAMPH1_01G11173 possibly involved in the methylation of H3K36 (Ashl, (72) in INRA156Δ*H2A.Z*#3). INRA156Δ*H2A.Z*#1 carried an additional mutation in a predicted oxidoreductase. Similarly, in addition to a mutation in Ashl, INRA156Δ *H2A.Z*#3 had another one in a gene annotated as hypothetical protein with no evident putative functional domain or homology identified elsewhere (FGRAMPH1_01G03975) that is located immediately downstream of the modified *H2A.Z* locus in the genome. This gene is also affected by a nonsense mutation in INRA812Δ*H2A.Z*, which also contains a mutation in FGRAMPH1_01G27197 encoding a hypothetical protein that contains the interpro domain IPR037651 found in the SWR1-complex protein 3 domain (Swc3). Finally, in INRA156Δ*H2A.Z*#2, mutations occurred in histone H3 and *Swr1* genes, the latter being the catalytic subunit of the SWR1 complex involved in the deposition of H2A.Z. A mutation in *Swr1* is also found in the add-

back mutant INRA349Δ*H2A.Z::H2A.Z*, in addition to the frameshift mutation in *Rpd3s* inherited from its parental strain INRA349Δ*H2A.Z*.

Discussion

Histone variant H2A.Z is found ubiquitously in fungi, plant and animal species. In the present study, we knocked out H2A.Z from *F. graminearum* genome using three different strains: INRA156, INRA349, and INRA812. A total of six H2A.Z deleted mutants were obtained, and all exhibited heterogenous phenotypes. All mutants exhibited deficiencies in macroconidia production and germination; growth and response to osmotic/ionic stress, but with varying intensities. The ability to produce viable perithecia and subsequently germinating ascospores was suppressed in the absence of H2A.Z for two ΔH2A.Z mutants out of the five tested. Finally, the production of the mycotoxins DON and 15-ADON was nearly suppressed except for two mutants in which it was stimulated. Moreover, complementation with native H2A.Z failed to restore wild-type phenotype. We found that each one of genetically modified strain we engineered contained one or more compensatory mutation elsewhere in the genome, compared to the wild-type parental strain. Thus, H2A.Z is essential in *F. graminearum*, and the occurrence of compensatory mutations rescued the lethal phenotype.

Compensatory mutations are defined as beneficial in a particularly deleterious context, and are otherwise undesirable as they usually come as the cost to at least some fitness. Their implications in terms of adaptation and evolution are of great importance. Yet, the underlying mechanisms are largely misunderstood. Usually, such mutations occur for proteins that are in the same functional module (74), and the outcome of such rescue depends on the genomic and environmental backgrounds (75). In our study, the six H2A.Z deletion mutants harbor different mutations, but some of them in the same genes. Davis and Colleagues (2007) proposed that evolution through compensatory mutations is not entirely random at the protein level and can partially be predicted. Although our results may, at the first glance, seem in agreement with this statement, the numerous failed attempts and unusually long recovery times we faced to obtain these mutants more likely indicate that many events occurred before a suitable mutation could be selected.

Several mutations were found in genes encoding histone modifiers: an essential subunit of the histone deacetylase Rpd3s complex (FGRAMPH1_01G23597) and the methyltransferase Ashl (FGRAMPH1_01G11173) both related to H3K36; and the Jarid1 H3K4 demethylase (FGRAMPH1_01G18925). Functions of methylated H3K36 are multiple and thus the subject of intense investigation (reviewed in (76)); methylation of H3K36 is typically associated with active transcription. A proposed mechanism is that SET2-mediated methylation of H3K36 towards the 3' end of genes mediates general histone deacetylation through the recruitment of Rpd3s to slow

down transcription elongation at the end of genes (77). In mammalian embryos, it was suggested that deposition of acetylated H2A.Z (a mark of active transcription) and methylated H3K36 are precisely orchestrated to allow fine-tuned expression of developmental genes (78). In our study, we found that a frameshift mutation in Rdp3s can rescue Δ*H2A.Z* mutants in two distinct strains, INRA349 and INRA156, with the likely consequence of losing the ability to undergo the conformational changes necessary to its activity (79). In this case, transcription elongation speed would increase at the end of genes to provide partial compensation for the loss of H2A.Z (and thus of the activation counterpart of a fine-tuned process). In this scenario, when H2A.Z was added back in such background, a mutation to slow down the incorporation of H2A.Z was required to somehow re-equilibrate the transcriptional balance (a single amino-acid change in Swr1p in our INRA349ΔH2AZ::H2AZ mutant). In fungi, the methyltransferase Ashl could also methylate H3K36 although strong experimental evidence remains to be provided (72). A mutation in Ashl, such as the amino-acid exchange observed in one of our mutants, may thus lead to reduced methylated H3K36 and by extension less Rdp3s recruited to deacetylate histones and slow down transcription. The strategy would therefore be the same as above: speeding up transcription to compensate for the loss of H2A.Z.

In mycotoxinogenic filamentous fungi such as *Fusarium* species, recent studies indicated that epigenetic marks play a critical role in the regulation of secondary metabolism. For example, in *F. graminearum*, H3K27me3 is found to decorate and repress numerous clusters of genes encoding the biosynthesis of secondary metabolism (2). In mouse embryonic stem cells, H2A.Z promotes the deposition of H3K27me3, here having a role of negative fine-tuner of gene expression (80). Two of our mutants (INRA156Δ*H2A.Z*#1 and #2) produced higher levels of DON and 15-ADON than wild type. Loss of H2A.Z may have led to de-repressing the genes involved in their biosynthesis by not depositing H3K27me3. In INRA156Δ*H2A.Z*#1, exhibiting the highest levels of toxins produced, mutations are found in a putative oxidoreductase (amino-acid exchange) and the H3K4 demethylase Jarid1 (frameshift). In *Fusarium fujikuroi*, methylated H3K4 is deposited by SET1 and positively regulates, among other traits, secondary metabolism (81). In this mutant, active transcription is certainly promoted by maintaining methylated H3K4. The effect on genes involved in secondary metabolism may be “synergistic” with the defective recruitment of the silencing mark H3K27me3, leading to enhanced toxin production. Incidentally, INRA156Δ*H2A.Z*#1 seem to display increased resistance to oxidative stress, a property that may be related to the presence of a mutation in a putative oxidoreductase.

Taking all phenotypes as a whole, the mutant INRA156Δ*H2A.Z*#2 may be the one for which rescue may have been the most successful as far as the phenotyped traits are concerned. Compared to our other mutants, the observed defects were of weaker intensity. Two compensatory mutations were detected: one in *H3* and another one in *Swr1*. As mentioned above, SWR1 is a major actor of the deposition of the dimer H2A.Z-H2B to replace a dimer H2A-

H2B, notably *via* interactions with $(H3\text{-}H4)_2$. Mutations in both *Swr1* and H3 may prevent protein interaction and subsequent removal of H2A-H2B, thus stabilizing durably the histone octamer in the absence of H2A.Z and rescuing part of the deficiency. Similarly, a nonsense mutation in *Swc3*, encoding a member of the SWR1 complex, may also contribute to enhance stability of nucleosomes and prevent lethality.

Finally, mutations were also detected in two genes encoding hypothetical proteins. We predict that they have a role in the same functional module as H2A.Z. Both encode predicted proteins with no obvious homologue in eukaryotes other than fungi. One of them is located immediately downstream of H2A.Z, and the possibility that proximity rather than function caused the mutation cannot, however, be excluded.

As a whole, in the present study, the observed phenotypes are the result of interactions between two or more mutations, under the influence of the genetic background. One feature emerges, lack of H2A.Z is lethal in *F. graminearum*, but its extraordinary plasticity allows compensation within the short time frame of a lab transformation. Our study proposes several targets and pathways mediated by H2A.Z, advancing the search for H2A.Z functional network.

Materials and Methods

Fungal strains and culture media

Wild-type *F. graminearum* strains INRA156 (INRA-MycSA collection), INRA349 (CBS185.32; CBS-KNAW Collection, the Netherlands), INRA812 (PH-1 strain FGSC9075; Fungal Genetics Stock Center, the USA) were propagated on Potato Dextrose Agar (PDA; DIFCO, France) plates at 25°C. INRA156 can efficiently undergo sexual reproduction but produces only moderate to low levels of DON and 15-ADON, whereas INRA349 always fails to produce perithecia but consistently produces very high levels of DON and 15-ADON; INRA812 is the sequenced reference strain widely used in *Fusarium* related studies (can reproduce sexually and produces only very low levels of DON and 15-ADON). Radial growth assays were performed on complete medium (82) supplemented prior solidification with NaCl, KCl, H_2O_2, or not supplemented. When needed, conidia were prepared by inoculating agar plugs in CMC medium (15 g/L carboxylmethyl cellulose, 1 g/L yeast extract, 0.5 g/L $MgSO_4.7H_2O$, 1 g/L NH_4NO_3, 1 g/L KH_2PO_4) (83), incubating at 150 rpm and 25°C for three to five days, and harvesting by filtration through Sefar Nitex 03-100 (100 µm, SEFAR AG - Switzerland). Toxin production was measured in liquid synthetic medium (MS, 84). For spore germination assays, glucose in MS medium was replaced by sucrose.

Generation of gene deletion and complementation mutants

Wild-type *F. graminearum* strains INRA156, INRA349, and INRA812 were used as parental

strains of deletion mutants. All primers and amplification conditions used are given Table S2. Upstream and downstream flanking regions of H2A.Z (793 bp and 951 bp, respectively; gene id FGRAMPH1_01G03973) were amplified by PCR (primer pairs 3r-3UTR-H2AZ-R / 3UTR-H2AZ-HY-F and 5UTR-H2AZ-GRO-R / 5f-UTR-H2AZ-F, respectively) from wild-type INRA349 genomic DNA. The hygromycin-resistance cassette, containing *hph* gene under the control of the *N. crassa CPC1* promoter and the *A. nidulans trpC* terminator, (2.1 kb) was amplified by PCR using primer pair neoHY-8-finF /neoHY-1-debutR and pBSK(-) NeoHygroR plasmid as template. Fragment assembly was achieved by multiple recombination of overlapping sequences in the *S. cerevisiae* strain FY1679 (genotype MATa/MATα, ura3-52/ura3-52, trp1Δ63/TRP1, leu2Δ/LEU2, his3Δ200/HIS3, GAL2/GAL2) transformed with the three PCR products and the pRS426 plasmid digested with BamHI and HindIII (85). SC-Ura medium containing 0.67 % yeast nitrogen base without amino acids was used for selection. A second round of PCR was carried out using successfully transformed FY1679 DNA as template to amplify two overlapping fragments (primer pairs 3UTR-H2AZ-N-R / NP_SplitHY_R and NP_SplitGRO_F / 5UTR-H2AZ-N-F) to be used for transformation into *F. graminearum* for the targeted replacement of H2A.Z (split-marker recombination approach (86); Figure S1A). PCR products were purified and transformed into protoplasts of INRA156, INRA349, and INRA812, according to a previously published protocol (17). H2A.Z was then added back to the deletion mutant derived from INRA349 by targeted replacement of the integrated hygromycin-resistance cassette with the H2A.Z gene under the control of its native promoter and trpC terminator fused to a geneticin-resistance cassette (as described in (17). Briefly, after individual amplification of all fragments (see Table S2), purified products were transformed into protoplasts derived from INRA349-ΔH2A.Z. Successfully selected transformants were purified by two rounds of monoconidial isolation.

Whole genome sequencing and data analysis

Genomic DNA was extracted from 50 mg of freeze-dried mycelium, from all wild-type and mutant strains, as previously described (87). Libraries were prepared from 500 ng of gDNA using the Westburg NGS DNA Library Prep Kit for Illumina (cat. # WB9024, Westburg, The Netherlands) following manufacturer's instructions. Sequencing in paired mode, 2x100 bp, was performed by the GenomEast platform, a member of the 'France Génomique' consortium (ANR-10-INBS-0009) on an Illumina HiSeq 4000 (reads were deposited at SRA under the BioProject accession number PRJNA580372). Raw reads were pre-processed with Trimmomatic v0.39 (88). The software CLC Genomics Workbench 10 (QIAGEN Bioinformatics, Denmark) was used for all subsequent bioinformatic analyses. Briefly, high-quality read pairs were mapped (including a re-alignment step) onto *F. graminearum* PH-1 reference genome. Coverage analysis was performed to confirm the correct insertion of the deletion/complementation cassette in all mutants. Variants were called

(ploidy = 1 and variant probability = 0.9), and those found in wild-type were substracted from their corresponding mutants. Each remaining variant was manually curated to ensure no false positive was retained.

Radial growth assays

A total of 100 spores or a plug (3 mm in diameter, taken from the periphery of a 7 day-old PDA culture plate) were cultured at 25°C in dark up to eight days on 94*16mm plates containing CM, or CM with 1M NaCl/ KCl, or CM with 5 mM/15 mM H_2O_2. Experiments were done in triplicate. Growth area of each petri dish was analyzed by ImageJ 1.x image processing program (89). Growth rate was measured by curve fitting through all points with function *fitlm* of Matlab R2019a (Mathworks).

Conidiation and germination rate assays

For sporulation assays, one mycelial plug (8 mm in diameter) of each strain, taken from the periphery of a 7 day-old colony on PDA was inoculated in a 50 mL tube with screw filter cap containing 10 mL of CMC medium, and incubated for up to 10 days at 25°C and 180 rpm in dark in an Infors Multitron (INFORS-HT). Spores were counted in a Thoma cell counting chamber under microscope every 24 hours. For germination assays, 10^6 five-day old spores were incubated in 10 mL MS sucrose liquid medium at 25°C for up to eight hours. Germination rates were counted under microscope every hour. Experiments were done in triplicate.

Sexual reproduction assays

Self-fertilization were performed on carrot agar plates as previously described (90). Perithecia were observed under microscope two days after sexual induction. Ascospores were harvested by placing the carrot agar plates upside down and allowing the mature perithecia to discharge ascospores on petri dish covers. Ascospores were collected by adding 1mL of sterile MilliQ water and observed under microscope. A volume of 10 µL of each suspension was inoculated in liquid CM to check whether ascospores could grow or not. Experiments were done in triplicate.

Type B trichothecene analysis

Eight milliliters of MS liquid medium were inoculated with $8x10^4$ conidia prepared in CMC medium and incubated at 25°C in the dark for up to 20 days. TCTB were extracted and analyzed by HPLC-DAD according to a previously published protocol (17).

Acknowledgments

The authors thank M.-N. Verdal-Bonnin, S. Chereau, C. Ducos, and L. Pinson-Gadais for their

expertise and technical assistance. Work was funded by ANR Grant ANR-18-CE91-0006 to N.P. Z.C. held a doctoral fellowship from the China Scholarship Council.

References

1. R. S. Goswami, H. C. Kistler, Heading for disaster: Fusarium graminearum on cereal crops. *Mol. Plant Pathol.* **5**, 515–525 (2004).

2. L. R. Connolly, K. M. Smith, M. Freitag, The Fusarium graminearum Histone H3 K27 Methyltransferase KMT6 Regulates Development and Expression of Secondary Metabolite Gene Clusters. *PLoS Genet.* **9**, e1003916 (2013).

3. B. D. Giaimo, F. Ferrante, A. Herchenröther, S. B. Hake, T. Borggrefe, The histone variant H2A.Z in gene regulation. *Epigenetics Chromatin* **12**, 37 (2019).

4. A. Balzer, D. Tardieu, J. Bailly, P. Guerre, The trichothecenes: Toxins nature, natural occurrence in food and feeds, and ways of struggle. *Rev. Med. Veterinaire* **155**, 299–314 (2004).

5. J. C. Sutton, Epidemiology of wheat head blight and maize ear rot caused by Fusarium graminearum. *Can. J. Plant Pathol.* **4**, 195–209 (1982).

6. M. McMullen, R. Jones, D. Gallenberg, Scab of Wheat and Barley: A Re-emerging Disease of Devastating Impact. *Plant Dis.* **81**, 1340–1348 (1997).

7. A. E. Desjardins, T. M. Hohn, S. P. McCormick, Trichothecene biosynthesis in Fusarium species: chemistry, genetics, and significance. *Microbiol. Rev.* **57**, 595–604 (1993).

8. M. Kimura, T. Tokai, N. Takahashi-Ando, S. Ohsato, M. Fujimura, Molecular and genetic studies of fusarium trichothecene biosynthesis: pathways, genes, and evolution. *Biosci. Biotechnol. Biochem.* **71**, 2105–2123 (2007).

9. M. Figueroa, K. E. Hammond-Kosack, P. S. Solomon, A review of wheat diseases—a field perspective. *Mol. Plant Pathol.* **19**, 1523–1536 (2018).

10. N. J. Alexander, R. H. Proctor, S. P. McCormick, Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in Fusarium. *Toxin Rev.* **28**, 198–215 (2009).

11. S. P. McCormick, A. M. Stanley, N. A. Stover, N. J. Alexander, Trichothecenes: From Simple to Complex Mycotoxins. *Toxins* **3**, 802–814 (2011).

12. P. Marín, N. Magan, C. Vázquez, M. T. González-Jaén, Differential effect of environmental conditions on the growth and regulation of the fumonisin biosynthetic gene FUM1 in the maize pathogens and fumonisin producers Fusarium verticillioides and Fusarium proliferatum. *FEMS Microbiol. Ecol.* **73**, 303–311 (2010).

13. F. Jiao, A. Kawakami, T. Nakajima, Effects of different carbon sources on trichothecene production and Tri gene expression by Fusarium graminearum in liquid culture. *FEMS Microbiol. Lett.* **285**, 212–219 (2008).

14. D. M. Gardiner, K. Kazan, J. M. Manners, Nutrient profiling reveals potent inducers of

trichothecene biosynthesis in Fusarium graminearum. *Fungal Genet. Biol. FG B* **46**, 604–613 (2009).

15. M. Pasquali, *et al.*, A Fusarium graminearum strain-comparative proteomic approach identifies regulatory changes triggered by agmatine. *J. Proteomics* **137**, 107–116 (2016).

16. K. Audenaert, E. Callewaert, M. Höfte, S. De Saeger, G. Haesaert, Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by Fusarium graminearum. *BMC Microbiol.* **10**, 112 (2010).

17. M. Montibus, *et al.*, The bZIP Transcription Factor Fgap1 Mediates Oxidative Stress Response and Trichothecene Biosynthesis But Not Virulence in Fusarium graminearum. *PLoS ONE* **8**, e83377 (2013).

18. T. J. Richmond, J. T. Finch, B. Rushton, D. Rhodes, A. Klug, Structure of the nucleosome core particle at 7 Å resolution. *Nature* **311**, 532 (1984).

19. K. Luger, M. L. Dechassa, D. J. Tremethick, New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nat. Rev. Mol. Cell Biol.* **13**, 436 (2012).

20. J. E. Audia, R. M. Campbell, Histone Modifications and Cancer. *Cold Spring Harb. Perspect. Biol.* **8** (2016).

21. H. S. Malik, S. Henikoff, Phylogenomics of the nucleosome. *Nat. Struct. Mol. Biol.* **10**, 882–891 (2003).

22. C. Bonisch, S. B. Hake, Histone H2A variants in nucleosomes and chromatin: more or less stable? *Nucleic Acids Res.* **40**, 10719–10741 (2012).

23. E. Bernstein, S. B. Hake, The nucleosome: a little variation goes a long wayThis paper is one of a selection of papers published in this Special Issue, entitled 27th International West Coast Chromatin and Chromosome Conference, and has undergone the Journal's usual peer review process. *Biochem. Cell Biol.* **84**, 505–507 (2006).

24. A. van Daal, E. M. White, S. C. Elgin, M. A. Gorovsky, Conservation of intron position indicates separation of major and variant H2As is an early event in the evolution of eukaryotes. *J. Mol. Evol.* **30**, 449–455 (1990).

25. T. H. Thatcher, M. A. Gorovsky, Phylogenetic analysis of the core histones H2A, H2B, H3, and H4. *Nucleic Acids Res.* **22**, 174–179 (1994).

26. K. Luger, A. W. Mäder, R. K. Richmond, D. F. Sargent, T. J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).

27. R. K. Suto, M. J. Clarkson, D. J. Tremethick, K. Luger, Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat. Struct. Biol.* **7**, 1121–1124 (2000).

28. R. March-Díaz, J. C. Reyes, The beauty of being a variant: H2A.Z and the SWR1 complex in plants. *Mol. Plant* **2**, 565–577 (2009).

29. J. D. Jackson, V. T. Falciano, M. A. Gorovsky, A likely histone H2A.F/Z variant in Saccharomyces cerevisiae. *Trends Biochem. Sci.* **21**, 466–467 (1996).

30. A. van Daal, S. C. Elgin, A histone variant, H2AvD, is essential in Drosophila melanogaster. *Mol. Biol. Cell* **3**, 593–602 (1992).

31. C. L. Hatch, W. M. Bonner, The human histone H2A.Z gene. Sequence and regulation. *J. Biol. Chem.* **265**, 15211–15218 (1990).

32. J. A. Downs, *et al.*, Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol. Cell* **16**, 979–990 (2004).

33. M.-C. Keogh, *et al.*, A phosphatase complex that dephosphorylates gammaH2AX regulates DNA damage checkpoint recovery. *Nature* **439**, 497–501 (2006).

34. N. J. Krogan, *et al.*, High-definition macromolecular composition of yeast RNA-processing complexes. *Mol. Cell* **13**, 225–239 (2004).

35. M. Kalocsay, N. J. Hiller, S. Jentsch, Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol. Cell* **33**, 335–343 (2009).

36. Y. Xu, *et al.*, Histone H2A.Z Controls a Critical Chromatin Remodeling Step Required for DNA Double-Strand Break Repair. *Mol. Cell* **48**, 723–733 (2012).

37. O. Gursoy-Yuzugullu, M. K. Ayrapetov, B. D. Price, Histone chaperone Anp32e removes H2A.Z from DNA double-strand breaks and promotes nucleosome reorganization and DNA repair. *Proc. Natl. Acad. Sci.* **112**, 7507–7512 (2015).

38. H. E. Alatwi, J. A. Downs, Removal of H2A.Z by INO80 promotes homologous recombination. *EMBO Rep.* **16**, 986–994 (2015).

39. J. T. Marques, *et al.*, Loqs and R2D2 act sequentially in the siRNA pathway in Drosophila. *Nat. Struct. Mol. Biol.* **17**, 24–30 (2010).

40. R. B. Deal, S. Henikoff, The INTACT method for cell type-specific gene expression and chromatin profiling in Arabidopsis thaliana. *Nat. Protoc.* **6**, 56–68 (2011).

41. T. A. Soboleva, M. Nekrasov, D. P. Ryan, D. J. Tremethick, Histone variants at the transcription start-site. *Trends Genet.* **30**, 199–209 (2014).

42. R. Faast, *et al.*, Histone variant H2A.Z is required for early mammalian development. *Curr. Biol. CB* **11**, 1183–1187 (2001).

43. N. Iouzalen, J. Moreau, M. Méchali, H2A.ZI, a new variant histone expressed during Xenopus early development exhibits several distinct features from the core histone H2A. *Nucleic Acids Res.* **24**, 3947–3952 (1996).

44. X. Liu, B. Li, null GorovskyMA, Essential and nonessential histone H2A variants in Tetrahymena thermophila. *Mol. Cell. Biol.* **16**, 4305–4311 (1996).

45. M. Adam, F. Robert, M. Larochelle, L. Gaudreau, H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Mol. Cell. Biol.* **21**, 6270–6279 (2001).

46. J. D. Jackson, M. A. Gorovsky, Histone H2A.Z has a conserved function that is distinct

from that of the major H2A sequence variants. *Nucleic Acids Res.* **28**, 3811–3816 (2000).

47. H. van Attikum, O. Fritsch, S. M. Gasser, Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. *EMBO J.* **26**, 4113–4125 (2007).

48. C. B. Gerhold, S. M. Gasser, INO80 and SWR complexes: relating structure to function in chromatin remodeling. *Trends Cell Biol.* **24**, 619–631 (2014).

49. A. Lusser, J. T. Kadonaga, Chromatin remodeling by ATP-dependent molecular machines. *BioEssays News Rev. Mol. Cell. Dev. Biol.* **25**, 1192–1200 (2003).

50. C. L. Smith, C. L. Peterson, ATP-dependent chromatin remodeling. *Curr. Top. Dev. Biol.* **65**, 115–148 (2005).

51. M. S. Kobor, *et al.*, A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol.* **2**, E131 (2004).

52. N. J. Krogan, *et al.*, A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol. Cell* **12**, 1565–1576 (2003).

53. G. Mizuguchi, ATP-Driven Exchange of Histone H2AZ Variant Catalyzed by SWR1 Chromatin Remodeling Complex. *Science* **303**, 343–348 (2004).

54. A. J. Morrison, X. Shen, Chromatin remodelling beyond transcription: the INO80 and SWR1 complexes. *Nat. Rev. Mol. Cell Biol.* **10**, 373–384 (2009).

55. A. Tosi, *et al.*, Structure and subunit topology of the INO80 chromatin remodeler and its nucleosome complex. *Cell* **154**, 1207–1219 (2013).

56. C. R. Clapier, B. R. Cairns, The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.* **78**, 273–304 (2009).

57. M. Chen, K. Licon, R. Otsuka, L. Pillus, T. Ideker, Decoupling epigenetic and genetic effects through systematic analysis of gene position. *Cell Rep.* **3**, 128–137 (2013).

58. M. Ku, *et al.*, H2A.Z landscapes and dual modifications in pluripotent and multipotent stem cells underlie complex genome regulatory functions. *Genome Biol.* **13**, R85 (2012).

59. S. V. Kumar, H2A.Z at the Core of Transcriptional Regulation in Plants. *Mol. Plant* **11**, 1112–1114 (2018).

60. D. P. Ryan, D. J. Tremethick, The interplay between H2A.Z and H3K9 methylation in regulating HP1α binding to linker histone-containing chromatin. *Nucleic Acids Res.* **46**, 9353–9366 (2018).

61. M. P. Creyghton, *et al.*, H2AZ Is Enriched at Polycomb Complex Target Genes in ES Cells and Is Necessary for Lineage Commitment. *Cell* **135**, 649–661 (2008).

62. Y. Li, *et al.*, The HDF1 histone deacetylase gene is important for conidiation, sexual reproduction, and pathogenesis in Fusarium graminearum. *Mol. Plant-Microbe Interact. MPMI* **24**, 487–496 (2011).

63. Y. Liu, *et al.*, Histone H3K4 methylation regulates hyphal growth, secondary metabolism

and multiple stress responses in Fusarium graminearum. *Environ. Microbiol.* **17**, 4615–4630 (2015).

64. Q. Dong, *et al.*, Histone variant H2A.Z antagonizes the positive effect of the transcriptional activator CPC1 to regulate catalase-3 expression under normal and oxidative stress conditions. *Free Radic. Biol. Med.* **121**, 136–148 (2018).

65. N. Ponts, L. Pinson-Gadais, M.-N. Verdal-Bonnin, C. Barreau, F. Richard-Forget, Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of Fusarium graminearum: DON-ADON accumulation is modulated by oxidative stress. *FEMS Microbiol. Lett.* **258**, 102–107 (2006).

66. N. Ponts, Mycotoxins are a component of Fusarium graminearum stress-response system. *Front. Microbiol.* **6** (2015).

67. K.-Y. Seong, *et al.*, Global gene regulation by Fusarium transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis. *Mol. Microbiol.* **72**, 354–367 (2009).

68. B. H. Davis, A. F. Y. Poon, M. C. Whitlock, Compensatory mutations are repeatable and clustered within proteins. *Proc. R. Soc. B Biol. Sci.* **276**, 1823–1827 (2009).

69. J. E. Stajich, *et al.*, FungiDB: an integrated functional genomics database for fungi. *Nucleic Acids Res.* **40**, D675-681 (2012).

70. E. Y. Basenko, *et al.*, FungiDB: An Integrated Bioinformatic Resource for Fungi and Oomycetes. *J. Fungi* **4**, 39 (2018).

71. J.-S. Lee, A. Shilatifard, A site to remember: H3K36 methylation a mark for histone deacetylation. *Mutat. Res.* **618**, 130–134 (2007).

72. M. Freitag, Histone Methylation by SET Domain Proteins in Fungi. *Annu. Rev. Microbiol.* **71**, 413–439 (2017).

73. M. H. P. West, W. M. Bonner, Histone 2A, a heteromorphous family of eight protein species. *Biochemistry* **19**, 3238–3245 (1980).

74. J. I. Rojas Echenique, S. Kryazhimskiy, A. N. Nguyen Ba, M. M. Desai, Modular epistasis and the compensatory evolution of gene deletion mutants. *PLOS Genet.* **15**, e1007958 (2019).

75. M. Filteau, *et al.*, Evolutionary rescue by compensatory mutations is constrained by genomic and environmental backgrounds. *Mol. Syst. Biol.* **11** (2015).

76. E. J. Wagner, P. B. Carpenter, Understanding the language of Lys36 methylation at histone H3. *Nat. Rev. Mol. Cell Biol.* **13**, 115–126 (2012).

77. H. Woo, S. D. Ha, S. B. Lee, S. Buratowski, T. Kim, Modulation of gene expression dynamics by co-transcriptional histone methylations. *Exp. Mol. Med.* **49**, e326–e326 (2017).

78. A. Bošković, *et al.*, Analysis of active chromatin modifications in early mammalian embryos reveals uncoupling of H2A.Z acetylation and H3K36 trimethylation from embryonic genome activation. *Epigenetics* **7**, 747–757 (2012).

79. K. Ruan, *et al.*, Histone H4 acetylation required for chromatin decompaction during DNA

replication. *Sci. Rep.* **5**, 12720 (2015).

80. Y. Wang, *et al.*, Histone variants H2A.Z and H3.3 coordinately regulate PRC2-dependent H3K27me3 deposition and gene expression regulation in mES cells. *BMC Biol.* **16**, 107 (2018).

81. S. Janevska, *et al.*, Elucidation of the Two H3K36me3 Histone Methyltransferases Set2 and Ash1 in Fusarium fujikuroi Unravels Their Different Chromosomal Targets and a Major Impact of Ash1 on Genome Stability. *Genetics* **208**, 153–171 (2018).

82. P. Ilgen, B. Hadeler, F. J. Maier, W. Schäfer, Developing kernel and rachis node induce the trichothecene pathway of Fusarium graminearum during wheat head infection. *Mol. Plant-Microbe Interact. MPMI* **22**, 899–908 (2009).

83. R. A. Cappellini, J. L. Peterson, Macroconidium Formation in Submerged Cultures by a Non-Sporulating Strain of Gibberella zeae. *Mycologia* **57**, 962–966 (1965).

84. A.-L. Boutigny, *et al.*, Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and Tri gene expression in Fusarium liquid cultures. *Mycol. Res.* **113**, 746–753 (2009).

85. P. D. Collopy, *et al.*, High-throughput construction of gene deletion cassettes for generation of Neurospora crassa knockout strains. *Methods Mol. Biol. Clifton NJ* **638**, 33–40 (2010).

86. N. Catlett, B.-N. Lee, O. Yoder, B. Turgeon, Split-Marker Recombination for Efficient Targeted Deletion of Fungal Genes. *Fungal Genet. Rep.* **50**, 9–11 (2003).

87. J. P. Javerzat, V. Bhattacherjee, C. Barreau, Isolation of telomeric DNA from the filamentous fungus Podospora anserina and construction of a self-replicating linear plasmid showing high transformation frequency. *Nucleic Acids Res.* **21**, 497–504 (1993).

88. A. Bolger, *et al.*, The genome of the stress-tolerant wild tomato species *Solanum pennellii*. *Nat. Genet.* **46**, 1034–1038 (2014).

89. C. A. Schneider, W. S. Rasband, K. W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675 (2012).

90. B. Cavinder, U. Sikhakolli, K. M. Fellows, F. Trail, Sexual Development and Ascospore Discharge in Fusarium graminearum. *J. Vis. Exp. JoVE* (2012) https:/doi.org/10.3791/3895 (August 27, 2019).

91. R Development Core Team, *a language and environment for statistical computing: reference index* (R Foundation for Statistical Computing, 2010) (October 18, 2019).

Figures and Tables

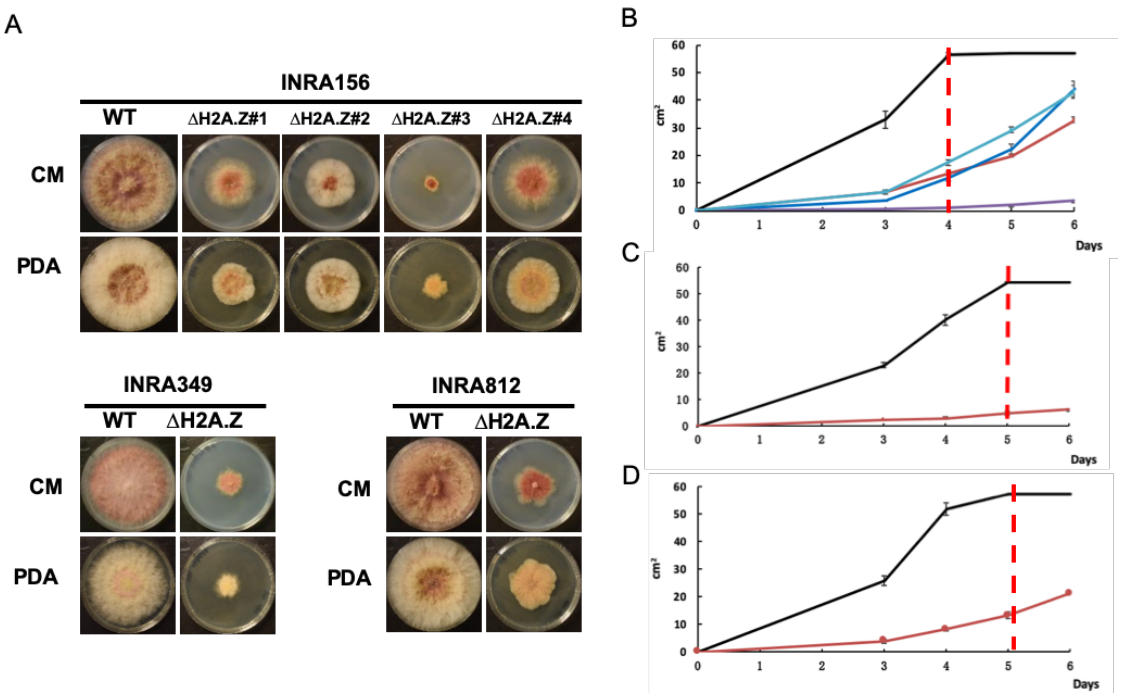


Figure 1. Comparison of radial growth phenotypes between wild-type strains (INRA349, INRA156, INRA812) and their ΔH2A.Z mutants, grown from a central 3 mm-diameter plug on CM or PDA Petri dishes for up to six days at 25°C in the dark. ***(****A)* Macroscopic aspect after 5 days of culture. All ΔH2A.Z show reduced radial growth. *(B, C, and D)* Kinetics of radial growth monitored for six days for wild-type and mutants for strains INRA156 *(B)*, INRA349 *(C)*, and INRA812 *(D)*. Black curves: wild-type; Colored curves: ΔH2A.Z mutants (for *(B)*, red = ΔH2A.Z#1, dark blue = ΔH2A.Z#2, purple = ΔH2A.Z#3, light blue = ΔH2A.Z#4). The red dotted line means growth in WT has reached the edge of the Petri dish, and comparison cannot be made past that point. Up to then, differences between WT and mutants were always significant (student's t-test, *p*-value < 0.001) Displayed values are average of three replicates ± standard deviation.

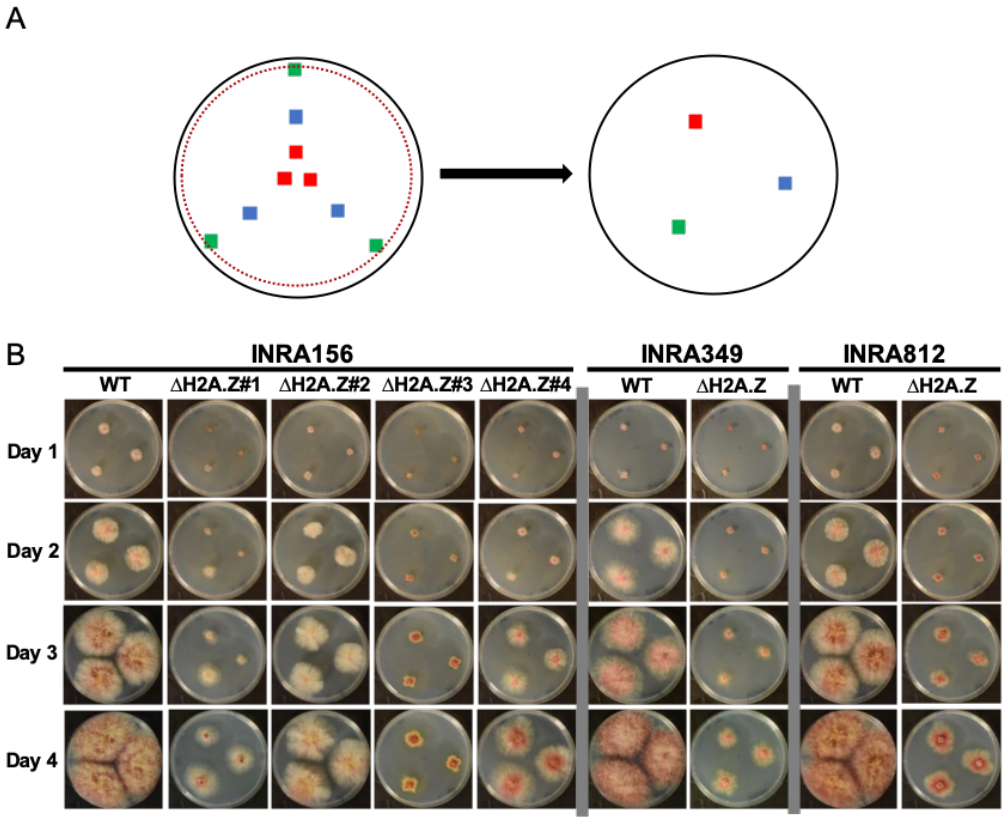


Figure 2. Growth restart test for WT and ΔH2A.Z strains on solid agar. *(A)* Schematic representation of the experimental set up. Three 3 mm-diameter plugs were excised from the center (red), middle (blue), and edge (green) of a pre-culture on CM plates and transferred to a new Petri dish to growth at 25°C in the dark for up to four days. The dashed line shows the growth front of the pre-culture at the moment of sampling. *(B)* Top view of mycelia showing that no difference is visible according to the sampling zone, except for INRA156ΔH2A.Z#1 and (to a lesser extent) INRA349ΔH2A.Z that show growth lags in less active mycelium (towards the center of the donor plate).

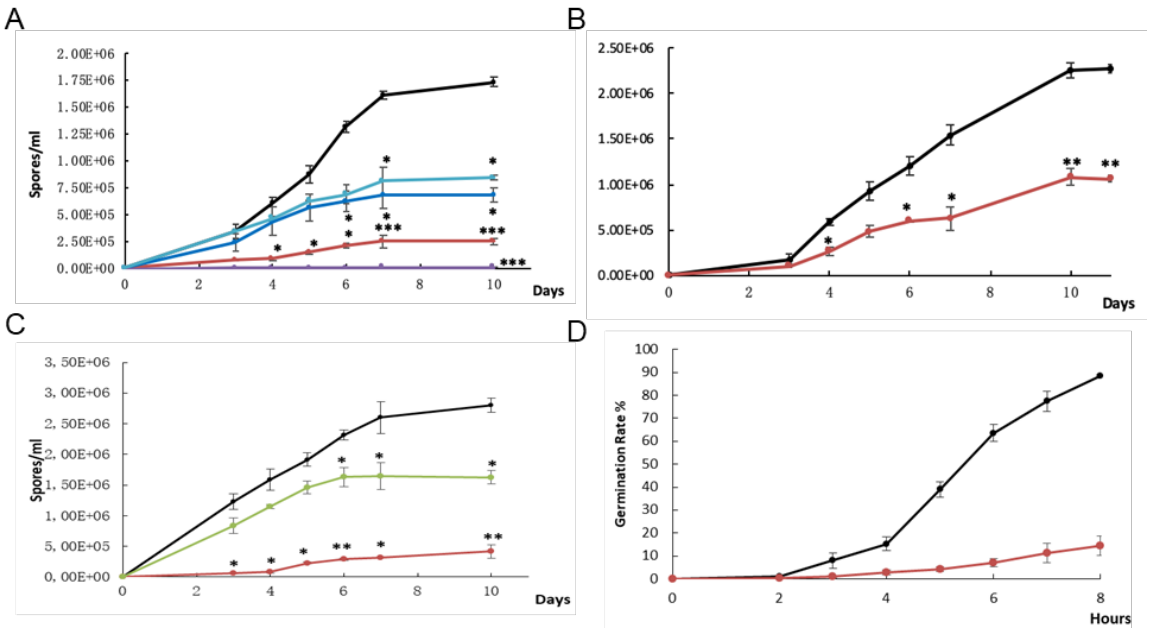


Figure 3. Kinetics of sporulation and germination WT *vs.* ΔH2A.Z. *(A, B, C)* Kinetics of sporulation for *(A)* INRA156 genetic background, *(B)* INRA812 genetic background, and *(C)* INRA 349 genetic background. For each strain, macroconidia were produced in a spore-inducing medium for up to 10 days with daily counting. Displayed values are means ± standard deviation. Stars * indicate significance (student's t-test): **p*-value < 0.05; ***p*-value < 0.01; ****p*-value < 0.001; *(D)* Kinetics of germination for 10^6 5 day-old spores obtained from INRA349 WT and ΔH2A.Z, with hourly counting. Black curves: wild-type; Colored curves: ΔH2A.Z mutants (for *(B)*, red = ΔH2A.Z#1, dark blue = ΔH2A.Z#2, purple = ΔH2A.Z#3, light blue = ΔH2A.Z#4). Displayed values are means ± standard deviation. Significant defect in germination could be observed in ΔH2A.Z INRA349 *vs.* WT starting at 4 hours and up (4 hours: *p*-value < 0.05; 5 to 8 hours: *p*-value < 0.001).

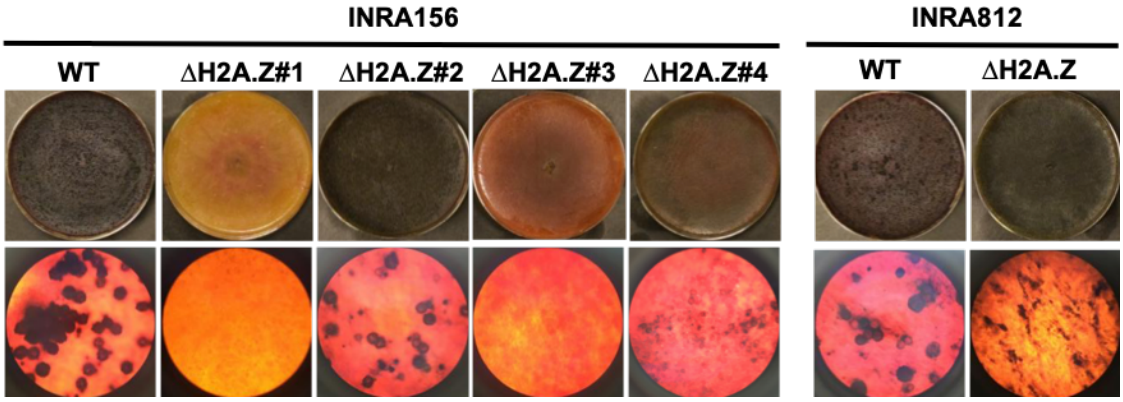


Figure 4. Formation of perithecia on carrot agar by INRA156 and INRA812 WT and ΔH2A.Z mutants. Top lane: macroscopic view; bottom lane: details under microscope (x40). Pictures were taken four days after induction of sexual differentiation.

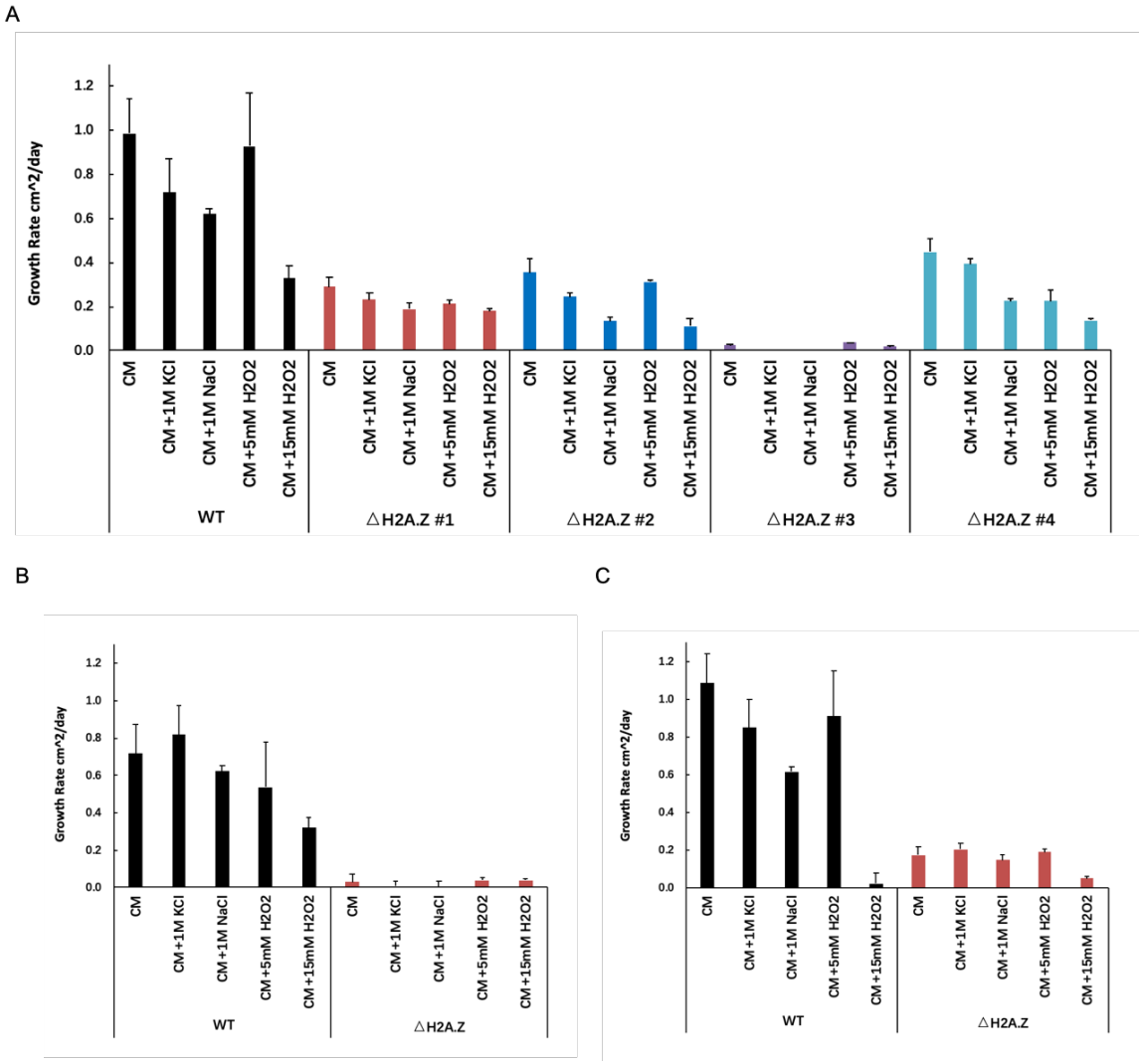


Figure 5. Abiotic stress affect growth rate differently in WT and mutants strains. Growth rates during linear phase were measured in cm^2/day. for WT and mutants grown under standard condition (CM), osmotic/ionic stress (CM+1M NaCl/KCl), or oxidative stress (CM+5mM/15mM H_2O_2). A plug of each sample was incubated on each petri dish for up to six days at 25°C in the dark and growth rates measured from corresponding growth curves (Fig.S2). Bars are mean values for three replicates ± standard deviation *(A)* INRA156 and mutants. *(B)* INRA349 and mutant. *(C)* INRA812 and mutant.

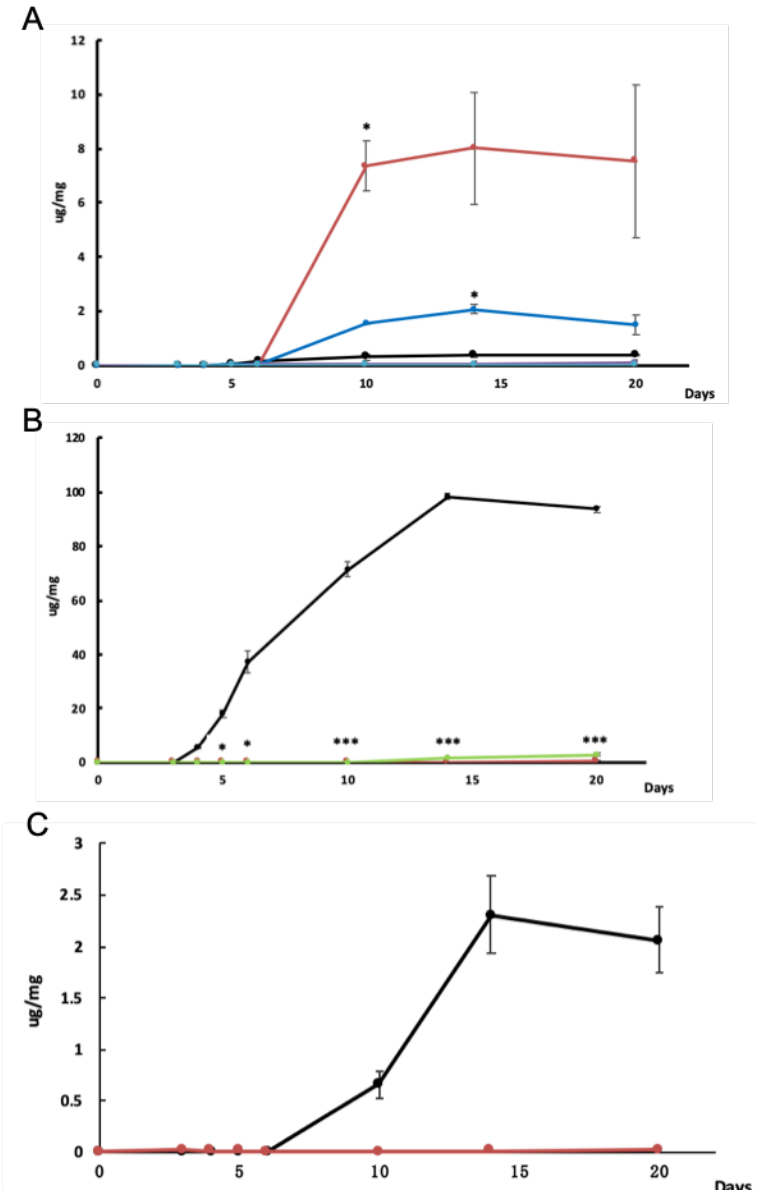


Figure 6. Kinetics of DON+15-ADON production in WT *vs.* mutant strains in MS glucose liquid medium (in micrograms of toxins per milligram of dry biomass; average of three biological replicates ± standard deviation). Black curves: wild-type; Colored curves: ΔH2A.Z mutants (for *(B)*, red = ΔH2A.Z#1, dark blue = ΔH2A.Z#2, purple = ΔH2A.Z#3, light blue = ΔH2A.Z#4). Stars * indicate significance (student's t-test): **p*-value < 0.05, ***p*-value < 0.01, ****p*-value < 0.001. *(A)* INRA156 WT and mutants. *(B)* INRA349 WT and mutants. *(C)* INRA812 WT and mutants.

Table 1. List of compensatory mutations in ΔH2A.Z and ΔH2A.Z::H2A.Z mutants.

Parental strain	Mutant	Gene ID	Protein	Amino acid change
INRA349	ΔH2A.Z	FGRAMPH1_01G23597	essential subunit of the histone deacetylase rpd3s complex	Ile673fs
	ΔH2A.Z ::H2A.Z	FGRAMPH1_01G18675	helicase Swr1	His852Pro
INRA156	ΔH2A.Z #1	FGRAMPH1_01G16577	oxidoreductase Yusz	Asp88Tyr
		FGRAMPH1_01G18925	histone demethylase Jarid1	Val81fs
	ΔH2A.Z #2	FGRAMPH1_01G14931	histone H3	Pro17Thr
		FGRAMPH1_01G18675	helicase Swr1p	Leu850del
	ΔH2A.Z #3	FGRAMPH1_01G03975	hypothetical protein	Glu34Lys
		FGRAMPH1_01G11173	histone-lysine n-methyltransferase Ash1l	His537Tyr
	ΔH2A.Z #4	FGRAMPH1_01G23597	essential subunit of the histone deacetylase rpd3s complex	Val116fs
INRA812	ΔH2A.Z	FGRAMPH1_01G03975	hypothetical protein	Asp13Asn
		FGRAMPH1_01G27197	hypothetical protein, putative Swc3	Arg478*

Supplemental Figures and Tables

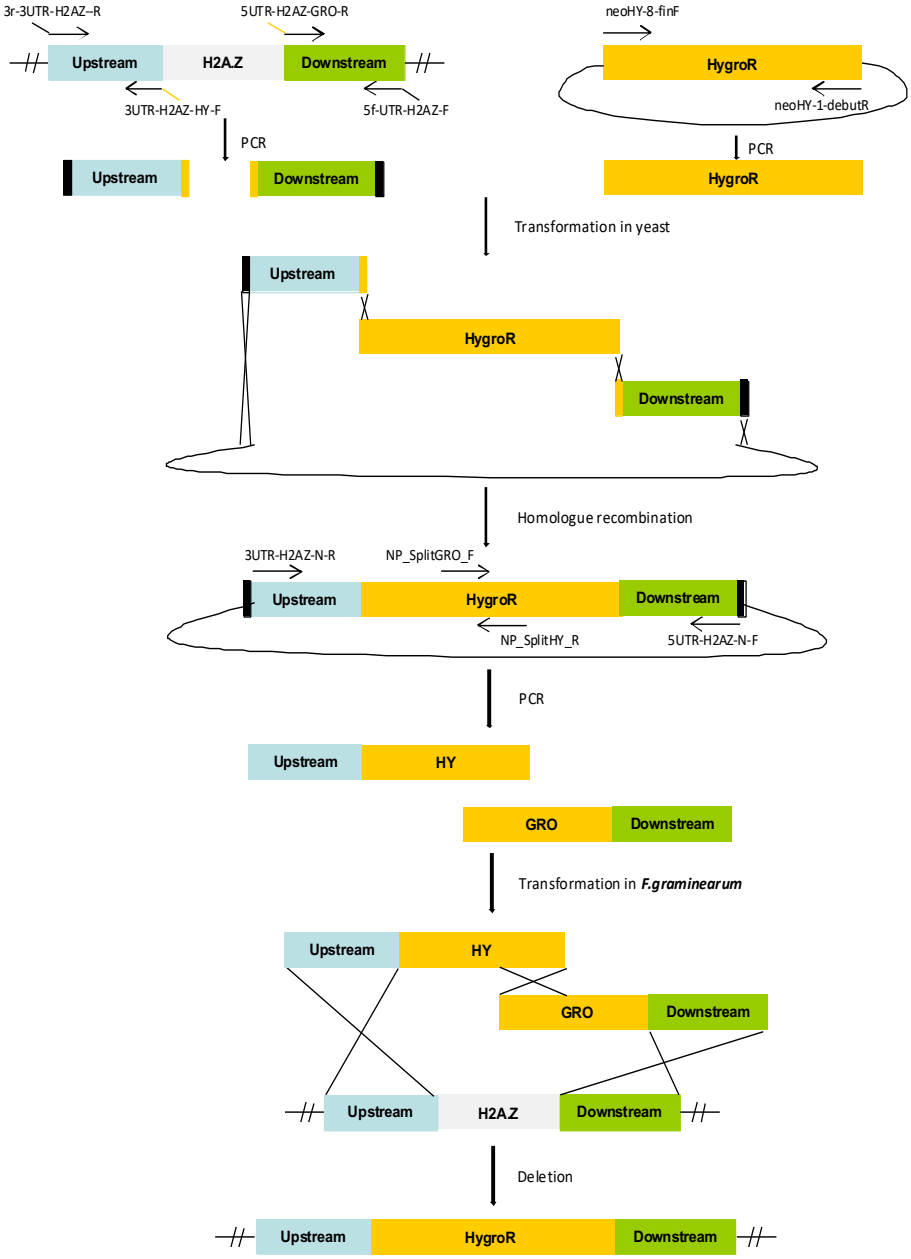


Figure S1. Split-marker approach used for the deletion of H2A.Z in *F. graminearum*. *(A)* The upstream and downstream flanking regions of H2A.Z, HygroR cassette were amplified from wild type *F. graminearum* genomic DNA and plasmid DNA of pBlueScriptSK(-)_NeoHygroR, respectively. (B) Transformation in the yeast. The three fragments were assembled by plasmid pRS426. *(C)* Extraction of yeast genomic DNA and amplification of fragments for split-mark. *(D)* Transformation in *F. graminearum*. H2A.Z was replaced by HygroR cassette.

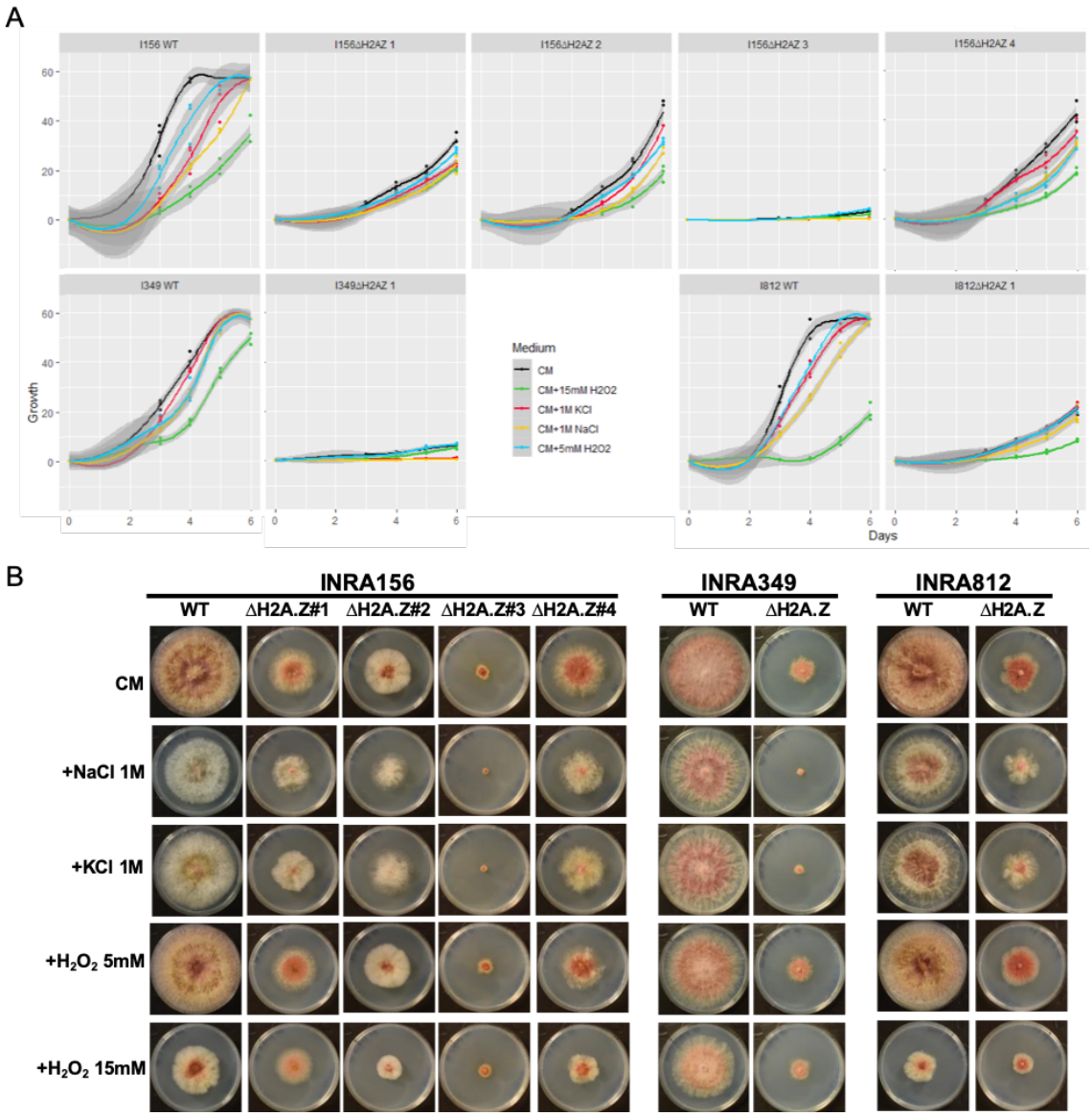


Figure S2. Radial growth for *F. graminearum* strains WT and mutants ΔH2A.Z grown under standard (CM) or stress (CM+1M NaCl/KCl, CM+5mM/15mM H_2O_2) conditions. Plugs from pre-cultures were incubated for up to six days at 25°C in the dark. Cultures were done in triplicate. *(A)* Growth curves were plotted with R version 3.6.1 (91). *(B)* Macroscopic view of morphological effects of abiotic stresses on WT and mutants.

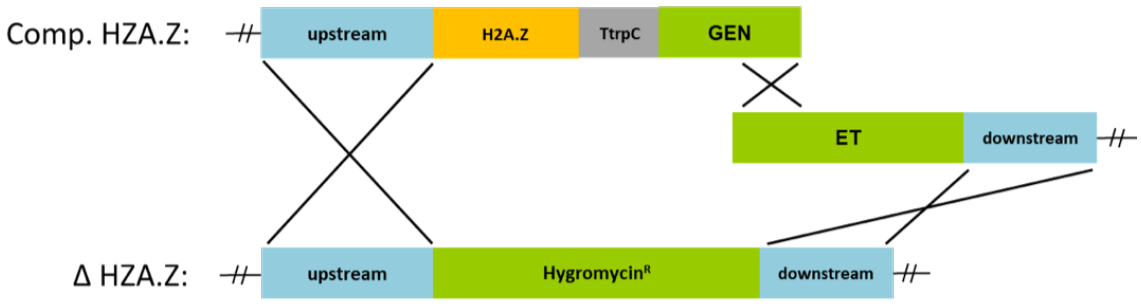


Figure S3. Schematic representation of the split-marker strategy used to add back H2A.Z to INRA349ΔH2A.Z.

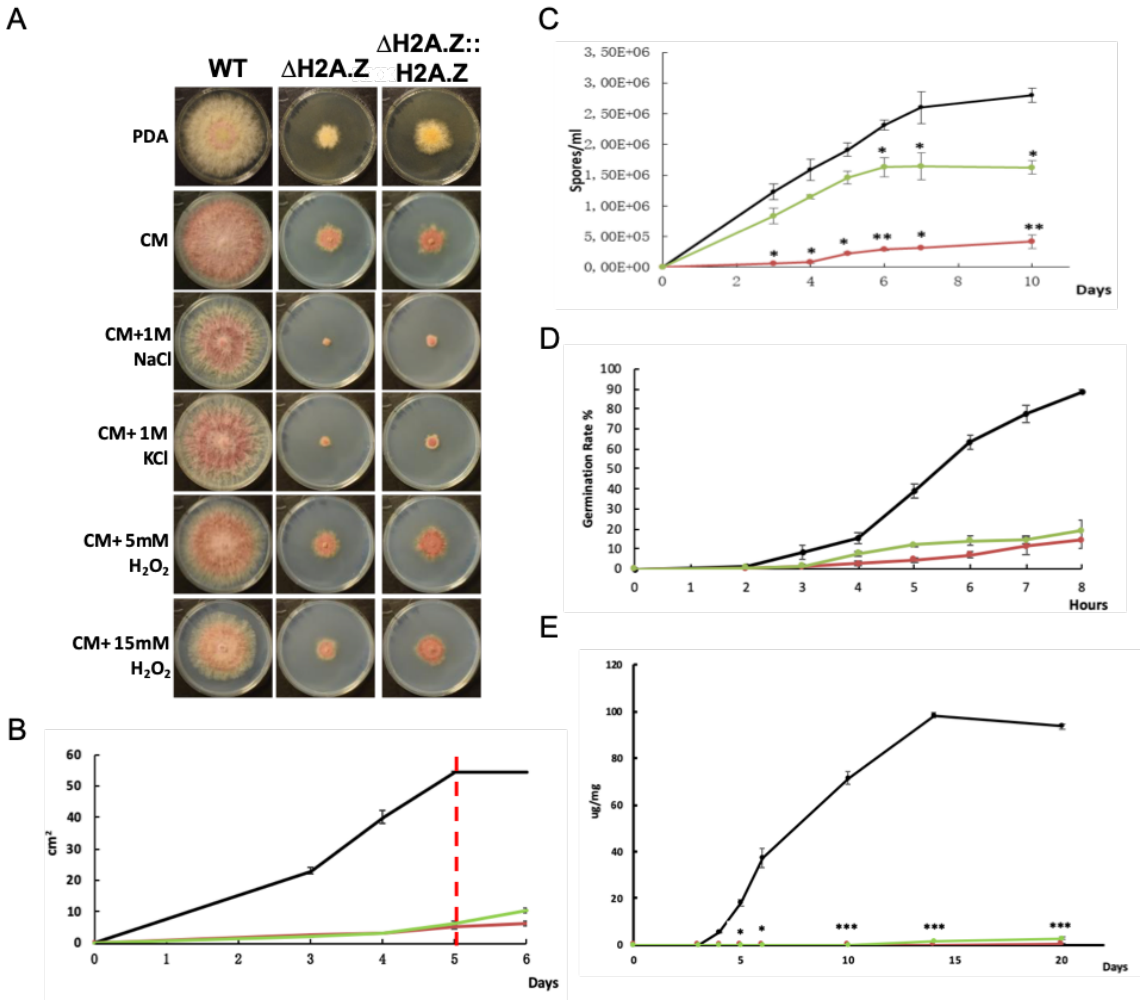


Figure S4. Phenotyping of INRA349ΔH2A.Z::H2A.Z add-back mutant. *(A)* Radial growth under standard (PDA or CM) or stress (CM+1M NaCl/KCl, CM+5mM/15mM H_2O_2) conditions. Plugs from pre-cultures were incubated for up to six days at 25°C in the dark. Cultures were done in triplicate. *(B)* Kinetics of radial growth. The red dotted line means growth in WT has reached the edge of the Petri dish, and comparison cannot be made past that point. (C) Kinetics of sporulation. (D) Kinetics of germination. (E) Kinetics of accumulation of DON+15-ADON. *(B to E)* Black = WT; red = ΔH2A.Z; green = ΔH2A.Z::H2A.Z. Displayed values are average of three replicates ± standard deviation.

Table S1. Results of radial growth rate of *F. graminearum* strains WT and mutants under standard (PDA or CM) or stress (CM + 1M NaCl/KCl, CM + 5 mM/15 mM H_2O_2) conditions computed by curve fitting through all points with function *fitlm* of Matlab R2019a (Mathworks). SE: standard error; R2: coefficient.

Strain	Condition	Growth rate (cm^2/day)	SE	R^2	*p*-value
INRA156 WT	CM	0.9855	0.1554	0.9096	3.16E-03
	CM + 1 M KCl	0.7185	0.1532	0.8462	9.47E-03
	CM + 1 M NaCl	0.6192	0.0277	0.9921	2.36E-05
	CM + 5 mM H_2O_2	0.9262	0.2425	0.7848	1.88E-02
	CM + 15 mM H_2O_2	0.3287	0.0584	0.8880	4.90E-03
INRA156 Δ*H2A.Z*#1	CM	0.2904	0.0432	0.9188	2.54E-03
	CM + 1 M KCl	0.2327	0.0304	0.9360	1.57E-03
	CM + 1 M NaCl	0.1889	0.0275	0.9219	2.35E-03
	CM + 5 mM H_2O_2	0.2119	0.0158	0.9782	1.80E-04
	CM + 15 mM H_2O_2	0.1798	0.0086	0.9910	3.08E-05
INRA156 Δ*H2A.Z*#2	CM	0.3545	0.0635	0.8863	5.04E-03
	CM + 1 M KCl	0.2460	0.0159	0.9836	1.01E-04
	CM + 1 M NaCl	0.1356	0.0161	0.9465	1.10E-03
	CM + 5 mM H_2O_2	0.3133	0.0102	0.9958	6.77E-06
	CM + 15 mM H_2O_2	0.1116	0.0343	0.7263	3.11E-02
INRA156 Δ*H2A.Z*#3	CM	0.0229	0.0037	0.9036	3.60E-03
	CM + 1 M KCl	0.0013	0.0015	0.1583	4.35E-01
	CM + 1 M NaCl	0.0011	0.0014	0.1360	4.72E-01
	CM + 5 mM H_2O_2	0.0365	0.0000	1.0000	2.24E-31
	CM + 15 mM H_2O_2	0.0208	0.0047	0.8281	1.18E-02
INRA156 Δ*H2A.Z*#4	CM	0.4469	0.0645	0.9232	2.27E-03
	CM + 1 M KCl	0.3937	0.0221	0.9875	5.87E-05
	CM + 1 M NaCl	0.2262	0.0106	0.9913	2.82E-05
	CM + 5 mM H_2O_2	0.2254	0.0466	0.8538	8.44E-03
	CM + 15 mM H_2O_2	0.1378	0.0066	0.9908	3.20E-05
INRA349 WT	CM	0.7176	0.1086	0.9161	2.72E-03
	CM + 1 M KCl	0.8185	0.0274	0.9955	7.50E-06
	CM + 1 M NaCl	0.6230	0.0324	0.9893	4.29E-05
	CM + 5 mM H_2O_2	0.5371	0.1418	0.7821	1.93E-02
	CM + 15 mM H_2O_2	0.3192	0.0421	0.9350	1.62E-03
INRA349 Δ*H2A.Z*	CM	0.0297	0.0142	0.5231	1.04E-01
	CM + 1 M KCl	0.0071	0.0037	0.4818	1.26E-01
	CM + 1 M NaCl	0.0045	0.0022	0.5100	1.11E-01
	CM + 5 mM H_2O_2	0.0406	0.0086	0.8491	9.01E-03
	CM + 15 mM H_2O_2	0.0385	0.0146	0.6331	5.84E-02
INRA349 Δ*H2A.Z ::H2A.Z*	CM	0.0443	0.0114	0.7903	1.78E-02
	CM + 1 M KCl	0.0142	0.0070	0.5043	1.14E-01
	CM + 1 M NaCl	0.0054	0.0026	0.5136	1.09E-01
	CM + 5 mM H_2O_2	0.0468	0.0075	0.9078	3.29E-03
	CM + 15 mM H_2O_2	0.0341	0.0045	0.9356	1.59E-03
INRA812 WT	CM	1.0862	0.1439	0.9344	1.65E-03
	CM + 1 M KCl	0.8484	0.0946	0.9526	8.55E-03
	CM + 1 M NaCl	0.6170	0.0332	0.9886	4.93E-05
	CM + 5 mM H_2O_2	0.9090	0.0125	0.9993	2.13E-07
	CM + 15 mM H_2O_2	0.0224	0.0072	0.7058	3.63E-02
INRA812 Δ*H2A.Z*	CM	0.1745	0.0342	0.8667	6.98E-03
	CM + 1 M KCl	0.2033	0.0232	0.9506	9.30E-04
	CM + 1 M NaCl	0.1466	0.0198	0.9321	1.77E-03
	CM + 5 mM H_2O_2	0.1912	0.0201	0.9575	6.88E-04
	CM + 15 mM H_2O_2	0.0499	0.0130	0.7861	1.86E-02

Table S2. Primers used for the deletion and complementation of H2A.Z in *F.graminearum*. Nucleotides marked in blue represent the link sequence between each fragment. The fragments marked with stars (*) were amplified by GoTaq® DNA polymerase, others were amplified with KAPA HiFi DNA polymerase. **Ta**: annealing temperature used for PCR.

Mutant	Transformation	Amplification fragment	Primer	Sequence 5'→3'	Ta °C
H2A.Z Deletion	in yeast	H2A.Z upstream*	3r-3UTR-H2AZ-R	GCGGATAACAATTTCACACAGGAAACAGCAGCTTGATGAAGAAATCCCATGG	63*
			3UTR-H2AZ-HY-F	GGACACCGGAGACAGGGCGGCCGAGGAATCGAAGTGGCAGAA	
		H2A.Z downstream*	5UTR-H2AZ-GRO-R	GCGTTTCGGGTTTACCTCTTCTAGCCTATCGGGGTTTAGTTTTGCT	60*
			5f-UTR-H2AZ-F	GTAACGCCAGGGTTTTCCCAGTCACGACGGAAATAACACAGCAGCGCCT	
		Hygromycin^{R}	neoHY-8-finF	CTAGAAGAGGTAAACCCGAAACG	68
			neoHY-1-debutR	GGCCGCCCTGTCTCCGGTGTCCCT	
	in *F. graminearum* wild type strains	Overlapping fragment 1	3UTR-H2AZ-N-R	CCACAGCGGGACATGATCAT	64
			NP_SplitHY_R	TGCCAGTGATACACATGGGG	
		Overlapping fragment 2	NP_SplitGRO_F	TCTGCAACCAAATCCACCCT	66
			5UTR-H2AZ-N-F	TTCATGAACAGACCCGACGA	
H2A.Z Complementation	in yeast	H2A.Z upstream +H2A.Z	Y-5F_F1	AACGCCAGGGTTTTCCCAGTCACGACGCACTTCTCGAACTCCTCCGA	66
			H2AZ-TtrpC_R	TGACATGGAGCTATTAAATCACTATTAACCCTCGAGCGCCTTGG	
		H2A.Z downstream	3F-GenR_F	AGGGTAGCCACGATTCGAAGAGAACAGGTCTCCTATC	62
			Y-3F_R	GGATAACAATTTCACACAGGAAACAGCAGCGATGTCGGCAAACTTCCTCT	
		TtrpC*	TtrpC-H2AZ_F	CCAAGGCGCTCGAGGGTTAATAGTGATTTAATAGCTCCATGTCA	60*
			TtrpC-GenR_F	CTAGATATACCTGTCAGACACTCAAGAGCGGATTCCTCAGTCT	
		Genticin^{R}	GenR-TtrpC_F	AGACTGAGGAATCCGCTCTTGAGTGTCTGACAGGTATATCTAGC	72
			GenR-3F_R	GATAGGAGACCTGTTCTCTTCGAATCGTGGCTACCCT	
	in *F. graminearum* H2A.Z-deleted strain	Overlapping fragment 1	Y-5F_F1	AACGCCAGGGTTTTCCCAGTCACGACGCACTTCTCGAACTCCTCCGA	68
			MM_SplitET_F	TTGGGTGGAGAGGCTATTCG	
		Overlapping fragment 2	MM_SplitGEN_R	GAATCCAGAAAAGCGGCCAT	65
			Y-3F_R	GGATAACAATTTCACACAGGAAACAGCAGCGATGTCGGCAAACTTCCTCT	

Chapter 3

Results Part 2: Side Project

Foreword

To investigate the roles of other epigenetic marks in *F. graminearum*, I participated to a side project aiming at understanding the roles of HP1, DIM2, and DIM5 (KMT1) in *Fusarium*, which are involved in the establishment and maintenance of heterochromatin in *Neurospora crassa* (Rountree and Selker 2010). Combining our findings with parallel results obtained from our collaboration lab in Vienna (L. Studt, BOKU, Austria), we found that strong epistatic relationships modulate the roles of these proteins.

3.1 Materials and Methods

Preliminary remark: *all media are presented in the research paper in Chapter 2 and their composition and specifics for culturing will not be repeated here.*

3.1.1 Fungal strains

The side project was carried out on mutants previously created in the lab including FgΔ*Dim5*, FgΔ*Dim2* and FgΔHP1 **(Silou, Master Thesis, 2014; Table S1)**. The method (split-marker approach) used for the creation of mutants (replacement of coding sequence with hygromycin-resistance cassette) was similar to the one used to delete H2A.Z in *F. graminearum* **(see Figure S1 in the Research Paper in Chapter 2)**.

3.1.2 Extraction of total RNA

Samples for total RNA extraction were isolated from 5-day old MS culture by vacuum filtration (Supelco VisiPrep system). Mycelia were rinsed twice with sterile distilled water and immediately flash frozen in liquid nitrogen. Samples were stored at -80°C until further use. About 20 mg frozen mycelium were grinded for 2*25s at 6500 rpm and a pause of five seconds between cycles at a temperature between 0°C and 4°C with a Precellys/Cryolis (Bertin), in the presence of TRIzol 1 mL and ø0.45-0.5mm glass beads. After incubation at room temperature for 5 min, beads and cell debris were pelleted by centrifugation at 16,100 g for 15 min at 4°C. The supernatant was extracted with 200 µL of chloroform (pH = 5). An additional centrifugation was performed at 4°C and 16,100 g for 15 min, followed by the recovery of the supernatant in a clean tube. One volume of isopropanol was used to precipitate the RNA with the help of centrifugation at 4°C and 16,100 g for 10 min. After two washes with freshly prepared 80 % ethanol, RNA pellet was air-dried then dissolved in 70 µl nuclease-free water. The integrity of the extracted RNA was verified on a 2 % agarose gel (e-gel EX invitrogen) after quantification by UV spectrophotometry. All samples were submitted to DNAse treatment according to the manufacturer's instructions (Ambion, cat#1906). To verify the absence of residual gDNA, qPCR targeting the housekeeping gene *β-tubulin* that contains an intron was performed using the QuantiFast SYBR® Green PCR Kit (QIAGEN, Vento, Netherlands). Briefly, reactions were carried out in a total volume of 10 µL containing RNA 2

ng/µL, 1 µM each of the forward and reverse primers, and 1×QuantiFast SYBR Green Master Mix. Amplifications were performed on a Roche® LightCycler® 2.0 thermocycler using the following cycling conditions: [Pre-incubation 95°C-5min, Amplification 45× (95°C-10s, 58°C-40s), Melting curve 65°C-15s, Cooling 4°C-30s]. If residual gDNA was detected, additional DNAse treatment would be performed. Total RNA samples were stored at -80°C until use.

3.1.3 Preparation of first-strand cDNA

Total RNA was reverse-transcribed using the SuperScript® IV First Strand Synthesis System kit (Invitrogen®) according to the supplier's recommendations. cDNA was synthesized in a 21 µL reaction containing 50 ng/µL RNA, 50 µM oligo$(dT)_{20}$ primer, 10mM dNTP mix, 5X SSIV Buffer, 0.1M DTT, RNaseOUT™ 40U/µL, and SSIV RT 200U transcriptase. The steps of the reverse transcription are summarized in **Table S2**. Once the program was completed, RNase H (Invitrogen®) was added to degrade the RNA/cDNA heteroduplexes. The cDNAs produced were single stranded and short-term stored at 4°C until use.

3.1.4 Quantitative PCR

The qPCR assays were performed with the QuantiFast SYBR® Green PCR Kit (QIAGEN, Vento, Netherlands) according to the supplier's recommendations (cDNA=5ng RNA equivalent). The analyzes were carried out on a Roche® LightCycler® 480 system equipped with lightcycler® software 480 software 1.5.1.61 (Roche®, Germany) using the following cycling conditions: [Pre-incubation 95°C-5min, Amplification 45× (95°C-10s, Ta-40s), Melting curve 65°C-15s, Cooling 4°C-30s]. For each gene, an external calibration curve was made using a pool of cDNA samples with a dilution gradient of 20 ng, 2 ng, 0.2 ng, 0.02 ng, 0.002 ng of equivalent RNA. The slope of each calibration curve was used as reaction efficiency of the corresponding gene. The summary of the target genes were listed in **Table S3**. Each sample was set up in triplicates. Quantitative analysis was made on the Roche Light Cycler Quantification software using the ΔΔCt method.

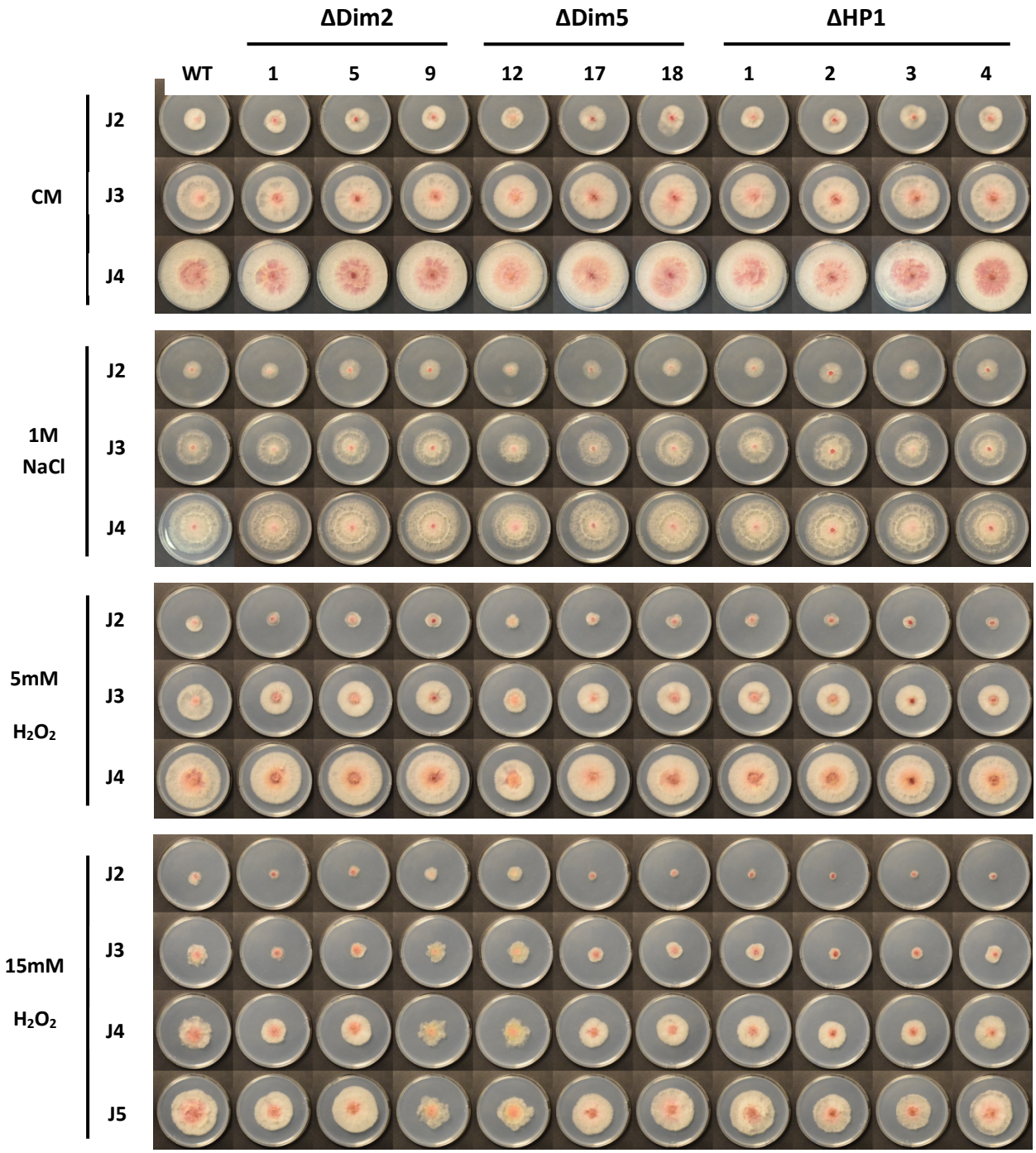


Figure 18. Comparison of radial growth between INRA605 wild-type strain and mutants under standard condition (CM), osmotic stress (CM+1M NaCl) and oxidative stress (CM+5mM/15mM H_2O_2). A 5mm diameter plug of each sample were incubated on a petri dish for up to 5 days at 25°C in the dark.

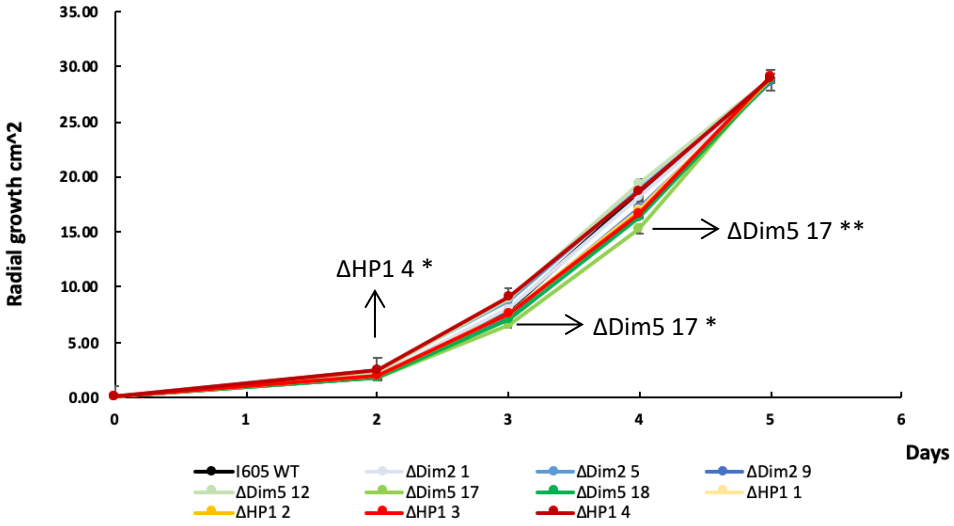


Figure 19 Radial growth curve of INRA605 wild-type strain and mutants under standard condition (CM). 100 spores of each sample were incubated on a petri dish for up to 5 days at 25°C in the dark. Bars denote standard errors from three repeats. P-value <0.05 *, p-value <0.01 ** (student's t-test).

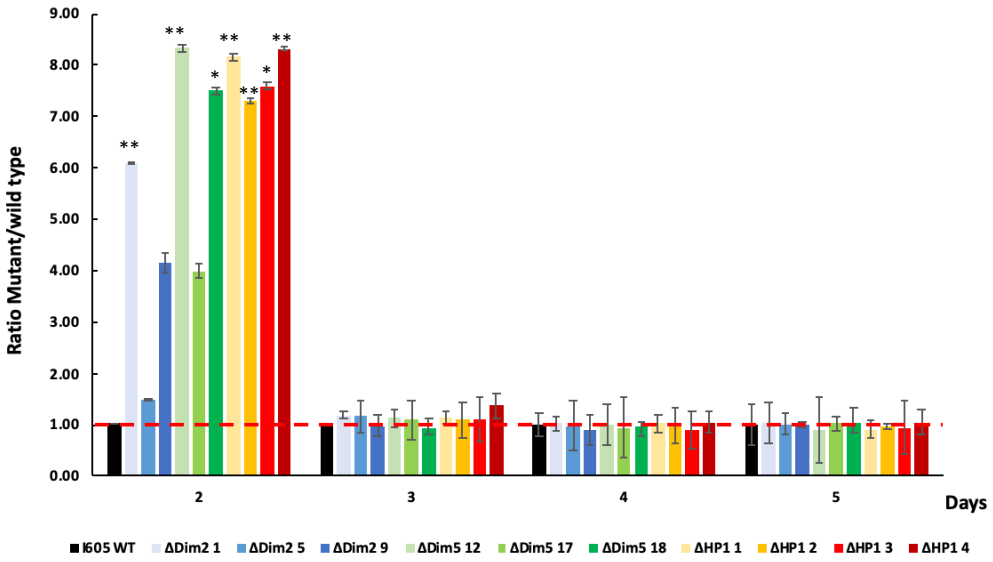
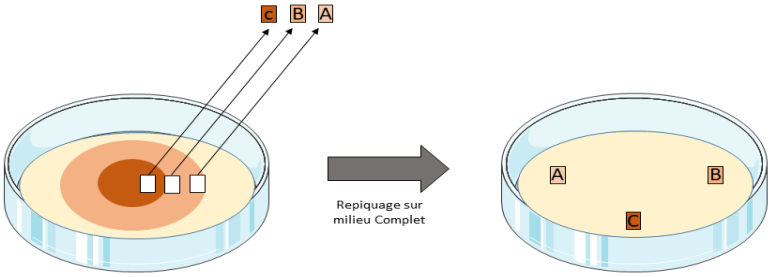


Figure 20. Ratio of radial growth between mutant and WT under osmotic stress caused by 1M NaCl on CM when inoculated with 100 spores. Each bar represents the quotient of the mean of the biological triplicate of the mutants on the wild type strain. The reference is the WT (=1). P-value <0.05 *, p-value <0.01 ** (student's t-test).

A



B

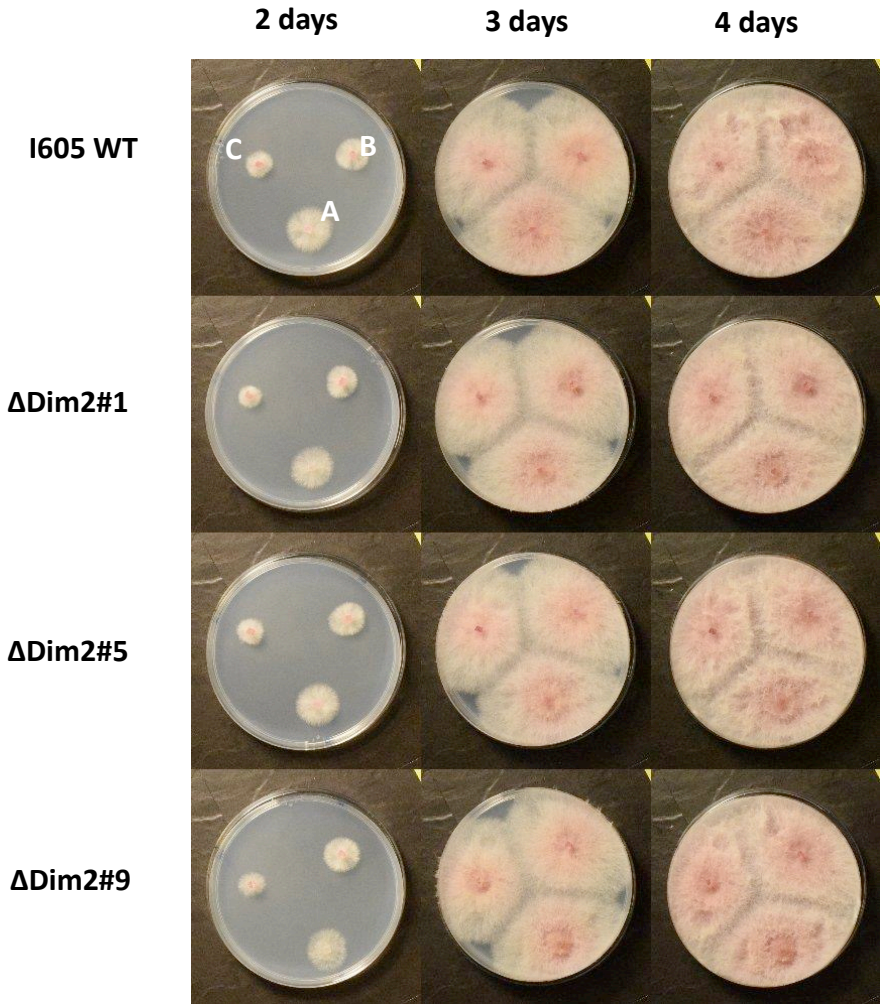


Figure 21. Kinetics of growth recovery in CM medium of INRA605 wild-type strain and ΔDim2 mutants. **(A)** Methods to measure the recover ability of mycelium with different age; **(B)** No significant difference of growth between the WT strain and mutants can be observed. Identical results were obtained for other mutants.

3.2 Results

3.2.1 Radial growth

To evaluate the effect of Dim2p, Dim5p, and HP1 deletion on the radial growth of *F. graminearum*, 100 spores of each sample were cultivated on CM for up to 5 days at 25°C in the dark. Pictures were taken every 24 hours and the growing surfaces were measured with ImageJ 1.x software (Schneider, Rasband, and Eliceiri 2012). Growth was detected after two days post-inoculation. In a general manner, no significant difference could be observed between wild-type and mutants, with the exception of ΔHP1#4 and ΔDim5#17. The former exhibited an early growth slightly faster than wild type (2 dpi; *p*-value < 0.05), while the growth of ΔDim5#17 at the third (p-value<0.05) and forth (p-value<0.01) day was slightly but significantly slower than wild type **(Figure 18 and Figure 19)**. We hypothesize that the different growth speed may be due to a faster / slower mycelium extension or germination rate. However, when we inoculated the samples with implants to test mycelium growth, there was no significant difference between wild-type and mutants (data not shown). Further experiment will be carried out to compare germination rates. Meanwhile, we observed an earlier pigmentation in the mutants **(Figure 18)**. This observation is somehow in line with a previous study, where expression of AUR gene required for the production of the pigment aurofusarin was greatly enhanced in HP1-deleted mutants of *F. graminearum* (Reyes-Dominguez et al. 2012).

Spores and implants of ΔDim2, ΔDim5, ΔHP1 mutant were also cultivated on CM supplemented with NaCl 1M to test their ability to respond to osmotic stress. When inoculated with spores, there was significant early promotion of growth in mutants compared to wild-type on the second day, especially in ΔHP1. Faster growth can also be observed in the mutants from implants at the same time point, however, none was significant. This may result from a less sensitivity of spores or a faster growth recovery of mycelium to osmotic stress at the beginning of inoculation. Subsequently, there was no difference of radial growth between wild type and mutants **(Figure 18 and Figure 20)**.

In the presence of oxidative stress caused by 5 mM or 15 mM H_2O_2, no spore from any strain was not able to grow at any of the two concentrations used in both mutants and wild type strain (data not shown), while there was growth when inoculated from implants. In the latter case, at the concentration of 5 mM, we did not observe any significant difference in mutants compared to wild-type, except for ΔDim5#12 that showed higher sensitivity. At the

concentration of 15 mM, mutants seemed more sensitive than wild-type strain (data not shown). However, due to the contamination of some petri dishes, the measurement should be re-done before drawing conclusions.

3.2.2 Growth restart tests

To analyze the capacity of mycelium to restart growth, three implants of each strain with different ages were inoculated for up to 4 days (see the modalities described in the Research Paper). For both wild-type and mutants, the growth of the youngest mycelium was the fastest, while the oldest mycelium grew the slowest. However, no significant different between wild-type and the ΔHP1, ΔDim2, ΔDim5 mutants can be observed **(Figure 21)**. Therefore, higher growth rates in CM+ 1M NaCl was not due to a faster growth recovery, but lower sensitivity to osmotic stress at the beginning of incubation.

3.2.3 Asexual reproduction: conidiation

To determine whether the ability to produce asexual spore is affected by deletion of *Dim2*, *Dim5* or *HP1* in *F. graminearum*, condiation assays were carried out. For each mutant, an 8 mm-diameter plug was incubated in CMC medium for up to 10 days at 25°C and 180 rpm in the dark. Conidia were counted daily with a Thoma cell counting chamber, under microscope. As depicted in **Figure 22**, the production of conidia reached a maximum level for both wild-type and mutants in nine days. At the beginning of the kinetics, there was is a slight inhibition of conidiation in mutants lacking *Dim2*. After four days, the mutant ΔDim2#1 begun to produce more conidia than wild-type, with a significant difference at the age of 8 days. By contrast, conidiation was significantly inhibited at the later stage of incubation (8-10 days) in mutants ΔDim2#5 and ΔDim2#9. In ΔDim5#12 and ΔDim5#18, the level of conidiation was similar with wild-type. However, a significant inhibition could be observed in ΔDim5#17, with a *p*-value lower than 0.01 and 0.001 at the age of 8 days and 9 days, respectively. Absence of HP1 in the four mutants had no effect on the ability of conidiation. Parallel experiment has been performed in our collaborative lab in Vienna for ΔDim5#12, ΔDim5#17 and ΔHP1, but with different condition (MBS media, 20°C and 140rpm).

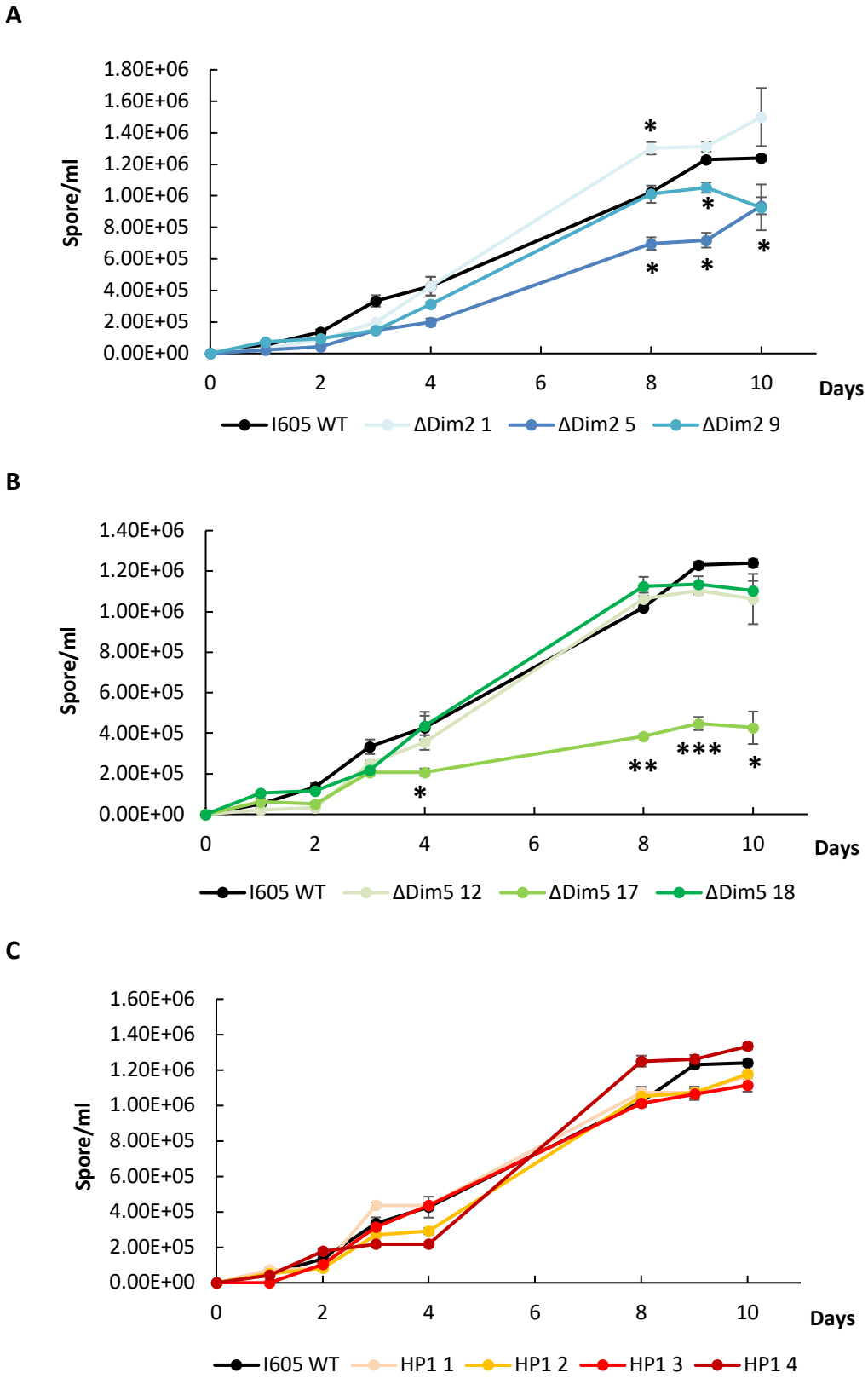


Figure 22. Comparison of sporulation between INRA605 wild-type strain and ΔDim2 **(A),** ΔDim2 **(B),** ΔHP1 **(C)** mutants. For each sample, a 5mm implant was incubated in 10 mL of CMC liquid medium for up to 10 days at 25°C and 180rpm in the dark. Spores were counted by Thoma under microscope every day. Bars denote standard errors from three repeats. P-value<0.05 *, p-value<0.01 **, p-value<0.001 *** (student's t-test).

Results indicated that three days after inoculation, there was no significant difference between wild-type and mutants, which is similar with our observation. As they did not prolong the time of incubation, we could not compare the later stage. Meanwhile, they measured the germination rate of INRA605 wild-type and ΔHP1 1 (liquid fusarium complete media, at 20°C and 180 rpm for 8 hours). Compared to wild-type, deletion of HP1 led to a reduction of germination rate of 15 % (A.K. Atanasoff-Kardjalieff, personal communication).

3.2.4 Sexual reproduction

The sexual cycle of *F. graminearum* is considered as the primarily inoculum for the infection of plants. Additionally, *F. graminearum* is homothallic and thus perithecia can be formed without the need of outcrosses (Leslie and Summerell 2006). To see whether Dim2, Dim5 and HP1 contribute to *F. graminearum* sexual development , the INRA605 wild type strain and all the mutants were grown on carrot agar at 25°C under white light to induce the formation of perithecia. Four days after treatment of 2.5% TWEEN® 60, perithecia of the wild type and all the mutants could be observed on petri dishes, accompanied by the production of red pigment. Under microscope, we found that per field of observation, the studied mutants had more perithecia than the wild type, with the exception of ΔHP1#4 which produced less (**Figure 23,** visual evaluation). However, mutants produced more perithecia also produced a great number of micro-perithecia, which may be delayed in maturation and unable to make or discharge ascospores. Therefore, it may also lead to a less effective sexual reproduction. Further experiment will be designed and carried out to measure the ability of discharge and activity of the ascospores.

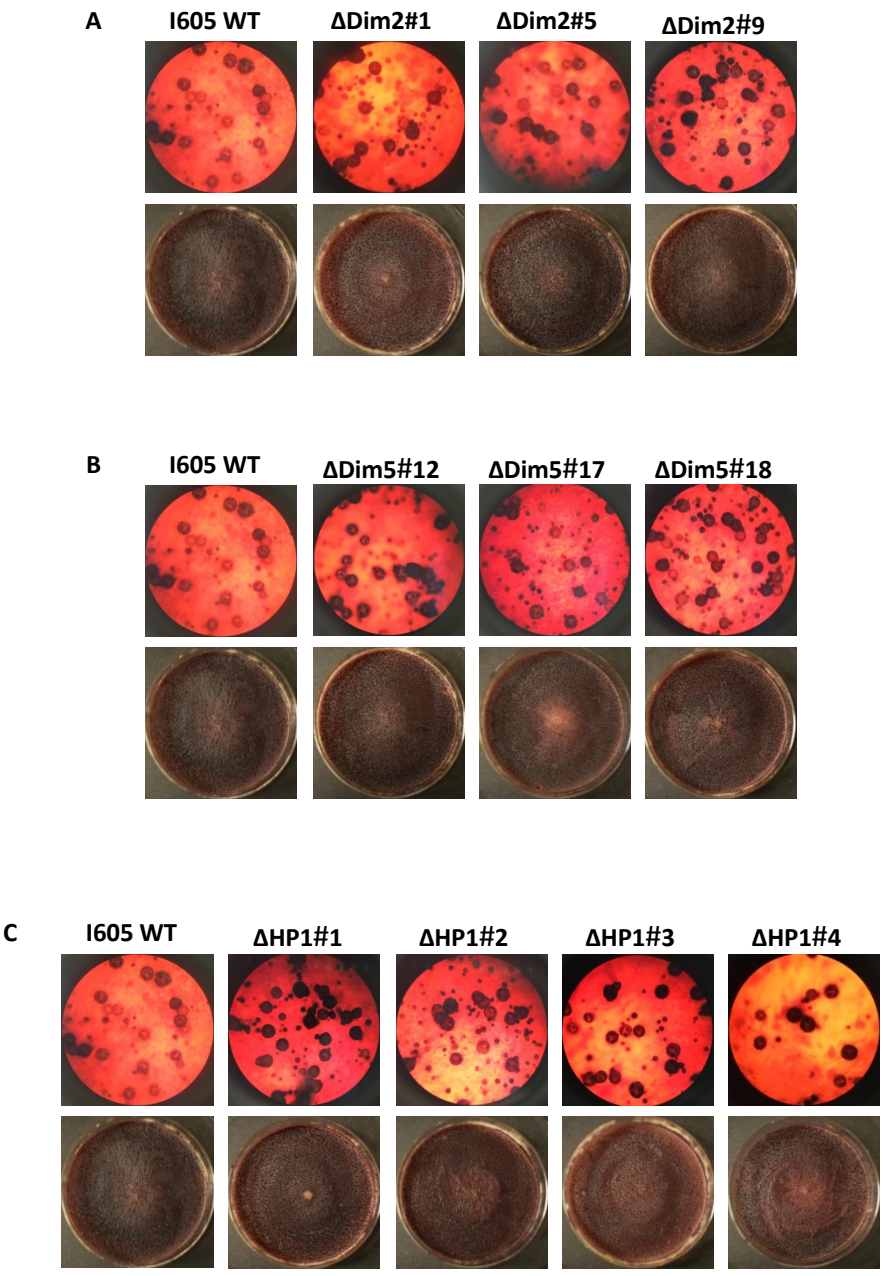


Figure 23 Perithecium formation by INRA605 wild type strains, its Dim2 deleted **(A),** Dim5 deleted **(B)** and HP1 deleted mutants under microscope and on carrot agar. Pictures were taken after 4 days of 2.5% TWEEN® 60 **treatment**

3.2.5 DON/15-ADON production

100 spores of wild type and mutants were cultivated in 8 ml MS liquid culture at 25°C in the dark. TCTB was extracted by acetyl acetate and analyzed by HPLC-DAD at three time points: 5, 10, 15 days after inoculation. There was no significant difference of biomass accumulation between the wild type and mutants (data not shown). The production of DON and 15-ADON was significantly inhibited in all mutants compared to wild-type strain (*p*-value<0.001 at the age of 10 days and 15 days), particularly the mutants without HP1 **(Figure 24)**.

To go further, total RNA was extracted from 5-day old mycelium and RT-qPCR was performed with several targeted genes involved in SM biosynthesis including *Tri4*, *AurO* and *AurF*. Two housekeeping gene β-tubulin and ef1α were used to normalize the results of qPCR. **Figure 25** represents the ratio of gene expression in mutants to the wild type. If the ratio is equal to 1, there is no difference of expression between wild type and mutants; If the ratio is lower or higher than 1, the gene expression in the mutants is inhibited or overexpressed, respectively. As indicated in the figure, the mean expression of Tri4 gene was less than halved in both Dim2, Dim5 or HP1 deleted mutants compared to the wild type as the ratio is lower than 0.5, which is in agreement with the results of HPLC-DAD. However, a significant difference can only be observed in ΔDim2 (P-value<0.05). It may be due to the larger variance between the replications in ΔDim5 and ΔHP1.

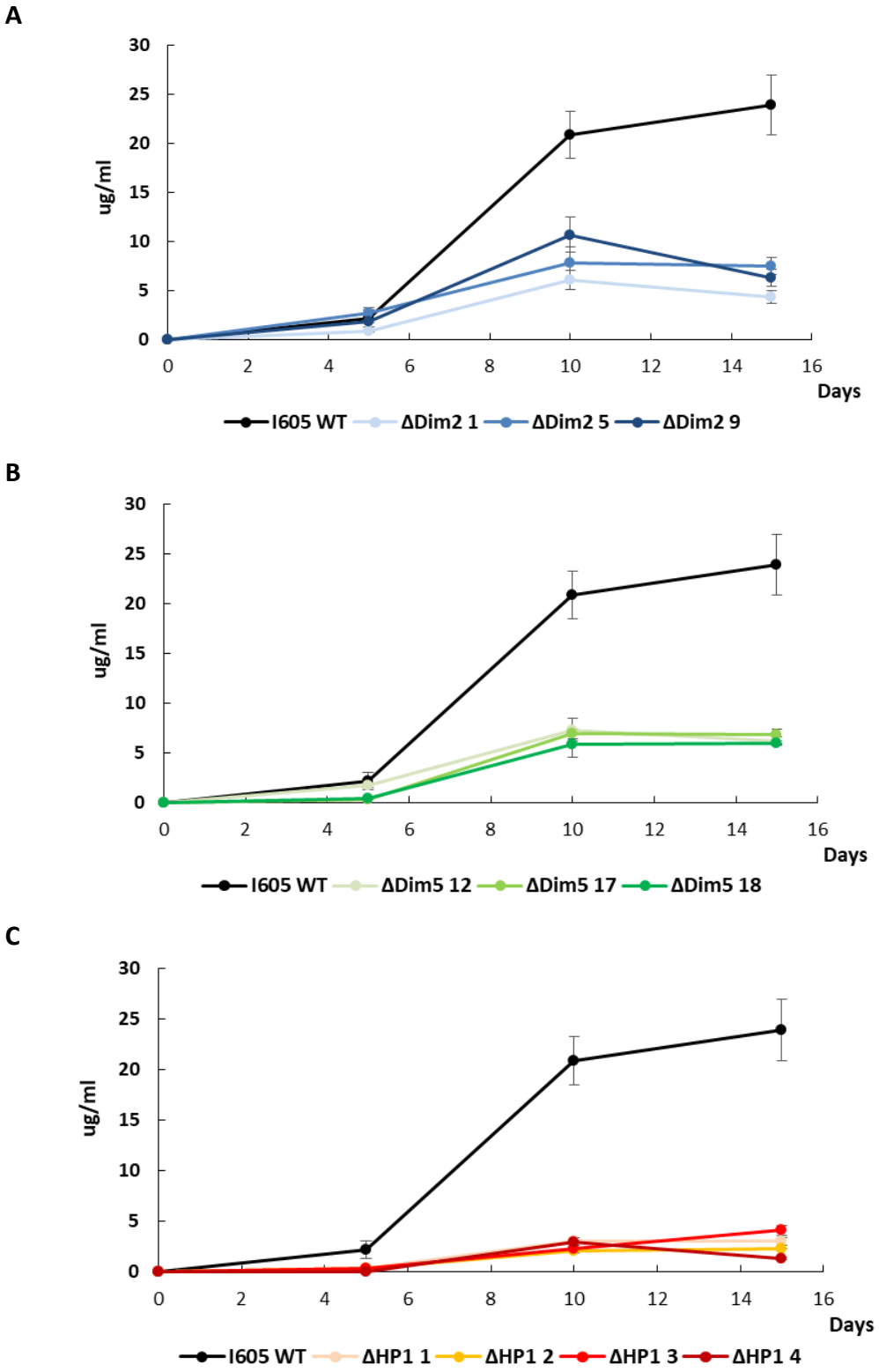


Figure 24. Production of DON+15-ADON in INRA605 wild-type strain and mutants. 1E+06 spores were inoculated in MS glucose liquid medium for up to 15 days at 25°C in the dark. TCTB were extracted and measured by ethyl acetate and HPLC-DAD at the age of 5, 10 and 15 days respectively. The black bars represent the standard errors from three repeats. At the age of 10 and 15 days, significant difference can be observed in all the mutants compared to the wild type (p-value<0.001).

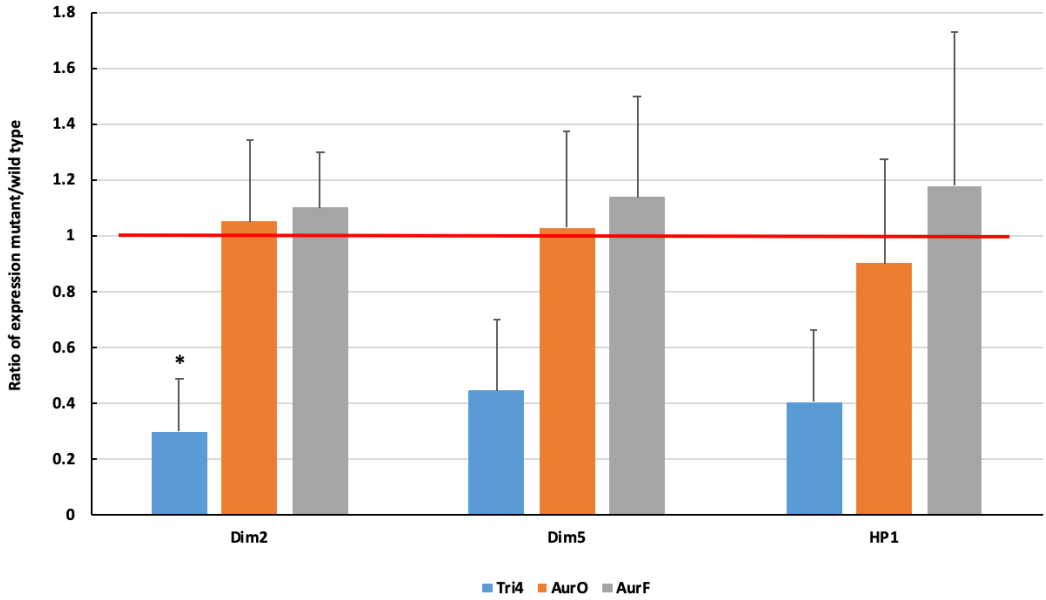


Figure 25. Ratio of the expression of target genes (*FgTri4, FgAurO, FgAurF*) in mutants (FgΔDim2, FgΔDim5, FgΔHP1) to the expression in wild type. Ratio=1 (red line) means the expression is the same in mutants and wild type; ratio > 1 means the target genes are overexpressed in mutants; ratio < 1 means the expression is inhibited in the mutants. The asterisk indicates a significant difference (P <0.05, student's t-test), and the black bars represent the standard errors.

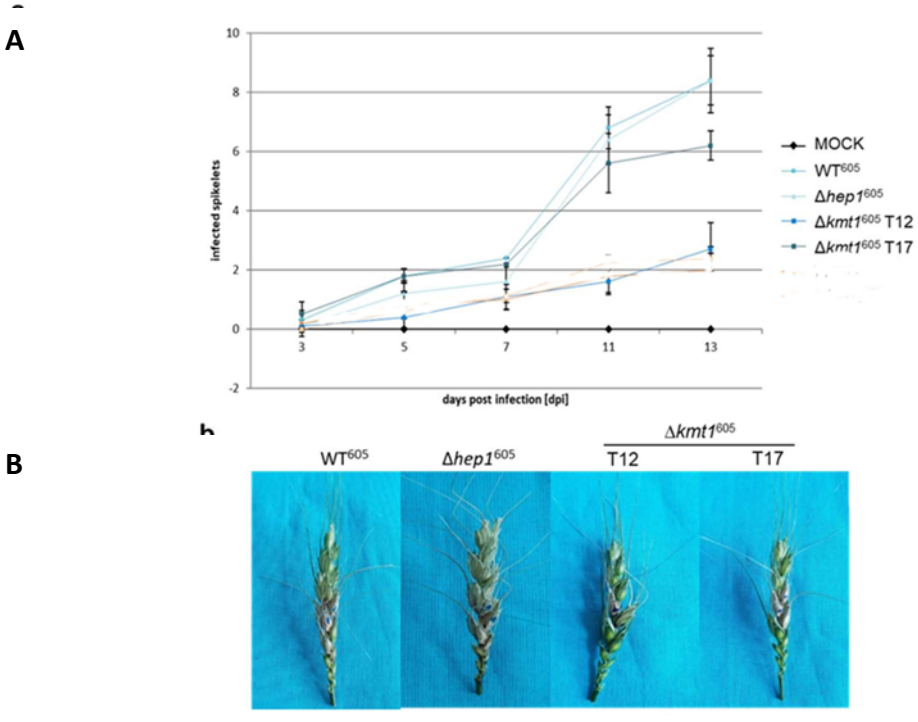


Figure 26 *In planta* study to measure the virulence of strains INRA605 WT, ΔHP1#1, ΔDim5#12 and ΔDim5#17. **(A)** Number of ears infected by each strain after 13 days of inoculation. MOCK is the negative control; **(B)** Symptoms of the wheat ear after 13 days of infection by different strains.

AurO and *AurF* are genes involved in the biosynthesis pathway of the pigment aurofusarin, which gives an orange/red color to *Fusarium*. It is a homodimeric naphthoquinone and is often associated with naturally infected wheat where the compound can occur in high levels (Westphal et al. 2018). In contrast to *Tri4*, expression of *AurO* and *AurF* increased in ΔDim2 and ΔDim5 even though not significant difference. Meanwhile, deletion of HP1 in *F. graminearum* led to a slight down-regulation of *AurO* and an up-regulation of *AurF*.

3.3 Discussion

Heterochromatic domains are transcriptionally silent and characterized by hypoacetylation and hypermethylation of lysines in H3 and H4 (Holbert and Marmorstein 2005). The histone marker H3K9me3 is mediated by the histone methyltransferase Dim5. It can be recognized by one of the principal component of heterochromatin HP1 through its chromodomain at the N-terminus which is necessary for transcriptional repression (Cryderman et al. 1998; Wang et al. 2000; Fanti and Pimpinelli 2008). In addition to the association with pericentric heterochromatin, HP1 is also found at many euchromatic sites where it can promote repression in a H3K9 methylation-dependent or independent process. This system is conserved in numerous organisms, including filamentous fungi such as *Neurospora crassa*, where it has been shown to direct DNA methylation (Freitag et al. 2004; Selker 2004; Reyes-Dominguez et al. 2010). In *F. graminearum*, DNA methylation level is lower than 1 % (N. Ponts, personal communication). Meanwhile, according to the previous study, it seems that SM gene clusters are primarily silenced by the H3K27me3 marker (Connolly, Smith, and Freitag 2013). However, the mechanisms and roles for the establishment and maintenance of constitutive heterochromatin remain unclear.

Here, we evaluated the influence of HP1, Dim5p as well as the DNA methyltransferase Dim2 deletion in the *F. graminearum* wild-type strain INRA605. Results indicated that absence of HP1, Dim2 or Dim5 did not affect the radial growth of *F. graminearum* under the standard condition. Under the osmotic stress caused by 1M NaCl, a significant faster growth in all the mutants can be observed at the first day of inoculation with spores. When inoculated with plugs, similar but slight faster also observed in the mutants. As the recovery ability of implants between wild type and mutants were identical, the promotion of radial growth in mutants may be due to the less sensitivity to osmotic stress at the beginning of inoculation. According to the previous study, the high osmolarity glycerol (HOG) response pathway is

required for growth under hyperosmotic conditions. Hog1 encodes a mitogen-activated protein kinase involved in osmoregulation, deletion of Hog1 as well as other two related genes Pbs2 and Ssk2 led to a slower hyphal growth, loss of female fertility and failure in accumulating compatible solutes in response to NaCl treatment in *F. graminearum* (Zheng et al. 2012). Absence of HP1, Dim2 and Dim5 would have a positive effect on the expression of these genes, accelerating the growth on the first day of culture. A threshold of the ability of HOG pathway may exists to inhibit the faster growth at the later stage, as there was no difference of sensitivity between the wild type and mutants in response to osmotic stress.

Both wild type and mutants have the ability of sexual reproduction. Even though mutants produced more perithecia, but they were mainly composed of microperithecia, which may lead to a delay of perithecia maturation and furthermore, a lack of ascospore production and discharge.

Remarkably, deletion of HP1, Dim2 and Dim5 has a significant negative influence on the production of DON and 15-ADON but a positive influence on aurofusarin biosynthesis in strain I605. This is consistent with the study carried out by (Reyes-Dominguez et al. 2012). However, a reverse phenotype was observed in I812-ΔHP1 (experiment was performed in our collaborative lab in Vienna) and I171-ΔHP1, where HP1 probably functions as a positive regulator for SMs in I812 and I171 wild type, meaning that effects of HP1 on SMs profiles vary between genetic backgrounds (N. Ponts and J. Strauss, personal communication). It might be due to the existence of epistatic interactions (Schuldiner *et al*. 2005; Snitkin and Segrè 2011; Storz 2018) or transposable elements (Shaaban *et al.* 2010).
(See details in Chapter 4, Section 4.4)

3.4 Supplemental data

Table S1. *F.graminearum* strains used in this study including INRA605 wild types strain, 3 Dim2-deleted mutants, 3 Dim5-deleted mutants and 4 HP1-deleted mutants.

Wild type strain	Mutant	No.
INRA605	ΔFgDim2	1
		5
		9
	ΔFgDim5	12
		17
		18
	ΔFgHP1	1
		2
		3
		4

Table S2. Reverse transcription program used for the synthesis of first-strand cDNA**.**

Step	Time	Temperature	Number of cycle
Anneal primer to template RNA	5 minutes	65°C	1×
Incubation of the reverse transcriptase	5 minutes	50°C	
Inactivation of the reverse transcriptase	10 minutes	80°C	
Incubation of RNase H	20 minutes	37°C	

Table S3. Target genes and sequences of primers used for qPCR. **Ta:** annealing temperature of qPCR.

Target gene	Primer	Sequence 5'→3'	Ta °C
β-tubulin	β-tub261F	GGTAACCAAATCGGTGCTGCTTT	58
	β-tub536R	GATTGACCGAAACGAAGTTG	
Histone 3	FgH3-F	AAGAAGCCTCACCGCTACAA	60
	FgH3-R	TCGAAGAAGGAGACGAGGTA	
Ef1α	Ef1A-F	TGTCTACAAGATTGGCGG	61
	Ef1A-R	CTTGCAGGCAATGTGG	
Citrate Synthase	FgCIT-F	GGCTCACCGAGTTCAAGAAG	60
	FgCIT-R	CTTCTCTTGGGCAAAAGTGC	
Tri4	EZ-TRI4-F2	TTGAGCTTTATGACACCACCA	60
	EZ-TRI4-R2	ACAAAGCCTTGAGAACCTTGAC	
AurO	Auro-F	GTCGGCCCACTTCGGTCGCTCAA	55
	Auro-R	GCGCCATTGCTGCTTCTTT	
AurF	Aur-F-F	CCCTGGGCCTTGTAACTCTCA	55
	Aur-F-R	GCGCCATTGCTGCTTCTTT	

Chapter 4
Discussion

Hunger and malnutrition are pervasive problems that affect millions of people in the world, especially in developing countries. In the next 30 years, the global population is projected to rise from about 7 billion to 9.2 billion, demanding a 60 percent increase in global food production (FAO and Collette 2011). Mycotoxins are toxic SM of fungi belonging, essentially, to the *Aspergillus*, *Penicillium* and *Fusarium* genera, which can be produced on a wide range of agricultural commodities and under a diverse range of situations worldwide thus limiting food and feed production and, representing a significant risk to human and animal health (Robens and Cardwell 2003; Wu 2007; Bryden 2012). According to the FAO, around 50 % of worldwide crop harvests may be contaminated with mycotoxins (FAO and Collette 2011). On account of global climate changes, this situation may become increasingly serious (Medina *et al.* 2017). Therefore, sustainable ways to limit fungal diseases on plants and contamination of food with mycotoxins is a key step in defeating hunger and ensure food safety.

In Europe, nearly half of the food derived from cereals and unprocessed grains collected between 2007 and 2012 in 21 countries were contaminated with Type B trichothecenes, or TCTB, and especially deoxynivalenol, or DON (EFSA, 2013). In Europe, DON is predominantly produced by the fungal phytopathogen *F. graminearum* on growing kernels of barley and wheat, before harvest. It is the primary causal agent of FHB, which is considered as one of the most devastating plant disease (Windels 2000; Gilbert and Haber 2013). Chemical treatment has been used as the most common and effective means for the inhibition of *F. graminearum* growth in fields and removal of mycotoxins from contaminated commodities. However, excessive use and misuse of agrochemicals is efficiency limited and not eco-friendly (Kn *et al.* 2012). Thus, alternative approaches are needed to guaranty good quality of environment and longer exploitation of agricultural lands. In this context, it is crucial to understand the molecular mechanisms underlying the production of mycotoxins in fungi. In *F. graminearum*, the various steps of the TCTB biosynthetic pathway have been well described and involve so-called *Tri* genes. Many factors influence TCTB production potentially implying the intervention of various regulatory genes in response to environmental factors such as nutrient source, pH and light. However, the molecular events involved are indeed complex and remain, so far, widely misunderstood. More recently, it has also been proved that chromatin structure changes, through specific histone modifications, play a major role in the regulation of secondary metabolism in filamentous fungi. Therefore, my PhD project aims

at investigating epigenetic mechanisms that control SM biosyntheses in *F. graminearum*, with a focus on deciphering the roles of the evolutionary conserved histone variant H2A.Z.

4.1 H2A.Z is essential in *F. graminearum*

With around 80% amino acid sequence identity within different organisms, H2A.Z is considered as the most conserved histone variant, making up 5% to 10% of the total H2A protein pool in most organisms examined to date. Generally, abundances of H2A.Z increase when cells exit the cell cycle and DNA replication stops, such as during development (Piña and Suau, 1987). Even though controversies exist about the precise roles of H2A.Z, it has been shown to be associated with a variety of major biological processes, including transcription regulation, DNA repair, genomic integrity and so on. In mouse (Faast *et al.* 2001) and *Drosophila melanogaster* (van Daal and Elgin 1992), H2A.Z was shown to be essential. Up to now, the only one study targeted directly on the function of H2A.Z in filamentous fungi was carried out on *N. crassa*, in which lack of H2A.Z is not lethal and involved in response to oxidative stress (Dong *et al.* 2018).

To study the function of H2A.Z in *F. graminearum*, we decided to use a reverse genetics approach implementing a method called split-marker for creating mutants. In filamentous fungi, due to the low frequencies of homologous integration, targeted gene disruption can be troublesome (Bird and Bradshaw 1997; Idnurm *et al.* 2003). Split-marker approach was initially developed for rapid, gap repaired-mediated cloning in the budding yeast *S. cerevisiae* (Fairhead *et al.* 1996). Two truncated, overlapping selectable marker gene fragments are joined with sequences of interest, without the need for bacterial cloning using fusion PCR or yeast-mediated recombination for example, and the PCR products of fused sequences are used directly for fungal transformation, allowing a faster and more efficient method of generating disruption constructs (Fairhead *et al.* 1996). Only transformants in which the functional dominant marker gene replaces successfully the targeted gene will grow on a medium containing the selection agent (Chung and Lee 2015). Split-marker approach has been shown to largely increase the frequency of targeted gene disruption and homologous integration as high as 100% in *A. alternata* and *Cercospora* species, probably by decreasing the occurrence of multiple and tandem integrations (You, Lee, and Chung 2009; Lin *et al.*

2010; Chung and Lee 2015). After optimization, more recently, it has become a mainstream method for gene function analysis in *Fusarium* species with high efficiency (Liang *et al.* 2014). Indeed, in our lab, we do it routinely and successful mutants can be obtained in 2 weeks. There is usually no problem, and since removal of H2A.Z is not lethal in yeast and *Neurospora*, we did not expect to have problems there. We thus confidently began with the replacement of the whole H2A.Z locus in *F. graminearum* strain I349 by hygromycin B resistance gene, a selectable marker which is widely used for fungal transformation (Punt and van den Hondel 1992). Compared with previous work on knocking out of other genes in *F. graminearum* by split-marker in our lab, deletion of H2A.Z was much tougher, reflecting that it may be involved in certain central biological processes. To see whether the efficiency of perturbation depends on different genetic backgrounds, another three strains of *F. graminearum* were used for the construction of H2A.Z deleted mutants: I156, I812 and I605. After validation by PCR and southern blot, eventually, six deletion mutants were obtained in total, among which four were derived from I156, one was from I349 and another one was from I812. Two H2A.Z deleted mutants of I605 have also been obtained, however, off-targets can be detected according to the results of southern blot. Therefore, we did not use them for further study.

All mutants exhibit important deficiencies in sporulation, germination, radial growth and DON production. However, intensities in the observed effects strongly depend on the considered individuals **(Table 3).** For example, comparing to wild type, the radial growth of I156ΔH2A.Z#3 is inhibited much more than I156ΔH2A.Z#1, I156ΔH2A.Z#2 and I156ΔH2A.Z#4, whose phenotype should be theoretically the same. There is a significant promotion of DON production in I156ΔH2A.Z#1 and I156ΔH2A.Z#2, while similar with the wild type, I156ΔH2A.Z#3 and I156ΔH2A.Z#4 almost stopped to produce DON anymore.

We attempted to add back wild type H2A.Z in ΔH2A.Z mutant and found it was unexpectedly difficult, but we insisted to do it in the background of I1349ΔH2A.Z#1. Finally, two H2A.Z-adding back mutants were obtained. However, it could not rescue the phenotypes of I1349ΔH2A.Z#1. Therefore, we investigated in more depth the genotypes of our mutants. Whole genome sequencing (WGS) showed that, although H2A.Z has been totally removed from the genome and the deletion cassette had been inserted at H2A.Z *locus* only,

compensatory mutations occur at other *loci* in each mutant regardless of the considered genetic background (see **Table 1** of the Research Paper in Chapter 2). Strikingly, one extra mutation was detected in the H2A.Z add-back mutants. Therefore, we draw the conclusion that H2A.Z is essential in *F. graminearum*. It is the occurrence of compensatory mutations that rescued part of the lethality caused by H2A.Z deletion.

Table 3. Summary of ΔH2A.Z phenotypes. **–** means there is a inhibition compared to wild type (**---**, dramatic; **--** moderate; **-** slight); **+** means there is a promotion compared to wild type (**++**, moderate; **+**, slight); = means the phenotype between mutant and wild type is similar; **nd** means data not determined.

		INRA156				INRA349	INRA812
		ΔH2A.Z#1	ΔH2A.Z#2	ΔH2A.Z#3	ΔH2A.Z#4	ΔH2A.Z	ΔH2A.Z
Sporulation		---	--	---	--	---	--
Germination		nd	nd	nd	nd	---	nd
Sexual reproduction		---	-	---	--	=	---
Radial growth	Standard condition	--	--	---	--	---	--
	Ability of resuming growth	-	=	=	=	-	=
	Osmotic stress	=	=	-	=	-	=
	Oxidative stress	+	=	=	-	+	=
DON/15-ADON production		++	+	--	--	--	--

4.2 Suppressions induced by deletion of H2A.Z

Eukaryotic cells show remarkable buffering systems, known as genetic robustness, to maintain normal developmental outcomes against external perturbations (Mather 1953; El-Brolosy and Stainier 2017).

Genetic robustness can be explained by two mechanisms in particular, **suppression** and **rewiring of genetic networks**.

- **Suppression**

Detrimental gene perturbations, particularly in plastic and rapidly proliferating organisms such as fungi, other genetic perturbations in one or more genes may occur modulating the affected pathway, thereby partially or fully compensating the final outcome. This phenomenon is referred to as suppression and the mutation being suppressed is usually called “query”. In model organisms, suppression interactions are generally divided into two classes: **(1) genomic suppressors** which are secondary mutations in the genome, or known as compensatory mutations, that bypass a mutant phenotype; **(2) dosage suppression**

interactions in which overexpression of a suppressor gene rescues a mutant phenotype (Tautz 1992; Zhang 2012; Teng *et al.* 2013; El-Brolosy and Stainier 2017; Jolanda van Leeuwen *et al.* 2017).

- **Rewiring of genetics networks**

Genetic networks include protein–protein interaction, metabolic, signaling and transcription-regulatory networks. Here, disruption of a particular gene's function in a network may affect the expression of other genes within the same network (Barabási and Oltvai 2004).

Results obtained during my PhD indicate that, in *F. graminearum*, deletion of the query gene H2A.Z is lethal unless mutations of genomic suppressors at other sites occur that rescue the phenotype. As indicated in **Table 1** of the Research Paper (see Chapter 2), all mutations occur in chromatin-involved genes. Besides, mutations in Rpd3s HDAC complex and the hypothetical protein FGRAMPH1_01G03975 are detected twice in two genetic backgrounds. There are two possibilities: (1) the occurrence of mutation is not random, it only happens in specific genes; (2) the occurrence is random, but there is a directional selection. Usually, the outcome of evolutionary rescue by suppressor mutation highly depends on the initial contexts in which the deleterious mutation occurs including the genotype, the environment, or a combination thereof (Filteau *et al.* 2015; Storz 2018). In our case, the deletion was performed on three *F. graminearum* strains with different genetic backgrounds, which may explain the diversity of mutants.

4.2.1 Suppressions by histone H3 and *Swr1* genes

Suppressor mutations are known to be more likely to cluster at sites close to the original deleterious mutation than others (Davis, Poon, and Whitlock 2009). Usually, query and suppressor genes encode functionally related members of the same protein complex, same pathway, or different, but related pathway (Jolanda van Leeuwen *et al.* 2017; Rojas Echenique *et al.* 2019). In our analysis, single amino acid exchange is detected in histone H3 and Swr1p in I156ΔH2A.Z#2, which are both tightly associated with chromatin structure.

As mentioned earlier, Swr1p is part of the SWR1 complex and contributes to the deposition of H2A.Z. In our mutant I156ΔH2A.Z#2, the leucine found at position 850, *i.e.,* within the SNF2 domain, in wild-type is missing. When we added back H2A.Z in I1349ΔH2A.Z#1, which already has a frameshift mutation in subunit of the histone deacetylase rpd3s, an additional

mutation occurs in Swr1. In yeast, SWR1-catalyzed H2A.Z deposition in *vitro* occurs in a stepwise fashion. Hyper-stimulation of ATPase activity of SWR1 complex requires the co-occurrence of H2A-H2B and H2A.Z-H2B free dimers on nucleosomes (Luk *et al.* 2010). Still in yeast, another two studies indicate that H2A.Z deletion is deleterious to cells. And many of the phenotypes of ΔH2A.Z are due to SWR1 complex's activity rather than to the absence of H2A.Z itself (Halley *et al.* 2010; Morillo-Huesca *et al.* 2010). Further experiments carried out by Morillo-Huesca *et al*. (2010) find that genetic instability, sensitivity to drugs impairing different cellular processes and genome-wide transcriptional mis-regulation caused by H2A.Z deletion in yeast can be partially or totally suppressed if SWR1 is not formed, if it forms but cannot bind to chromatin or if it binds to chromatin but lacks histone replacement activity (Morillo-Huesca *et al.* 2010). Taken together, we think that similar mechanism can be used to explain our results in *F. graminearum,* even though details of the mechanism still need further investigation. In I156ΔH2A.Z#2, the compensatory mutation happened in Swr1p destroys the activity of SWR1 complex and rescues partially the phenotype. In fact, construction of Swr1 deleted mutants is ongoing in our lab. Up to now, only one viable transformant was obtained, but it has not been validated by southern blot or WGS yet.

An additional single amino exchange at the N-terminus of H3 was also found in I156ΔH2A.Z#2. Experiments reveals that in vertebrates, when H2A.Z is paired with H3, it forms an unusually stable nucleosome, probably due to the acetylation of histones (Park et al. 2004; Thambirajah et al. 2006; C. Jin and Felsenfeld 2007). Thus, mutation in H3 and Swr1p may function together to compensate the nucleosome instability in I156ΔH2A.Z#2.

4.2.2 Suppressions by H2A.Z-related PTMs

Several compensatory mutations happen in genes encoding proteins related with histone PTMs, including frame shifts in essential subunit of the Rpd3s HDAC complex (I349ΔH2A.ZΔ#2 and I156ΔH2A.Z#4), histone demethylase Jarid1 (I156ΔH2A.Z#1) and single amino acid exchange in histone-lysine n-methyltransferase Ash1l (I156ΔH2A.Z#3). In plants, yeast and mammals, H2A.Z has been proved to be associated with various PTMs such as H3K4me3, H3K9me3 and H3K27me3 to alter the chromatin structure. In *Fusarium* species, recent studies indicated that absence of HP1 which is linked with H3K9me3 led to both up and down regulation of several secondary metabolic gene clusters including these involved in the DON production (Reyes-Dominguez *et al.* 2010; 2012). H3K27me3 histone mark

represses 14 % of its genome, including genes involved in secondary metabolic pathways (Connolly, Smith, and Freitag 2013). Similar with other species, we think H2A.Z may also collaborate with histone PTMs in *F. graminearum* to perform their functions. The frequent presences of suppressor mutations in genes encoding HDAC or enzymes related with histone methylation that offset the influence of H2A.Z deletion further confirm our hypothesis.

- **Mutation in Rpd3s HDAC complex**

Remarkably, frameshift mutation of Rpd3s is the single suppressor mutation found for both I349ΔH2A.Z#2 and I156ΔH2A.Z#4, while in other mutants there are two or more additional mutations. As showed in the research paper, the phenotype of I156ΔH2A.Z#4 was much closer to the I156 wild type than I156ΔH2A.Z#1, I156ΔH2A.Z#2 and I156ΔH2A.Z#3. Thereby, compensation by Rpd3s complex could be more efficient than others, indicating a particularly close relationship with H2A.Z. In yeast, SET2-methylated H3K36 can be recognized by Rpd3s HDAC complex within transcribed sequences. This erases transcription elongation-associated histone acetylation and serves to repress the occurrence of spurious transcription initiation from cryptic start sites within open reading frames. One key subunit of Rpd3s complex is the Eaf3 protein (Carrozza *et al.* 2005). Interestingly, Eaf3p is also a component of the promoter-targeted NuA4 HAT complex (Eisen *et al.* 2001). Eaf3p may act as a "buffer" subunit to ensure the normal expression of genes in different regions by keeping the equilibrium between HATs and HDACs. As illustrated in the review, in human and yeast, the deposition of H2A.Z on nucleosome by SWR1 is greatly enhanced by prior acetylation of chromatin by NuA4 (Doyon and Côté 2004; J. Jin et al. 2005; Auger et al. 2008; Altaf et al. 2010). As a feedback of H2A.Z deletion in *F. graminearum*, mutation occurs in the subunit of Rpd3s complex to rescue the deficiency **(Figure 27)**.

- **Mutations in Ash1lp and Jarid1p**

According to bibliography, we found that in fact, another two suppressors, histone demethylase Jarid1 and histone-lysine n-methyltransferase Ash1lp, also show close association with H2A.Z **(Figure 27)**. Jarid1p is specific for H3K4me3, while Ash1lp targets on H3K36me2 at subtelomeric regions to inactive gene expression (Harmeyer *et al.* 2017; Bicocca *et al.* 2018). In *N. crassa*, Ash1p-marked chromatin can be further modified by methylation of H3K27, and Ash1p catalytic activity modulates the accumulation of

H3K27me2/3 both positively and negatively (Bicocca *et al.* 2018). In *Arabidopsis*, human and mouse embryonic stem cells, H2A.Z preferentially associated with H3K4me3 and H3K27me3 at promoters or enhancers respectively to control gene transcription (Santos-Rosa *et al.* 2002; Creyghton *et al.* 2008; Ku *et al.* 2012; Hu *et al.* 2013; Dai *et al.* 2017). We believe that mutations in Jarid1p or Ash1lp mimic functions of H2A.Z to compensate for the consequences of H2A.Z deletion.

- **Other hypothesis**

Another mechanism of suppression is related with the nonsense-mediated mRNA Decay (NMD) pathway. In yeast, nonsense or frameshift mutations of query genes with PTC, often trigger loss-of-function suppressor mutations in NMD2 or NAM7, which encode members of the NMD pathway, therefore, stabilizing mutant mRNA (Schuldiner *et al.* 2005; J. van Leeuwen *et al.* 2016). More recently, (Ma *et al.* 2019) found that in zebrafish, mRNA bearing a PTC promptly actives a GCR, or dosage suppression that involves Upf3a (a member of NMD pathway) and components of the COMPASS complex. Furthermore, in the same paper, they indicated the process is accompanied by an enhancement of histone H3K4me3 at the TSS regions of the homologous genes of query to promote the transcription of the suppressor genes, which revealed the relationship between histone markers and GCR. We speculate that genetic suppression mediated by NMD pathway also relies on PTMs, may be linked directly with H2A.Z as well, since H2A.Z and H3K4me3 often co-localize at TSS regions.

4.2.3 Suppressions by transcription factor

In I156ΔH2A.Z#3, there is a frame shift mutation in a transcription factor which seems to be involved in the development, reproduction and pathogenesis. Indeed, alteration of the expression of transcription factor which related with the query gene could compensate the defect to a certain extent (Magtanong *et al.* 2011). However, phenotypes of I156ΔH2A.Z#3, including radial growth, conidium and ascospore formation, and DON production, are more affected than I156ΔH2A.Z#1, I156ΔH2A.Z#2 and I156ΔH2A.Z#4, even though they derived from the same genetic background. In fact, in addition to the mutation detected in the transcription factor, there are another three suppressor mutations in I156ΔH2A.Z#3. Interactions may exist between these mutations, making the mutant more complex to study.

4.2.4 Predict novel gene functions

The functional relationship observed between a query mutant and its suppressor can be exploited to assign gene function to previously uncharacterized genes (J. van Leeuwen *et al.* 2016). Three hypothetical proteins, FGRAMPH1_01G03975, FGRAMPH1_01G27197 and FGRAMPH1_01G26173, are detected carrying mutations in H2A.Z deleted mutants. Among which, single amino acid exchange in FGRAMPH1_01G03975 occurs in I156ΔH2A.Z#3 and I812-H2A.ZΔ1. According to the annotated gene models made in the reference strain I812, we found that both FGRAMPH1_01G03975 and H2A.Z (FGRAMPH1_01G03973) are both located on chromosome 1. They are in close proximity to each other. Thus, mutations on FGRAMPH1_01G03975 may be just a consequence of its adjoining location with H2A.Z, rather than the functional relevance. The other two genes FGRAMPH1_01G27197 and FGRAMPH1_01G26173 are more likely to be involved in the same functional module as H2A.Z. However, further experiments should be designed and carried out for validation. For example, gene-deleted mutants can be created respectively to identify their roles in *F. graminearum.*

4.2.5 Genetic suppression may overlap with dosage suppression network

According to a systematic study on assembling global network of genetic suppression interactions in yeast, the genetic suppression network shows significant overlap with a dosage suppression network, including both positive and negative interactions (Magtanong *et al.* 2011; J. van Leeuwen *et al.* 2016). Here, even though we identified suppressor mutations in ΔH2A.Z mutants, the expression level of suppressor genes has not been measured yet. Meanwhile, to see whether dosage suppression exist in the mutants, it is necessary to perform RNA-sequencing to get a global view.

4.3 H2A.Z overexpression has no impact on phenotype

To see whether H2A.Z OE mutant exhibits reverse phenotype compared to ΔH2A.Z mutants, we attempted to insert pGPD, a strong commonly used constitutive yeast promoter before the H2A.Z sequence using split-marker approach. Repeated transformations were unsuccessful when we used a full length version of pGPD (2301 bp) and an 826 bp downstream fragment of H2A.Z. We thought it was the heavy exogenous insertion and short overlapping cassette which increased the difficulty of homologuous recombination in *F.*

graminearum. Therefore, we re-tried the experiment using a truncated version of pGPD (717 bp) and a longer upstream fragment (1131 bp). After validation by southern blot and whole genome sequencing, we obtained one OE:H2A.Z mutant in the background of I349 wild type. Expression analysis by qPCR indicated that H2A.Z is more than 2-fold overexpressed in the mutant than wild type. However, up to now, there is no phenotype that we could observe in the mutant compared to wild type. Several explanations are raised: **(1)** H2A.Z requires cofactors, for example, deposition of H2A.Z is mediated by SWR1 complex. Even though H2A.Z is overexpressed in the mutant, SWR1 is still limiting. According to the results of qPCR, the expression of Swr1 subunit in mutant is exactly identical with wild type, which agrees with our hypothesis. For further confirmation, qPCR or RNA-sequencing will be carried out to check the expression of other genes, especially those belonging to SWR1 complex and also the canonical histone H2B, as it forms dimer with H2A.Z; **(2)** H2A.Z may be already abundant enough in wild type and thereby overexpression only has tiny consequence; **(3)** Overexpression of H2A.Z in our mutant does not reach the threshold which could lead to the change of phenotype. A stronger promoter can be used to create new OE mutants with higher H2A.Z expression level.

4.4 Roles of heterochromatin markers depend on genetic backgrounds

TEs are DNA fragments which can be found in all eukaryotic genomes. For example, they account for almost 50% of the human genome They are able to insert into new chromosomal locations and duplicate themselves during the process (Bowen and Jordan 2002; Wessler 2006). In fungi, while TEs have been proposed to provide some beneficial functions to their hosts, *e.g.,* by promoting genetic diversity and accelerating adaptive evolution, their overall impact is considered deleterious (Gladyshev 2017). Various mechanisms have been developed to protect fungal genomes against TEs including repeat-induced point mutation (RIP), which occurs specifically during haploid dikaryotic stage of sexual reproductive cycle that occurs following fertilization and prior to meiosis (Selker 2004; Gladyshev 2017). RIP selectively mutate duplicated sequences in both DNA strands by inducing SNP mutations that converted C:G base pairs to T:A, leading to the introduction of nonsense or missense mutations which affected the expression of these sequences (Selker 2004; Hane *et al.* 2015). The process concomitants methylation of nearly all remaining cytosines. Meanwhile, the A:T rich regions serve as a recognition signal for the recruitment of the facultative

heterochromatin formation machinery including H3K9me3 and HP1 (Selker and Stevens 1985; Gladyshev 2017). The model was established in *N. crassa* and further research showed that this mechanism is conserved in fungi. For example, deletion of Dim5p, the methyltransferase which is responsible for H3K9me3, impairs sexual and asexual development as well as virulence (Gu *et al.* 2017). To investigate the network involved in establishment and maintenance of heterochromatin in *F. graminearum*, here, we evaluated the phenotype of *F. graminearum* mutants lack of HP1, Dim5p and the DNA methyltransferase Dim2p. All the mutants were derived from INRA605 wild type strain and constructed by split-marker approach before.

In general, HP1, Dim5p or Dim2p are dispensable for the asexual development of INRA605. However, several differences between biological repeats are detected. For example, the ability to produce conidia is significantly inhibited in ΔDim5p 17 compared to wild type, while the other two mutants without Dim5p exhibit same phenotype with wild type. It is possible that off-targets exist, the mutants should be sent for whole genome sequencing to verify.

Concerning the sexual reproduction, all the mutants produce more perithecia, but they are mainly composed of microperithecia, which may lead to a delay of perithecia maturation and furthermore, a lack of ascospore production and discharge. Parallel experiments are carried out in another wild type strain I171 in our lab. Strikingly, we found that deletion of HP1 enabled I171 to enter sexual cycle and produce healthy and maturated perithecia. WGS revealed a SNP in the velvet complex protein Ve1 of I171 (N. Ponts, personal communication). Ve1p is reported to be essential for the sexual development, as its deletion lead to a complete loss of perithecis formation (Kim *et al.* 2013). One of our hypotheses is that the heterochromatin state at *loci* involved in the development of *F. graminearum* can be maintained by HP1 and removed by the Ve1 complex. Thereby, incomplete function of Ve1 in I171 could not activate genes related to sexual reproduction, while deletion of HP1 blocks the heterochromatin formation and activates the gene (N. Ponts, personal communication).
According to Reyes-Dominguez *et al.* (2012), absence of HP1 results to a down regulation of DON production and an up regulation of AUR in *F. graminearum* (Reyes-Dominguez et al. 2012), which is in accordance with our results observed in both I605-ΔDim5p, ΔDim2p and ΔHP1. However, a reverse phenotype was observed in I812-ΔHP1 (experiment was

performed in our collaborative lab in Vienna) and I171-ΔHP1, where HP1 probably functions as a positive regulator for SMs in I812 and I171 wild type, meaning that effects of HP1 on SMs profiles vary between genetic backgrounds (N. Ponts and J. Strauss, personal communication).

We speculate that SMs biosynthesis in response to different nutrient sources is regulated by epigenetic markers, at least the heterochromatin mark HP1. In the model organism *A. nidulans*, TEs are shown to be involved in SM cluster regulation (Shaaban *et al.* 2010). In *F. graminearum*, transposable activity controlled by heterochromatin machinery may be also linked with SM profiles, which can explain the diverse roles of HP1 in distinct SM gene clusters or genetic backgrounds. Another possibility is the existence of epistatic interactions (Schuldiner *et al*. 2005; Snitkin and Segrè 2011; Storz 2018). The effects of *HP1* may be dependent on the presence of other genes in *F. graminearum*. Therefore, the overcome of HP1 deletion may depends on genetic background. To obtain a more integrated comprehension, similar investigation performed on other *Fusarium* species including *F. fujikuroi* and *F. mangiferae* is ongoing.

4.5 Conclusions and perspectives

The results from my PhD work are unexpected. The most noticeable observation is the genetic plasticity of *F. graminearum* facing detrimental gene perturbation which is probably the consequence of evolution and adaptation. Rewiring of genetic networks in response to H2A.Z deletion through suppression leads to the complexity and indistinctness of this project. Obviously, epigenetic markers interact with each other and work together systematically rather than independently to control the dynamic structure of chromatin, which further influence gene expression.

Combining our results with the roles of heterochromatin markers in *F. graminearum* obtained in the side project, and other previous studies on H2A.Z carried out in yeast, plant and animals, I propose a model **(Figure 27)** to help us to explain the appearance of compensatory mutations upon H2A.Z deletion in *F. graminearum* and make new hypothesizes. I think lack of H2A.Z in *F. graminearum* lead to a reorganization of epigenetic markers including H2A.Z-related PTMs and the remodeling complexes SWR1 and INO80

which mediate the exchange of H2A.Z from nucleosomes. For example, H2A.Z deletion may restrict the amount of H3K4me3 and H3/H4ac, which are known as active epigenetic markers. However, this kind of restriction can be partly counteracted by frame shifts in HDAC Rpd3s or histone demethylase Jarid1p **(See details in section 4.2.2)**. In *Arabidopsis*, it is reported that H2A.Z acts as a functional substitute for H3K9me3 in chromatin during the recruitment of HP1 (Cai *et al.* 2019). Therefore, personally, I think depletion of H2A.Z does not affect H3K9me3, or, at least, does not influence the occurrence of H3K9me3 in a direct way.

Up to now, the precise relationships between H2A.Z and other marks remain totally unclear in *F. graminearum*. However, fortunately, the occurrence of compensatory mutations enables us to find next targets worth studying such as Swr1p and various PTMs which may be involved in the H2A.Z function module. To clarify the network, further experiments are currently being carrying out.

The **short-term** perspectives include:

(1) Transcriptomic analysis of ΔH2A.Z, OE H2A.Z and Comp. H2A.Z mutants by RNA-seq to have an overview of the underlying genetic network rewiring;

(2) Creation and verification of ΔSwr1p and ΔSnf2p mutants (one transformant per mutant has already been obtained). If not lethal, we can use them for further H2A.Z-related study.

In a **long term**, we are going to:

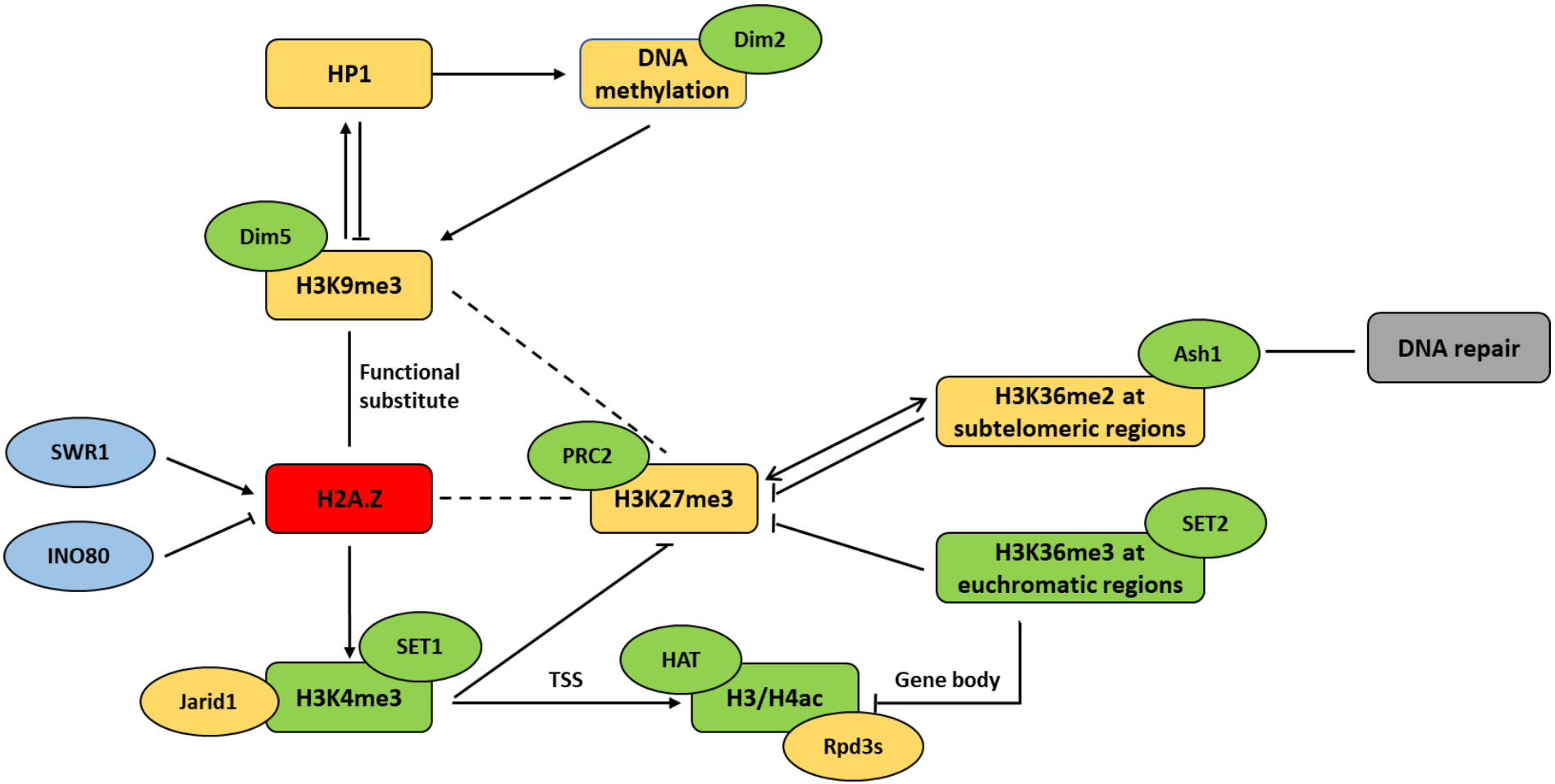
(1) Study genome-wide distribution of H2A.Z by ChIP-seq in wild type strain, OE:H2A.Z and ΔH2A.Z::H2A.Z mutants. The test of antibodies against *F. graminearum* H2A.Z is in planning.

(2) Study genome-wide distribution of histone marks which are already known to be involved in SM gene clusters of *Fusarium* or related with H2A.Z such as H3K4me3, H3K27me3 and H3K9me3 by ChIP-seq in ΔH2A.Z and OE:H2A.Z mutants. Hopefully, we are also going to identify 'novel' PTMs including the actual H2A.Z PTMs in *F. graminearum* by HPLC-MS/MS based proteomics approach.

(3) Predict functions of hypothetical proteins in which compensatory mutation happens upon H2A.Z deletion including FGRAMPH1_01G03975, FGRAMPH1_01G27197 and FGRAMPH1_01G26173 by reverse genetic approach.

We believe that our discoveries in the filamentous fungus *F. graminearum* could be presented as an experimental bridge between yeasts and higher organisms, and use it to decipher unresolved questions concerning H2A.Z.

A


HP1
DNA methylation
Dim2
Dim5
H3K9me3
Functional substitute
SWR1
INO80
H2A.Z
PRC2
H3K27me3
Ash1
DNA repair
H3K36me2 at subtelomeric regions
SET2
H3K36me3 at euchromatic regions
SET1
Jarid1
H3K4me3
TSS
HAT
H3/H4ac
Rpd3s
Gene body

B

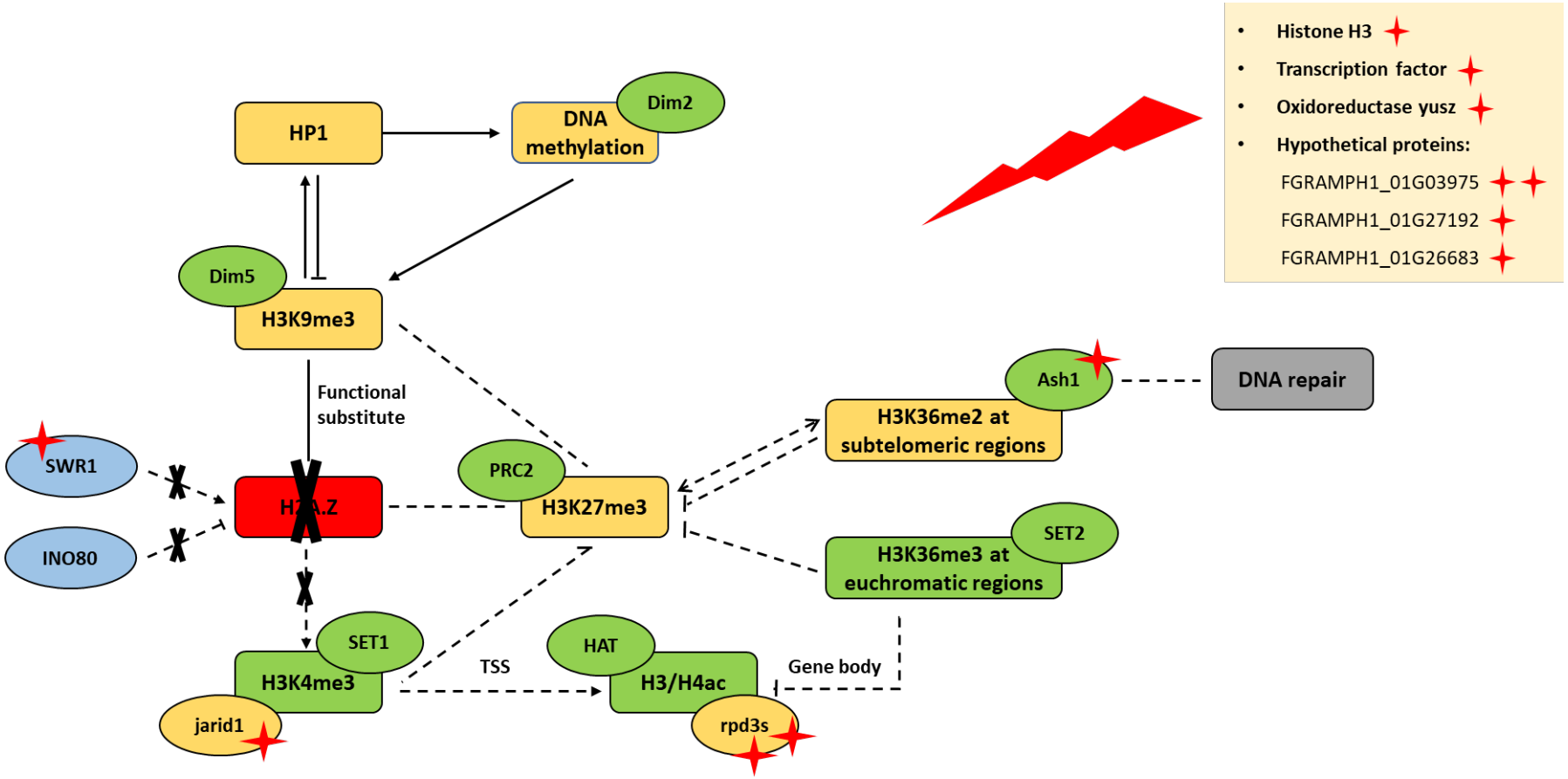


Figure 27. Hypothetical model in *F. graminearum* in response to H2A.Z deletion. **(A)** Association of H2A.Z with different epigenetic marks according to previous study in yeast, plants and animals. **(B)** Compensatory mutations and pathways which are probably affected due to the absence of H2A.Z. **Dashed lines:** controversial or hypothetical relationship, and pathways which may be affected by H2A.Z deletion. **Blocks:** active (green) or repressive (yellow) epigenetic markers, and DNA repair (grey). **Ovals:** writers (green) or erasers (yellow) of epigenetic markers, and complexes involved in the deposition or removal of H2A.Z (blue). **Red Stars:** compensatory mutations triggered by H2A.Z deletion in *F. graminearum*, number of stars represent the occurrence number of mutations on one gene.

References

"About Microbiology – Fungi." n.d. Accessed October 16, 2019. https://microbiologyonline.org/about-microbiology/introducing-microbes/fungi.

Aiken, Alexander M., Benedetta Allegranzi, J. Anthony Scott, Shaheen Mehtar, Didier Pittet, and Hajo Grundmann. 2014. "Antibiotic Resistance Needs Global Solutions." *The Lancet. Infectious Diseases* 14 (7): 550–51. https://doi.org/10.1016/S1473-3099(14)70709-1.

Alberti, Fabrizio, Gary D. Foster, and Andy M. Bailey. 2017. "Natural Products from Filamentous Fungi and Production by Heterologous Expression." *Applied Microbiology and Biotechnology* 101 (2): 493–500. https://doi.org/10.1007/s00253-016-8034-2.

Alberts. 2008. *Molecular Biology of the Cell (MBoC)*. Garland Science. https://www.molbiolcell.org/.

Alexander, Nancy J., Thomas M. Hohn, and Susan P. McCormick. 1998. "The TRI11 Gene of Fusarium Sporotrichioides Encodes a Cytochrome P-450 Monooxygenase Required for C-15 Hydroxylation in Trichothecene Biosynthesis." *Applied and Environmental Microbiology* 64 (1): 221–25.

Alexander, Nancy J., Susan P. McCormick, Troy M. Larson, and James E. Jurgenson. 2004. "Expression of Tri15 in Fusarium Sporotrichioides." *Current Genetics* 45 (3): 157–62. https://doi.org/10.1007/s00294-003-0467-3.

Alexander, Nancy J., Susan P. McCormick, Cees Waalwijk, Theo van der Lee, and Robert H. Proctor. 2011. "The Genetic Basis for 3-ADON and 15-ADON Trichothecene Chemotypes in Fusarium." *Fungal Genetics and Biology: FG & B* 48 (5): 485–95. https://doi.org/10.1016/j.fgb.2011.01.003.

Alshannaq, Ahmad, and Jae-Hyuk Yu. 2017. "Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food." *International Journal of Environmental Research and Public Health* 14 (6). https://doi.org/10.3390/ijerph14060632.

Altaf, Mohammed, Andréanne Auger, Julie Monnet-Saksouk, Joëlle Brodeur, Sandra Piquet, Myriam Cramet, Nathalie Bouchard, et al. 2010. "NuA4-Dependent Acetylation of Nucleosomal Histones H4 and H2A Directly Stimulates Incorporation of H2A.Z by the SWR1 Complex." *Journal of Biological Chemistry* 285 (21): 15966–77. https://doi.org/10.1074/jbc.M110.117069.

Arst, Herbert N., and David J. Cove. 1973. "Nitrogen Metabolite Repression in Aspergillus Nidulans." *Molecular and General Genetics MGG* 126 (2): 111–41. https://doi.org/10.1007/BF00330988.

Atanasova-Penichon, Vessela, Laurie Legoahec, Stéphane Bernillon, Catherine Deborde,

Mickaël Maucourt, Marie-Noëlle Verdal-Bonnin, Laetitia Pinson-Gadais, Nadia Ponts, Annick Moing, and Florence Richard-Forget. 2018. “Mycotoxin Biosynthesis and Central Metabolism Are Two Interlinked Pathways in Fusarium Graminearum, as Demonstrated by the Extensive Metabolic Changes Induced by Caffeic Acid Exposure.” *Applied and Environmental Microbiology* 84 (8): e01705-17. https://doi.org/10.1128/AEM.01705-17.

Audenaert, Kris, Adriaan Vanheule, Monica Höfte, and Geert Haesaert. 2013. “Deoxynivalenol: A Major Player in the Multifaceted Response of Fusarium to Its Environment.” *Toxins* 6 (1): 1–19. https://doi.org/10.3390/toxins6010001.

Audia, James E., and Robert M. Campbell. 2016. “Histone Modifications and Cancer.” *Cold Spring Harbor Perspectives in Biology* 8 (4). https://doi.org/10.1101/cshperspect.a019521.

Auger, Andréanne, Luc Galarneau, Mohammed Altaf, Amine Nourani, Yannick Doyon, Rhea T. Utley, Dominique Cronier, Stéphane Allard, and Jacques Côté. 2008. “Eaf1 Is the Platform for NuA4 Molecular Assembly That Evolutionarily Links Chromatin Acetylation to ATP-Dependent Exchange of Histone H2A Variants.” *Molecular and Cellular Biology* 28 (7): 2257–70. https://doi.org/10.1128/MCB.01755-07.

Avery, Oswald T, and MACLYN McCARTY. 1944. “STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES,” 23.

Baker, SP, and PA Grant. 2007. “The SAGA Continues: Expanding the Cellular Role of a Transcriptional Co-Activator Complex.” *Oncogene* 26 (37): 5329–40. https://doi.org/10.1038/sj.onc.1210603.

Bao, Yunhe, and Xuetong Shen. 2007. “SnapShot: Chromatin Remodeling Complexes.” *Cell* 129 (3): 632. https://doi.org/10.1016/j.cell.2007.04.018.

Barabási, Albert-László, and Zoltán N. Oltvai. 2004. “Network Biology: Understanding the Cell’s Functional Organization.” *Nature Reviews Genetics* 5 (2): 101–13. https://doi.org/10.1038/nrg1272.

Baral, Bikash, Amir Akhgari, and Mikko Metsä-Ketelä. 2018. “Activation of Microbial Secondary Metabolic Pathways: Avenues and Challenges.” *Synthetic and Systems Biotechnology* 3 (3): 163–78. https://doi.org/10.1016/j.synbio.2018.09.001.

Bargaje, Rhishikesh, Mohammad Parwez Alam, Ashok Patowary, Maharnob Sarkar, Tamer Ali, Shivani Gupta, Manali Garg, et al. 2012. “Proximity of H2A.Z Containing Nucleosome to the Transcription Start Site Influences Gene Expression Levels in the Mammalian Liver and Brain.” *Nucleic Acids Research* 40 (18): 8965–78. https://doi.org/10.1093/nar/gks665.

Barra, Jose L., Laïla Rhounim, Jean-Luc Rossignol, and Godeleine Faugeron. 2000. “Histone H1 Is Dispensable for Methylation-Associated Gene Silencing in Ascobolus Immersus and Essential for Long Life Span.” *Molecular and Cellular Biology* 20 (1): 61–69.

Bayram, Ozgür, Sven Krappmann, Min Ni, Jin Woo Bok, Kerstin Helmstaedt, Oliver Valerius, Susanna Braus-Stromeyer, et al. 2008. “VelB/VeA/LaeA Complex Coordinates Light Signal with Fungal Development and Secondary Metabolism.” *Science (New York, N.Y.)* 320 (5882): 1504–6. https://doi.org/10.1126/science.1155888.

Becker, Peter B., and Wolfram Hörz. 2002. “ATP-Dependent Nucleosome Remodeling.” *Annual Review of Biochemistry* 71: 247–73. https://doi.org/10.1146/annurev.biochem.71.110601.135400.

Bennett, J. W., and M. Klich. 2003. “Mycotoxins.” *Clinical Microbiology Reviews* 16 (3): 497–516. https://doi.org/10.1128/cmr.16.3.497-516.2003.

Berthiller, Franz, Michael Sulyok, Rudolf Krska, and Rainer Schuhmacher. 2007. “Chromatographic Methods for the Simultaneous Determination of Mycotoxins and Their Conjugates in Cereals.” *International Journal of Food Microbiology* 119 (1–2): 33–37. https://doi.org/10.1016/j.ijfoodmicro.2007.07.022.

Bicocca, Vincent T, Tereza Ormsby, Keyur K Adhvaryu, Shinji Honda, and Eric U Selker. 2018. “ASH1-Catalyzed H3K36 Methylation Drives Gene Repression and Marks H3K27me2/3-Competent Chromatin.” Edited by Jerry L Workman and Kevin Struhl. *ELife* 7 (November): e41497. https://doi.org/10.7554/eLife.41497.

Binder, E M, and R Krska. 2012. “ROMER LABS GUIDE TO MYCOTOXINS,” 127.

Binz, Thomas, Nisha D’Mello, and Paul A. Horgen. 1998. “A Comparison of DNA Methylation Levels in Selected Isolates of Higher Fungi.” *Mycologia* 90 (5): 785–90. https://doi.org/10.2307/3761319.

Bird, D., and R. Bradshaw. 1997. “Gene Targeting Is Locus Dependent in the Filamentous Fungus Aspergillus Nidulans.” *Molecular & General Genetics: MGG* 255 (2): 219–25. https://doi.org/10.1007/s004380050492.

Blackwell, B. A., O. E. Edwards, A. Fruchier, J. W. ApSimon, and J. D. Miller. 1996. “NMR Structural Studies of Fumonisin B1 and Related Compounds from Fusarium Moniliforme.” *Advances in Experimental Medicine and Biology* 392: 75–91. https://doi.org/10.1007/978-1-4899-1379-1_7.

Bowen, Nathan J., and I. King Jordan. 2002. “Transposable Elements and the Evolution of Eukaryotic Complexity.” *Current Issues in Molecular Biology* 4 (3): 65–76.

Brown, Daren W., Rex B. Dyer, Susan P. McCormick, David F. Kendra, and Ronald D. Plattner. 2004. “Functional Demarcation of the Fusarium Core Trichothecene Gene Cluster.” *Fungal Genetics and Biology: FG & B* 41 (4): 454–62. https://doi.org/10.1016/j.fgb.2003.12.002.

Brown, Daren W., Susan P. McCormick, Nancy J. Alexander, Robert H. Proctor, and Anne E. Desjardins. 2002. “Inactivation of a Cytochrome P-450 Is a Determinant of Trichothecene Diversity in Fusarium Species.” *Fungal Genetics and Biology: FG & B* 36 (3): 224–33.

Bryden, Wayne. 2012. “Food and Feed, Mycotoxins and the Perpetual Pentagram in a Changing Animal Production Environment.” *Animal Production Science* 52 (January): 383–97. https://doi.org/10.1071/AN12073.

Bucci, T. J., D. K. Hansen, and J. B. LaBorde. 1996. “Leukoencephalomalacia and Hemorrhage in the Brain of Rabbits Gavaged with Mycotoxin Fumonisin B1.” *Natural Toxins* 4 (1): 51–52.

Bushnell, W., Beth E. Hazen, Clara Pritsch, and Keith John Leonard. 2003. “Histology and Physiology of Fusarium Head Blight.” In .

Cai, Hanyang, Man Zhang, Mengnan Chai, Qing He, Xinyu Huang, Lihua Zhao, and Yuan Qin. 2019. “Epigenetic Regulation of Anthocyanin Biosynthesis by an Antagonistic Interaction between H2A.Z and H3K4me3.” *The New Phytologist* 221 (1): 295–308. https://doi.org/10.1111/nph.15306.

Calvo, Ana M. 2008. “The VeA Regulatory System and Its Role in Morphological and Chemical Development in Fungi.” *Fungal Genetics and Biology: FG & B* 45 (7): 1053–61. https://doi.org/10.1016/j.fgb.2008.03.014.

Calvo, Ana M., Richard A. Wilson, Jin Woo Bok, and Nancy P. Keller. 2002. “Relationship between Secondary Metabolism and Fungal Development.” *Microbiology and Molecular Biology Reviews: MMBR* 66 (3): 447–59, table of contents. https://doi.org/10.1128/mmbr.66.3.447-459.2002.

Capuano, Floriana, Michael Mülleder, Robert Kok, Henk J. Blom, and Markus Ralser. 2014. “Cytosine DNA Methylation Is Found in Drosophila Melanogaster but Absent in Saccharomyces Cerevisiae, Schizosaccharomyces Pombe, and Other Yeast Species.” *Analytical Chemistry* 86 (8): 3697–3702. https://doi.org/10.1021/ac500447w.

Caracuel, Zaira, M. Isabel G. Roncero, Eduardo A. Espeso, Clara I. González-Verdejo, Fe I. García-Maceira, and Antonio Di Pietro. 2003. “The PH Signalling Transcription Factor PacC Controls Virulence in the Plant Pathogen Fusarium Oxysporum.” *Molecular Microbiology* 48 (3): 765–79. https://doi.org/10.1046/j.1365-2958.2003.03465.x.

Carrozza, Michael J., Bing Li, Laurence Florens, Tamaki Suganuma, Selene K. Swanson, Kenneth K. Lee, Wei-Jong Shia, et al. 2005. “Histone H3 Methylation by Set2 Directs Deacetylation of Coding Regions by Rpd3S to Suppress Spurious Intragenic Transcription.” *Cell* 123 (4): 581–92. https://doi.org/10.1016/j.cell.2005.10.023.

“Chromatin Immunoprecipitation Sequencing (ChIP-Seq) Analyisis & Services | Diagenode.” n.d. Accessed October 16, 2019. https://www.diagenode.com/en/categories/chip-seq-

service.

Chung, Kuang-Ren, and Miin-Huey Lee. 2015. “Split-Marker-Mediated Transformation and Targeted Gene Disruption in Filamentous Fungi.” In , 175–80. https://doi.org/10.1007/978-3-319-10503-1_15.

Coleman-Derr, Devin, and Daniel Zilberman. 2012. “Deposition of Histone Variant H2A.Z within Gene Bodies Regulates Responsive Genes.” *PLoS Genetics* 8 (10): e1002988. https://doi.org/10.1371/journal.pgen.1002988.

Compere, S. J., and R. D. Palmiter. 1981. “DNA Methylation Controls the Inducibility of the Mouse Metallothionein-I Gene Lymphoid Cells.” *Cell* 25 (1): 233–40. https://doi.org/10.1016/0092-8674(81)90248-8.

Conlon, H., I. Zadra, H. Haas, H. N. Arst, M. G. Jones, and M. X. Caddick. 2001. “The Aspergillus Nidulans GATA Transcription Factor Gene AreB Encodes at Least Three Proteins and Features Three Classes of Mutation.” *Molecular Microbiology* 40 (2): 361–75. https://doi.org/10.1046/j.1365-2958.2001.02399.x.

Connolly, Lanelle R., Kristina M. Smith, and Michael Freitag. 2013. “The Fusarium Graminearum Histone H3 K27 Methyltransferase KMT6 Regulates Development and Expression of Secondary Metabolite Gene Clusters.” Edited by Hiten D. Madhani. *PLoS Genetics* 9 (10): e1003916. https://doi.org/10.1371/journal.pgen.1003916.

Cooper, Geoffrey M. 2000. “Chromosomes and Chromatin.” *The Cell: A Molecular Approach. 2nd Edition*. https://www.ncbi.nlm.nih.gov/books/NBK9863/.

Creyghton, Menno P., Styliani Markoulaki, Stuart S. Levine, Jacob Hanna, Michael A. Lodato, Ky Sha, Richard A. Young, Rudolf Jaenisch, and Laurie A. Boyer. 2008. “H2AZ Is Enriched at Polycomb Complex Target Genes in ES Cells and Is Necessary for Lineage Commitment.” *Cell* 135 (4): 649–61. https://doi.org/10.1016/j.cell.2008.09.056.

Cryderman, D. E., M. H. Cuaycong, S. C. Elgin, and L. L. Wallrath. 1998. “Characterization of Sequences Associated with Position-Effect Variegation at Pericentric Sites in Drosophila Heterochromatin.” *Chromosoma* 107 (5): 277–85. https://doi.org/10.1007/s004120050309.

Daal, A. van, and S. C. Elgin. 1992. “A Histone Variant, H2AvD, Is Essential in Drosophila Melanogaster.” *Molecular Biology of the Cell* 3 (6): 593–602.

Dai, Xiaozhuan, Youhuang Bai, Lihua Zhao, Xianying Dou, Yanhui Liu, Lulu Wang, Yi Li, et al. 2017. “H2A.Z Represses Gene Expression by Modulating Promoter Nucleosome Structure and Enhancer Histone Modifications in Arabidopsis.” *Molecular Plant* 10 (10): 1274–92. https://doi.org/10.1016/j.molp.2017.09.007.

Davis, Brad H., Art F.Y. Poon, and Michael C. Whitlock. 2009. “Compensatory Mutations Are Repeatable and Clustered within Proteins.” *Proceedings of the Royal Society B: Biological*

Sciences 276 (1663): 1823–27. https://doi.org/10.1098/rspb.2008.1846.

Dickey, Jennifer S., Christophe E. Redon, Asako J. Nakamura, Brandon J. Baird, Olga A. Sedelnikova, and William M. Bonner. 2009. "H2AX: Functional Roles and Potential Applications." *Chromosoma* 118 (6): 683–92. https://doi.org/10.1007/s00412-009-0234-4.

Diepeningen, Anne D. van, Abdullah M. S. Al-Hatmi, Balázs Brankovics, and G. Sybren de Hoog. 2014. "Taxonomy and Clinical Spectra of Fusarium Species: Where Do We Stand in 2014?" *Current Clinical Microbiology Reports* 1 (1): 10–18. https://doi.org/10.1007/s40588-014-0003-x.

Dodge, Jonathan E., Bernard H. Ramsahoye, Z. Galen Wo, Masaki Okano, and En Li. 2002. "De Novo Methylation of MMLV Provirus in Embryonic Stem Cells: CpG versus Non-CpG Methylation." *Gene* 289 (1–2): 41–48. https://doi.org/10.1016/s0378-1119(02)00469-9.

Dong, Qing, Yajun Wang, Shaohua Qi, Kexin Gai, Qun He, and Ying Wang. 2018. "Histone Variant H2A.Z Antagonizes the Positive Effect of the Transcriptional Activator CPC1 to Regulate Catalase-3 Expression under Normal and Oxidative Stress Conditions." *Free Radical Biology and Medicine* 121 (June): 136–48. https://doi.org/10.1016/j.freeradbiomed.2018.05.003.

Doyen, Cécile-Marie, Fabien Montel, Thierry Gautier, Hervé Menoni, Cyril Claudet, Marlène Delacour-Larose, Dimitri Angelov, et al. 2006. "Dissection of the Unusual Structural and Functional Properties of the Variant H2A.Bbd Nucleosome." *The EMBO Journal* 25 (18): 4234–44. https://doi.org/10.1038/sj.emboj.7601310.

Doyon, Yannick, and Jacques Côté. 2004. "The Highly Conserved and Multifunctional NuA4 HAT Complex." *Current Opinion in Genetics & Development* 14 (2): 147–54. https://doi.org/10.1016/j.gde.2004.02.009.

Draizen, Eli J., Alexey K. Shaytan, Leonardo Mariño-Ramírez, Paul B. Talbert, David Landsman, and Anna R. Panchenko. 2016. "HistoneDB 2.0: A Histone Database with Variants—an Integrated Resource to Explore Histones and Their Variants." *Database: The Journal of Biological Databases and Curation* 2016 (March). https://doi.org/10.1093/database/baw014.

Dreyer, Jacqueline, Heiko Eichhorn, Ernst Friedlin, Hubert Kürnsteiner, and Ulrich Kück. 2007. "A Homologue of the Aspergillus Velvet Gene Regulates Both Cephalosporin C Biosynthesis and Hyphal Fragmentation in Acremonium Chrysogenum." *Applied and Environmental Microbiology* 73 (10): 3412–22. https://doi.org/10.1128/AEM.00129-07.

Dutton, M. F. 1996. "Fumonisins, Mycotoxins of Increasing Importance: Their Nature and Their Effects." *Pharmacology & Therapeutics* 70 (2): 137–61.

Dyer, Rex B., Ronald D. Plattner, David F. Kendra, and Daren W. Brown. 2005. "Fusarium Graminearum TRI14 Is Required for High Virulence and DON Production on Wheat but Not

for DON Synthesis in Vitro." *Journal of Agricultural and Food Chemistry* 53 (23): 9281–87. https://doi.org/10.1021/jf051441a.

Dzikowska, Agnieszka, Magdalena Kacprzak, Rafał Tomecki, Michał Koper, Claudio Scazzocchio, and Piotr Weglenski. 2003. "Specific Induction and Carbon/Nitrogen Repression of Arginine Catabolism Gene of Aspergillus Nidulans--Functional in Vivo Analysis of the OtaA Promoter." *Fungal Genetics and Biology: FG & B* 38 (2): 175–86.

Ehrlich, K. C., and P. J. Cotty. 2002. "Variability in Nitrogen Regulation of Aflatoxin Production by Aspergillus Flavus Strains." *Applied Microbiology and Biotechnology* 60 (1–2): 174–78. https://doi.org/10.1007/s00253-002-1094-5.

Eisen, A., R. T. Utley, A. Nourani, S. Allard, P. Schmidt, W. S. Lane, J. C. Lucchesi, and J. Cote. 2001. "The Yeast NuA4 and Drosophila MSL Complexes Contain Homologous Subunits Important for Transcription Regulation." *The Journal of Biological Chemistry* 276 (5): 3484–91. https://doi.org/10.1074/jbc.M008159200.

Eisen, Jonathan A., Kevin S. Sweder, and Philip C. Hanawalt. 1995. "Evolution of the SNF2 Family of Proteins: Subfamilies with Distinct Sequences and Functions." *Nucleic Acids Research* 23 (14): 2715–23. https://doi.org/10.1093/nar/23.14.2715.

El-Brolosy, Mohamed A., and Didier Y. R. Stainier. 2017. "Genetic Compensation: A Phenomenon in Search of Mechanisms." *PLoS Genetics* 13 (7). https://doi.org/10.1371/journal.pgen.1006780.

Espeso, E. A., and M. A. Peñalva. 1992. "Carbon Catabolite Repression Can Account for the Temporal Pattern of Expression of a Penicillin Biosynthetic Gene in Aspergillus Nidulans." *Molecular Microbiology* 6 (11): 1457–65. https://doi.org/10.1111/j.1365-2958.1992.tb00866.x.

———. 1996. "Three Binding Sites for the Aspergillus Nidulans PacC Zinc-Finger Transcription Factor Are Necessary and Sufficient for Regulation by Ambient PH of the Isopenicillin N Synthase Gene Promoter." *The Journal of Biological Chemistry* 271 (46): 28825–30. https://doi.org/10.1074/jbc.271.46.28825.

Faast, R., V. Thonglairoam, T. C. Schulz, J. Beall, J. R. Wells, H. Taylor, K. Matthaei, P. D. Rathjen, D. J. Tremethick, and I. Lyons. 2001. "Histone Variant H2A.Z Is Required for Early Mammalian Development." *Current Biology: CB* 11 (15): 1183–87.

Fairhead, C., B. Llorente, F. Denis, M. Soler, and B. Dujon. 1996. "New Vectors for Combinatorial Deletions in Yeast Chromosomes and for Gap-Repair Cloning Using 'split-Marker' Recombination." *Yeast (Chichester, England)* 12 (14): 1439–57. https://doi.org/10.1002/(SICI)1097-0061(199611)12:14%3C1439::AID-YEA37%3E3.0.CO;2-O.

Fan, Jing, Kimberly A. Krautkramer, Jessica L. Feldman, and John M. Denu. 2015. "Metabolic

Regulation of Histone Post-Translational Modifications." *ACS Chemical Biology* 10 (1): 95–108. https://doi.org/10.1021/cb500846u.

Fanti, Laura, and Sergio Pimpinelli. 2008. "HP1: A Functionally Multifaceted Protein." *Current Opinion in Genetics & Development* 18 (2): 169–74. https://doi.org/10.1016/j.gde.2008.01.009.

FAO, and Linda Collette, eds. 2011. *Save and Grow: A Policymaker's Guide to the Sustainable Intensification of Smallholder Crop Production*. Rome: FAO.

Filteau, Marie, Véronique Hamel, Marie-Christine Pouliot, Isabelle Gagnon-Arsenault, Alexandre K Dubé, and Christian R Landry. 2015. "Evolutionary Rescue by Compensatory Mutations Is Constrained by Genomic and Environmental Backgrounds." *Molecular Systems Biology* 11 (10). https://doi.org/10.15252/msb.20156444.

Flaherty, Joseph E., Anna Maria Pirttilä, Burton H. Bluhm, and Charles P. Woloshuk. 2003. "PAC1, a PH-Regulatory Gene from Fusarium Verticillioides." *Applied and Environmental Microbiology* 69 (9): 5222–27. https://doi.org/10.1128/AEM.69.9.5222-5227.2003.

Foss, H. M., C. J. Roberts, K. M. Claeys, and E. U. Selker. 1995. "Abnormal Chromosome Behavior in Neurospora Mutants Defective in DNA Methylation." *Science (New York, N.Y.)* 267 (5196): 316. https://doi.org/10.1126/science.7824923.

"Four Common Histone Modifications-CUSABIO." n.d. Accessed October 16, 2019. https://www.cusabio.com/c-20829.html.

Freitag, Michael, Patrick C. Hickey, Tamir K. Khlafallah, Nick D. Read, and Eric U. Selker. 2004. "HP1 Is Essential for DNA Methylation in Neurospora." *Molecular Cell* 13 (3): 427–34. https://doi.org/10.1016/S1097-2765(04)00024-3.

"Fusarium Head Blight (Scab) of Wheat." n.d. *Badger Crop Doc* (blog). Accessed July 24, 2019. https://badgercropdoc.com/fusarium-head-blight-scab-of-wheat/.

Gardiner, Donald M., Kemal Kazan, and John M. Manners. 2009a. "Nutrient Profiling Reveals Potent Inducers of Trichothecene Biosynthesis in Fusarium Graminearum." *Fungal Genetics and Biology: FG & B* 46 (8): 604–13. https://doi.org/10.1016/j.fgb.2009.04.004.

———. 2009b. "Novel Genes of Fusarium Graminearum That Negatively Regulate Deoxynivalenol Production and Virulence." *Molecular Plant-Microbe Interactions: MPMI* 22 (12): 1588–1600. https://doi.org/10.1094/MPMI-22-12-1588.

Gardiner, Donald M., Sheree Osborne, Kemal Kazan, and John M. Manners. 2009. "Low PH Regulates the Production of Deoxynivalenol by Fusarium Graminearum." *Microbiology* 155 (9): 3149–56. https://doi.org/10.1099/mic.0.029546-0.

Gelderblom, W. C., C. M. Smuts, S. Abel, S. D. Snyman, M. E. Cawood, L. van der Westhuizen,

and S. Swanevelder. 1996. “Effect of Fumonisin B1 on Protein and Lipid Synthesis in Primary Rat Hepatocytes.” *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 34 (4): 361–69.

Giese, Henriette, Teis Esben Sondergaard, and Jens Laurids Sørensen. 2013. “The AreA Transcription Factor in Fusarium Graminearum Regulates the Use of Some Nonpreferred Nitrogen Sources and Secondary Metabolite Production.” *Fungal Biology* 117 (11–12): 814–21. https://doi.org/10.1016/j.funbio.2013.10.006.

Gilbert, Jeannie, and Steve Haber. 2013. “Overview of Some Recent Research Developments in Fusarium Head Blight of Wheat.” *Canadian Journal of Plant Pathology* 35 (2): 149–74. https://doi.org/10.1080/07060661.2013.772921.

Gladyshev, Eugene. 2017. “Repeat-Induced Point Mutation and Other Genome Defense Mechanisms in Fungi.” *Microbiology Spectrum* 5 (4). https://doi.org/10.1128/microbiolspec.FUNK-0042-2017.

Glenn, A. E. 2007. “Mycotoxigenic Fusarium Species in Animal Feed.” *Animal Feed Science and Technology*, Fusarium and their toxins: Mycology, occurrence, toxicity, control and economic impact, 137 (3): 213–40. https://doi.org/10.1016/j.anifeedsci.2007.06.003.

Goswami, Rubella S., and H. Corby Kistler. 2004. “Heading for Disaster: Fusarium Graminearum on Cereal Crops.” *Molecular Plant Pathology* 5 (6): 515–25. https://doi.org/10.1111/j.1364-3703.2004.00252.x.

Goyon, C. 1996. “Native DNA Repeats and Methylation in Ascobolus.” *Nucleic Acids Research* 24 (17): 3348–56. https://doi.org/10.1093/nar/24.17.3348.

Goyon, Christophe, Teresa Nogueira, and Godeleine Faugeron. 1994. “Perpetuation of Cytosine Methylation in Ascobolus Immersus Implies a Novel Type of Maintenance Methylase.” *Journal of Molecular Biology* 240 (August): 42–51. https://doi.org/10.1006/jmbi.1994.1416.

Grant, P. A., L. Duggan, J. Côté, S. M. Roberts, J. E. Brownell, R. Candau, R. Ohba, et al. 1997. “Yeast Gcn5 Functions in Two Multisubunit Complexes to Acetylate Nucleosomal Histones: Characterization of an Ada Complex and the SAGA (Spt/Ada) Complex.” *Genes & Development* 11 (13): 1640–50. https://doi.org/10.1101/gad.11.13.1640.

Greer, Eric L., and Yang Shi. 2012. “Histone Methylation: A Dynamic Mark in Health, Disease and Inheritance.” *Nature Reviews. Genetics* 13 (5): 343–57. https://doi.org/10.1038/nrg3173.

Grenier, B., and I. Oswald. 2011. “Mycotoxin Co-Contamination of Food and Feed: Meta-Analysis of Publications Describing Toxicological Interactions.” *World Mycotoxin Journal* 4 (3): 285–313. https://doi.org/10.3920/WMJ2011.1281.

Grenier, Bertrand, and Todd J. Applegate. 2013. “Modulation of Intestinal Functions

Following Mycotoxin Ingestion: Meta-Analysis of Published Experiments in Animals." *Toxins* 5 (2): 396–430. https://doi.org/10.3390/toxins5020396.

Grigoriev, Igor V., Daniel Cullen, Stephen B. Goodwin, David Hibbett, Thomas W. Jeffries, Christian P. Kubicek, Cheryl Kuske, et al. 2011. "Fueling the Future with Fungal Genomics." *Mycology* 2 (3): 192–209. https://doi.org/10.1080/21501203.2011.584577.

Grigoriev, Igor V., Roman Nikitin, Sajeet Haridas, Alan Kuo, Robin Ohm, Robert Otillar, Robert Riley, et al. 2014. "MycoCosm Portal: Gearing up for 1000 Fungal Genomes." *Nucleic Acids Research* 42 (D1): D699–704. https://doi.org/10.1093/nar/gkt1183.

Gruber-Dorninger, Christiane, Timothy Jenkins, and Gerd Schatzmayr. 2019. "Global Mycotoxin Occurrence in Feed: A Ten-Year Survey." *Toxins* 11 (7): 375. https://doi.org/10.3390/toxins11070375.

Gu, Qin, Tiantian Ji, Xiao Sun, Hai Huang, Hao Zhang, Xi Lu, Liming Wu, Rong Huo, Huijun Wu, and Xuewen Gao. 2017. "Histone H3 Lysine 9 Methyltransferase FvDim5 Regulates Fungal Development, Pathogenicity and Osmotic Stress Responses in Fusarium Verticillioides." *FEMS Microbiology Letters* 364 (19). https://doi.org/10.1093/femsle/fnx184.

Gu, Qin, Hafiz Abdul Samad Tahir, Hao Zhang, Hai Huang, Tiantian Ji, Xiao Sun, Liming Wu, Huijun Wu, and Xuewen Gao. 2017. "Involvement of FvSet1 in Fumonisin B1 Biosynthesis, Vegetative Growth, Fungal Virulence, and Environmental Stress Responses in Fusarium Verticillioides." *Toxins* 9 (2). https://doi.org/10.3390/toxins9020043.

Guarro, J. 2013. "Fusariosis, a Complex Infection Caused by a High Diversity of Fungal Species Refractory to Treatment." *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 32 (12): 1491–1500. https://doi.org/10.1007/s10096-013-1924-7.

Gunupuru, L. R., A. Perochon, and F. M. Doohan. 2017. "Deoxynivalenol Resistance as a Component of FHB Resistance." *Tropical Plant Pathology* 42 (3): 175–83. https://doi.org/10.1007/s40858-017-0147-3.

Haas, H., and G. A. Marzluf. 1995. "NRE, the Major Nitrogen Regulatory Protein of Penicillium Chrysogenum, Binds Specifically to Elements in the Intergenic Promoter Regions of Nitrate Assimilation and Penicillin Biosynthetic Gene Clusters." *Current Genetics* 28 (2): 177–83.

Haines, T. R., D. I. Rodenhiser, and P. J. Ainsworth. 2001. "Allele-Specific Non-CpG Methylation of the Nf1 Gene during Early Mouse Development." *Developmental Biology* 240 (2): 585–98. https://doi.org/10.1006/dbio.2001.0504.

Hake, Sandra B., Benjamin A. Garcia, Monika Kauer, Stephen P. Baker, Jeffrey Shabanowitz, Donald F. Hunt, and C. David Allis. 2005. "Serine 31 Phosphorylation of Histone Variant H3.3 Is Specific to Regions Bordering Centromeres in Metaphase Chromosomes." *Proceedings of*

the National Academy of Sciences of the United States of America 102 (18): 6344–49. https://doi.org/10.1073/pnas.0502413102.

Halley, Jeffrey E., Tommy Kaplan, Alice Y. Wang, Michael S. Kobor, and Jasper Rine. 2010. "Roles for H2A.Z and Its Acetylation in GAL1 Transcription and Gene Induction, but Not GAL1-Transcriptional Memory." *PLOS Biology* 8 (6): e1000401. https://doi.org/10.1371/journal.pbio.1000401.

Hamad, Bashar. 2010. "The Antibiotics Market." *Nature Reviews. Drug Discovery* 9 (9): 675–76. https://doi.org/10.1038/nrd3267.

Hane, James K., Angela H. Williams, Adam P. Taranto, Peter S. Solomon, and Richard P. Oliver. 2015. "Repeat-Induced Point Mutation: A Fungal-Specific, Endogenous Mutagenesis Process." In *Genetic Transformation Systems in Fungi, Volume 2*, edited by Marco A. van den Berg and Karunakaran Maruthachalam, 55–68. Fungal Biology. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-10503-1_4.

Hanson, B. Leif, Chad Alexander, Joel M Harp, and Gerard J Bunick. 2003. "Preparation and Crystallization of Nucleosome Core Particle." In *Methods in Enzymology*, 375:44–62. Chromatin and Chromatin Remodeling Enzymes, Part A. Academic Press. https://doi.org/10.1016/S0076-6879(03)75003-4.

Harmeyer, Kayla M., Nicole D. Facompre, Meenhard Herlyn, and Devraj Basu. 2017. "JARID1 Histone Demethylases: Emerging Targets in Cancer." *Trends in Cancer* 3 (10): 713–25. https://doi.org/10.1016/j.trecan.2017.08.004.

Hawksworth, David, and Robert Lücking. 2017. "Fungal Diversity Revisited: 2.2 to 3.8 Million Species." *Microbiology Spectrum* 5 (July). https://doi.org/10.1128/microbiolspec.FUNK-0052-2016.

Heller, Jens, and Paul Tudzynski. 2011. "Reactive Oxygen Species in Phytopathogenic Fungi: Signaling, Development, and Disease." *Annual Review of Phytopathology* 49: 369–90. https://doi.org/10.1146/annurev-phyto-072910-095355.

Henderson, Ian R., and Steven E. Jacobsen. 2007. "Epigenetic Inheritance in Plants." *Nature* 447 (7143): 418–24. https://doi.org/10.1038/nature05917.

Henikoff, Steven, and M. Mitchell Smith. 2015. "Histone Variants and Epigenetics." *Cold Spring Harbor Perspectives in Biology* 7 (1): a019364. https://doi.org/10.1101/cshperspect.a019364.

"Histone Modifications." 2013. *What Is Epigenetics?* (blog). 2013. http://www.whatisepigenetics.com/histone-modifications/.

Ho, Joshua W. K., Youngsook L. Jung, Tao Liu, Burak H. Alver, Soohyun Lee, Kohta Ikegami, Kyung-Ah Sohn, et al. 2014. "Comparative Analysis of Metazoan Chromatin Organization."

Nature 512 (7515): 449–52. https://doi.org/10.1038/nature13415.

Hoeijmakers, Wieteke A M, Adriana M Salcedo-Amaya, Arne H Smits, Kees-Jan Françoijs, Moritz Treeck, Tim-Wolf Gilberger, Hendrik G Stunnenberg, and Richárd Bártfai. 2013. "H2A.Z/H2B.Z Double-Variant Nucleosomes Inhabit the AT-Rich Promoter Regions of the Plasmodium Falciparum Genome." *Molecular Microbiology* 87 (5): 1061–73. https://doi.org/10.1111/mmi.12151.

Hohn, T. M., and P. D. Beremand. 1989. "Isolation and Nucleotide Sequence of a Sesquiterpene Cyclase Gene from the Trichothecene-Producing Fungus Fusarium Sporotrichioides." *Gene* 79 (1): 131–38. https://doi.org/10.1016/0378-1119(89)90098-x.

Hohn, T. M., and F. Vanmiddlesworth. 1986. "Purification and Characterization of the Sesquiterpene Cyclase Trichodiene Synthetase from Fusarium Sporotrichioides." *Archives of Biochemistry and Biophysics* 251 (2): 756–61. https://doi.org/10.1016/0003-9861(86)90386-3.

Hohn, Thomas M., Anne E. Desjardins, and Susan P. McCormick. 1995. "TheTri4 Gene OfFusarium Sporotrichioides Encodes a Cytochrome P450 Monooxygenase Involved in Trichothecene Biosynthesis." *Molecular and General Genetics MGG* 248 (1): 95–102. https://doi.org/10.1007/BF02456618.

Holbert, Marc A., and Ronen Marmorstein. 2005. "Structure and Activity of Enzymes That Remove Histone Modifications." *Current Opinion in Structural Biology* 15 (6): 673–80. https://doi.org/10.1016/j.sbi.2005.10.006.

Holliday, R., and J. E. Pugh. 1975. "DNA Modification Mechanisms and Gene Activity during Development." *Science (New York, N.Y.)* 187 (4173): 226–32.

Hota, Swetansu K., and Benoit G. Bruneau. 2016. "ATP-Dependent Chromatin Remodeling during Mammalian Development." *Development (Cambridge, England)* 143 (16): 2882–97. https://doi.org/10.1242/dev.128892.

Hu, Gangqing, Kairong Cui, Daniel Northrup, Chengyu Liu, Chaochen Wang, Qingsong Tang, Kai Ge, David Levens, Colyn Crane-Robinson, and Keji Zhao. 2013. "H2A.Z Facilitates Access of Active and Repressive Complexes to Chromatin in Embryonic Stem Cell Self-Renewal and Differentiation." *Cell Stem Cell* 12 (2): 180–92. https://doi.org/10.1016/j.stem.2012.11.003.

Hueza, Isis M., Paulo Cesar F. Raspantini, Leonila Ester R. Raspantini, Andreia O. Latorre, and Silvana L. Górniak. 2014. "Zearalenone, an Estrogenic Mycotoxin, Is an Immunotoxic Compound." *Toxins* 6 (3): 1080–95. https://doi.org/10.3390/toxins6031080.

Hussein, H. S., and J. M. Brasel. 2001. "Toxicity, Metabolism, and Impact of Mycotoxins on Humans and Animals." *Toxicology* 167 (2): 101–34.

Idnurm, Alexander, Dirk Warnecke, Ernst Heinz, and Barbara Howlett. 2003.

"Characterisation of Neutral Trehalase and UDP-Glucose:Sterol Glucosyltransferase Genes from the Plant Pathogenic Fungus Leptosphaeria Maculans." *Physiological and Molecular Plant Pathology* 62 (May): 305–13. https://doi.org/10.1016/S0885-5765(03)00074-2.

International Human Genome Sequencing Consortium. 2004. "Finishing the Euchromatic Sequence of the Human Genome." *Nature* 431 (7011): 931. https://doi.org/10.1038/nature03001.

Iyer, Lakshminarayan M., Dapeng Zhang, and L. Aravind. 2016. "Adenine Methylation in Eukaryotes: Apprehending the Complex Evolutionary History and Functional Potential of an Epigenetic Modification." *Bioessays* 38 (1): 27–40. https://doi.org/10.1002/bies.201500104.

Jamieson, D. J. 1998. "Oxidative Stress Responses of the Yeast Saccharomyces Cerevisiae." *Yeast (Chichester, England)* 14 (16): 1511–27. https://doi.org/10.1002/(SICI)1097-0061(199812)14:16<1511::AID-YEA356>3.0.CO;2-S.

Janevska, Slavica, Leonie Baumann, Christian M. K. Sieber, Martin Münsterkötter, Jonas Ulrich, Jörg Kämper, Ulrich Güldener, and Bettina Tudzynski. 2018. "Elucidation of the Two H3K36me3 Histone Methyltransferases Set2 and Ash1 in Fusarium Fujikuroi Unravels Their Different Chromosomal Targets and a Major Impact of Ash1 on Genome Stability." *Genetics* 208 (1): 153–71. https://doi.org/10.1534/genetics.117.1119.

Jiang, Jinhua, Xin Liu, Yanni Yin, and Zhonghua Ma. 2011. "Involvement of a Velvet Protein FgVeA in the Regulation of Asexual Development, Lipid and Secondary Metabolisms and Virulence in Fusarium Graminearum." *PLOS ONE* 6 (11): e28291. https://doi.org/10.1371/journal.pone.0028291.

Jiao, Feng, Akira Kawakami, and Takashi Nakajima. 2008. "Effects of Different Carbon Sources on Trichothecene Production and *Tri* Gene Expression by *Fusarium Graminearum* in Liquid Culture." *FEMS Microbiology Letters* 285 (2): 212–19. https://doi.org/10.1111/j.1574-6968.2008.01235.x.

Jin, Chunyuan, and Gary Felsenfeld. 2007. "Nucleosome Stability Mediated by Histone Variants H3.3 and H2A.Z." *Genes & Development* 21 (12): 1519–29. https://doi.org/10.1101/gad.1547707.

Jin, Jingji, Yong Cai, Tingting Yao, Aaron J. Gottschalk, Laurence Florens, Selene K. Swanson, José L. Gutiérrez, et al. 2005. "A Mammalian Chromatin Remodeling Complex with Similarities to the Yeast INO80 Complex." *The Journal of Biological Chemistry* 280 (50): 41207–12. https://doi.org/10.1074/jbc.M509128200.

Käfer, Etta. 1965. "Origins of Translocations in ASPERGILLUS NIDULANS." *Genetics* 52 (1): 217–32.

Katoh, H., K. Ohtani, H. Yamamoto, and K. Akimitsu. 2007. "Overexpression of a Gene

Encoding a Catabolite Repression Element in Alternaria Citri Causes Severe Symptoms of Black Rot in Citrus Fruit." *Phytopathology* 97 (5): 557–63. https://doi.org/10.1094/PHYTO-97-5-0557.

Kaygun, Handan, and William F. Marzluff. 2005. "Regulated Degradation of Replication-Dependent Histone MRNAs Requires Both ATR and Upf1." *Nature Structural & Molecular Biology* 12 (9): 794–800. https://doi.org/10.1038/nsmb972.

Kim, Hee-Kyoung, Seunghoon Lee, Seong-Mi Jo, Susan P. McCormick, Robert A. E. Butchko, Robert H. Proctor, and Sung-Hwan Yun. 2013. "Functional Roles of FgLaeA in Controlling Secondary Metabolism, Sexual Development, and Virulence in Fusarium Graminearum." *PloS One* 8 (7): e68441. https://doi.org/10.1371/journal.pone.0068441.

Kimura, M., I. Kaneko, M. Komiyama, A. Takatsuki, H. Koshino, K. Yoneyama, and I. Yamaguchi. 1998. "Trichothecene 3-O-Acetyltransferase Protects Both the Producing Organism and Transformed Yeast from Related Mycotoxins. Cloning and Characterization of Tri101." *The Journal of Biological Chemistry* 273 (3): 1654–61. https://doi.org/10.1074/jbc.273.3.1654.

Kimura, M., G. Matsumoto, Y. Shingu, K. Yoneyama, and I. Yamaguchi. 1998. "The Mystery of the Trichothecene 3-O-Acetyltransferase Gene. Analysis of the Region around Tri101 and Characterization of Its Homologue from Fusarium Sporotrichioides." *FEBS Letters* 435 (2–3): 163–68. https://doi.org/10.1016/s0014-5793(98)01061-8.

King, Robert, Martin Urban, Michael C. U. Hammond-Kosack, Keywan Hassani-Pak, and Kim E. Hammond-Kosack. 2015. "The Completed Genome Sequence of the Pathogenic Ascomycete Fungus Fusarium Graminearum." *BMC Genomics* 16 (July): 544. https://doi.org/10.1186/s12864-015-1756-1.

Kn, Chandrashekara, C. Chandrashekara, M. Chakravathi, and S. Manivannan. 2012. "Biological Control of Plant Disease." In , 147–66.

Krude, Torsten. 1995. "Chromatin: Nucleosome Assembly during DNA Replication." *Current Biology* 5 (11): 1232–34. https://doi.org/10.1016/S0960-9822(95)00245-4.

Ku, Manching, Jacob D. Jaffe, Richard P. Koche, Esther Rheinbay, Mitsuhiro Endoh, Haruhiko Koseki, Steven A. Carr, and Bradley E. Bernstein. 2012. "H2A.Z Landscapes and Dual Modifications in Pluripotent and Multipotent Stem Cells Underlie Complex Genome Regulatory Functions." *Genome Biology* 13 (10): R85. https://doi.org/10.1186/gb-2012-13-10-r85.

Kubena, L., T. Edrington, R. Harvey, S. Buckley, T. Phillips, G. Rottinghaus, and H. Casper. 1997. "Individual and Combined Effects of Fumonisin B1 Present in Fusarium Moniliforme Culture Material and T-2 Toxin or Deoxynivalenol in Broiler Chicks." *Poultry Science* 76 (9): 1239–47. https://doi.org/10.1093/ps/76.9.1239.

Kulis, Marta, Angelika Merkel, Simon Heath, Ana C. Queirós, Ronald P. Schuyler, Giancarlo Castellano, Renée Beekman, et al. 2015. “Whole-Genome Fingerprint of the DNA Methylome during Human B Cell Differentiation.” *Nature Genetics* 47 (7): 746–56. https://doi.org/10.1038/ng.3291.

Kuo, M. H., and C. D. Allis. 1998. “Roles of Histone Acetyltransferases and Deacetylases in Gene Regulation.” *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 20 (8): 615–26. https://doi.org/10.1002/(SICI)1521-1878(199808)20:8<615::AID-BIES4>3.0.CO;2-H.

Kurtz, H. J., and C. J. Mirocha. 1978. “Zearalenone (F 2) Induced Estrogenic Syndrome in Swine.” *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses Encycl Handb*. http://agris.fao.org/agris-search/search.do?recordID=US201302405339.

Lasserre, Julia, Ho-Ryun Chung, and Martin Vingron. 2013. “Finding Associations among Histone Modifications Using Sparse Partial Correlation Networks.” *PLOS Computational Biology* 9 (9): e1003168. https://doi.org/10.1371/journal.pcbi.1003168.

Lee, Theresa, You-Kyoung Han, Kook-Hyung Kim, Sung-Hwan Yun, and Yin-Won Lee. 2002. “Tri13 and Tri7 Determine Deoxynivalenol- and Nivalenol-Producing Chemotypes of Gibberella Zeae.” *Applied and Environmental Microbiology* 68 (5): 2148–54. https://doi.org/10.1128/AEM.68.5.2148-2154.2002.

Leeuwen, J. van, C. Pons, J. C. Mellor, T. N. Yamaguchi, H. Friesen, J. Koschwanez, M. M. U aj, et al. 2016. “Exploring Genetic Suppression Interactions on a Global Scale.” *Science* 354 (6312): aag0839–aag0839. https://doi.org/10.1126/science.aag0839.

Leeuwen, Jolanda van, Carles Pons, Charles Boone, and Brenda J. Andrews. 2017. “Mechanisms of Suppression: The Wiring of Genetic Resilience.” *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology* 39 (7). https://doi.org/10.1002/bies.201700042.

Leslie, John F., and Brett A. Summerell. 2006. *The Fusarium Laboratory Manual*. 1st ed. Ames, Iowa: Blackwell Pub.

Li, Fenghua, Dafeng Jiang, Fengjia Zheng, Jindong Chen, and Wei Li. 2015. “Fumonisins B_1, B_2 and B_3 in Corn Products, Wheat Flour and Corn Oil Marketed in Shandong Province of China.” *Food Additives & Contaminants. Part B, Surveillance* 8 (3): 169–74. https://doi.org/10.1080/19393210.2015.1028480.

Li, Yimin, Chenfang Wang, Wende Liu, Guanghui Wang, Zhensheng Kang, H. Corby Kistler, and Jin-Rong Xu. 2011. “The HDF1 Histone Deacetylase Gene Is Important for Conidiation, Sexual Reproduction, and Pathogenesis in Fusarium Graminearum.” *Molecular Plant-Microbe Interactions: MPMI* 24 (4): 487–96. https://doi.org/10.1094/MPMI-10-10-0233.

Liang, Liqin, Jianqiang Li, Lin Cheng, Jian Ling, Zhongqin Luo, Miao Bai, and Bingyan Xie. 2014. “A High Efficiency Gene Disruption Strategy Using a Positive–Negative Split Selection Marker and Electroporation for Fusarium Oxysporum.” *Microbiological Research* 169 (11): 835–43. https://doi.org/10.1016/j.micres.2014.03.004.

Lin, Ching-Hsuan, Siwy Ling Yang, Nan-Yi Wang, and Kuang-Ren Chung. 2010. “The FUS3 MAPK Signaling Pathway of the Citrus Pathogen Alternaria Alternata Functions Independently or Cooperatively with the Fungal Redox-Responsive AP1 Regulator for Diverse Developmental, Physiological and Pathogenic Processes.” *Fungal Genetics and Biology: FG & B* 47 (4): 381–91. https://doi.org/10.1016/j.fgb.2009.12.009.

Lister, Ryan, Ronan C. O’Malley, Julian Tonti-Filippini, Brian D. Gregory, Charles C. Berry, A. Harvey Millar, and Joseph R. Ecker. 2008. “Highly Integrated Single-Base Resolution Maps of the Epigenome in Arabidopsis.” *Cell* 133 (3): 523–36. https://doi.org/10.1016/j.cell.2008.03.029.

Lister, Ryan, Mattia Pelizzola, Robert H. Dowen, R. David Hawkins, Gary Hon, Julian Tonti-Filippini, Joseph R. Nery, et al. 2009. “Human DNA Methylomes at Base Resolution Show Widespread Epigenomic Differences.” *Nature* 462 (7271): 315–22. https://doi.org/10.1038/nature08514.

Liu, Na, Shiqi Li, Nan Wu, and Kin-Sang Cho. 2017. “Acetylation and Deacetylation in Cancer Stem-like Cells.” *Oncotarget* 8 (51): 89315–25. https://doi.org/10.18632/oncotarget.19167.

Liu, Si-Yang, Jian-Qing Lin, Hong-Long Wu, Cheng-Cheng Wang, Shu-Jia Huang, Yan-Feng Luo, Ji-Hua Sun, et al. 2012. “Bisulfite Sequencing Reveals That Aspergillus Flavus Holds a Hollow in DNA Methylation.” *PLOS ONE* 7 (1): e30349. https://doi.org/10.1371/journal.pone.0030349.

Liu, Ye, Na Liu, Yanni Yin, Yun Chen, Jinhua Jiang, and Zhonghua Ma. 2015. “Histone H3K4 Methylation Regulates Hyphal Growth, Secondary Metabolism and Multiple Stress Responses in Fusarium Graminearum.” *Environmental Microbiology* 17 (11): 4615–30. https://doi.org/10.1111/1462-2920.12993.

Lomberk, Gwen, Lori Wallrath, and Raul Urrutia. 2006. “The Heterochromatin Protein 1 Family.” *Genome Biology* 7 (7): 228. https://doi.org/10.1186/gb-2006-7-7-228.

López-Berges, Manuel S., Concepción Hera, Michael Sulyok, Katja Schäfer, Javier Capilla, Josep Guarro, and Antonio Di Pietro. 2013. “The Velvet Complex Governs Mycotoxin Production and Virulence of Fusarium Oxysporum on Plant and Mammalian Hosts.” *Molecular Microbiology* 87 (1): 49–65. https://doi.org/10.1111/mmi.12082.

“LSU Small Grains Data.” n.d. Accessed October 16, 2019.

http://wheat.lsu.edu/pestpics.shtml.

Luk, Ed, Anand Ranjan, Peter C. FitzGerald, Gaku Mizuguchi, Yingzi Huang, Debbie Wei, and Carl Wu. 2010. “Stepwise Histone Replacement by SWR1 Requires Dual Activation with Histone H2A.Z and Canonical Nucleosome.” *Cell* 143 (5): 725–36. https://doi.org/10.1016/j.cell.2010.10.019.

Lusser, Alexandra, and James T. Kadonaga. 2003. “Chromatin Remodeling by ATP-Dependent Molecular Machines.” *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 25 (12): 1192–1200. https://doi.org/10.1002/bies.10359.

M Arnaudo, Anna, Rosalynn Molden, and Benjamin A Garcia. 2011. “Revealing Histone Variant Induced Changes Via Quantitative Proteomics.” *Critical Reviews in Biochemistry and Molecular Biology* 46 (April): 284–94. https://doi.org/10.3109/10409238.2011.577052.

M Rösler, Sarah, Katharina Kramer, Iris Finkemeier, Hans-Ulrich Humpf, and Bettina Tudzynski. 2016. “The SAGA Complex in the Rice Pathogen Fusarium Fujikuroi: Structure and Functional Characterization.” *Molecular Microbiology* 102 (September). https://doi.org/10.1111/mmi.13528.

Ma, Li-Jun, David M Geiser, Robert H Proctor, Alejandro P Rooney, K O’Donnell, Frances Trail, Donald M Gardiner, John M Manners, and Kemal Kazan. 2013. “Fusarium Pathogenomics.” *Annual Review of Microbiology* 67 (September): 399–416. https://doi.org/10.1146/annurev-micro-092412-155650.

Ma, Zhipeng, Peipei Zhu, Hui Shi, Liwei Guo, Qinghe Zhang, Yanan Chen, Shuming Chen, Zhe Zhang, Jinrong Peng, and Jun Chen. 2019. “PTC-Bearing MRNA Elicits a Genetic Compensation Response via Upf3a and COMPASS Components.” *Nature* 568 (7751): 259–63. https://doi.org/10.1038/s41586-019-1057-y.

Magtanong, Leslie, Cheuk Hei Ho, Sarah L Barker, Wei Jiao, Anastasia Baryshnikova, Sondra Bahr, Andrew M Smith, et al. 2011. “Dosage Suppression Genetic Interaction Networks Enhance Functional Wiring Diagrams of the Cell.” *Nature Biotechnology* 29 (6): 505–11. https://doi.org/10.1038/nbt.1855.

Malagnac, Fabienne, Birgit Wendel, Christophe Goyon, Godeleine Faugeron, Denise Zickler, Jean-Luc Rossignol, Mario Noyer-Weidner, Peter Vollmayr, Thomas A. Trautner, and Jörn Walter. 1997. “A Gene Essential for De Novo Methylation and Development in Ascobolus Reveals a Novel Type of Eukaryotic DNA Methyltransferase Structure.” *Cell* 91 (2): 281–90. https://doi.org/10.1016/S0092-8674(00)80410-9.

Mannironi, C., W. M. Bonner, and C. L. Hatch. 1989. “H2A.X. a Histone Isoprotein with a Conserved C-Terminal Sequence, Is Encoded by a Novel MRNA with Both DNA Replication Type and PolyA 3’ Processing Signals.” *Nucleic Acids Research* 17 (22): 9113–26. https://doi.org/10.1093/nar/17.22.9113.

Manzoni, M., and M. Rollini. 2002. “Biosynthesis and Biotechnological Production of Statins by Filamentous Fungi and Application of These Cholesterol-Lowering Drugs.” *Applied Microbiology and Biotechnology* 58 (5): 555–64. https://doi.org/10.1007/s00253-002-0932-9.

Marasas, W. F. 1995. “Fumonisins: Their Implications for Human and Animal Health.” *Natural Toxins* 3 (4): 193–98; discussion 221.

Marasas, W. F., T. S. Kellerman, W. C. Gelderblom, J. A. Coetzer, P. G. Thiel, and J. J. van der Lugt. 1988. “Leukoencephalomalacia in a Horse Induced by Fumonisin B1 Isolated from Fusarium Moniliforme.” *The Onderstepoort Journal of Veterinary Research* 55 (4): 197–203.

Markell, S. G., and L. J. Francl. 2003. “Fusarium Head Blight Inoculum: Species Prevalence and Gibberella Zeae Spore Type.” *Plant Disease* 87 (7): 814–20. https://doi.org/10.1094/PDIS.2003.87.7.814.

Marzluf, G. A. 1997. “Genetic Regulation of Nitrogen Metabolism in the Fungi.” *Microbiology and Molecular Biology Reviews: MMBR* 61 (1): 17–32.

Marzluff, William F., and Kaitlin P. Koreski. 2017. “Birth and Death of Histone MRNAs.” *Trends in Genetics: TIG* 33 (10): 745–59. https://doi.org/10.1016/j.tig.2017.07.014.

Mather, Kenneth. 1953. “Genetical Control of Stability in Development.” *Heredity* 7 (3): 297–336. https://doi.org/10.1038/hdy.1953.41.

Maze, Ian, Kyung-Min Noh, Alexey A. Soshnev, and C. David Allis. 2014. “Every Amino Acid Matters: Essential Contributions of Histone Variants to Mammalian Development and Disease.” *Nature Reviews. Genetics* 15 (4): 259–71. https://doi.org/10.1038/nrg3673.

Mazurkiewicz, Jacek, J. Felix Kepert, and Karsten Rippe. 2006. “On the Mechanism of Nucleosome Assembly by Histone Chaperone NAP1.” *Journal of Biological Chemistry* 281 (24): 16462–72. https://doi.org/10.1074/jbc.M511619200.

McCormick, S. P., T. M. Hohn, and A. E. Desjardins. 1996. “Isolation and Characterization of Tri3, a Gene Encoding 15-O-Acetyltransferase from Fusarium Sporotrichioides.” *Applied and Environmental Microbiology* 62 (2): 353–59. https://doi.org/10113/25333.

McCormick, S. P., S. L. Taylor, R. D. Plattner, and M. N. Beremand. 1990. “Bioconversion of Possible T-2 Toxin Precursors by a Mutant Strain of Fusarium Sporotrichioides NRRL 3299.” *Applied and Environmental Microbiology* 56 (3): 702–6. https://doi.org/10113/24129.

McCormick, Susan P., Nancy J. Alexander, and Robert H. Proctor. 2006a. “Heterologous Expression of Two Trichothecene P450 Genes in Fusarium Verticillioides.” *Canadian Journal of Microbiology* 52 (3): 220–26. https://doi.org/10.1139/w05-124.

———. 2006b. “Fusarium Tri4 Encodes a Multifunctional Oxygenase Required for Trichothecene Biosynthesis.” *Canadian Journal of Microbiology* 52 (7): 636–42. https://doi.org/10.1139/w06-011.

McCormick, Susan P., April M. Stanley, Nicholas A. Stover, and Nancy J. Alexander. 2011. “Trichothecenes: From Simple to Complex Mycotoxins.” *Toxins* 3 (7): 802–14. https://doi.org/10.3390/toxins3070802.

McMullen, Marcia, Roger Jones, and Dale Gallenberg. 1997. “Scab of Wheat and Barley: A Re-Emerging Disease of Devastating Impact.” *Plant Disease* 81 (12): 1340–48. https://doi.org/10.1094/PDIS.1997.81.12.1340.

Medina, Angel, Asya Akbar, Alaa Baazeem, Alicia Rodriguez, and Naresh Magan. 2017. “Climate Change, Food Security and Mycotoxins: Do We Know Enough?” *Fungal Biology Reviews* 31 (3): 143–54. https://doi.org/10.1016/j.fbr.2017.04.002.

Merhej, J., A. L. Boutigny, L. Pinson-Gadais, F. Richard-Forget, and C. Barreau. 2010. “Acidic PH as a Determinant of TRI Gene Expression and Trichothecene B Biosynthesis in Fusarium Graminearum.” *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment* 27 (5): 710–17. https://doi.org/10.1080/19440040903514531.

Merhej, Jawad, Florence Richard-Forget, and Christian Barreau. 2011. “The PH Regulatory Factor Pac1 Regulates Tri Gene Expression and Trichothecene Production in Fusarium Graminearum.” *Fungal Genetics and Biology* 48 (3): 275–84. https://doi.org/10.1016/j.fgb.2010.11.008.

Merhej, Jawad, Martin Urban, Marie Dufresne, Kim Hammond-Kosack, Florence Richard-Forget, and Christian Barreau. 2011. “The Velvet Gene, FgVe1, Affects Fungal Development and Positively Regulates Trichothecene Biosynthesis and Pathogenicity in Fusarium Graminearum.” *Molecular Plant Pathology* 13 (October): 363–74. https://doi.org/10.1111/j.1364-3703.2011.00755.x.

Mihlan, Martina, Veronika Homann, Ta-Wei D. Liu, and Bettina Tudzynski. 2003. “AREA Directly Mediates Nitrogen Regulation of Gibberellin Biosynthesis in Gibberella Fujikuroi, but Its Activity Is Not Affected by NMR.” *Molecular Microbiology* 47 (4): 975–91. https://doi.org/10.1046/j.1365-2958.2003.03326.x.

Mondo, Stephen J., Richard O. Dannebaum, Rita C. Kuo, Katherine B. Louie, Adam J. Bewick, Kurt LaButti, Sajeet Haridas, et al. 2017. “Widespread Adenine N6-Methylation of Active Genes in Fungi.” *Nature Genetics* 49 (6): 964–68. https://doi.org/10.1038/ng.3859.

Montibus, Mathilde, Christine Ducos, Marie-Noelle Bonnin-Verdal, Jorg Bormann, Nadia Ponts, Florence Richard-Forget, and Christian Barreau. 2013. “The BZIP Transcription Factor Fgap1 Mediates Oxidative Stress Response and Trichothecene Biosynthesis But Not Virulence in Fusarium Graminearum.” Edited by Yin-Won Lee. *PLoS ONE* 8 (12): e83377.

https://doi.org/10.1371/journal.pone.0083377.

Moore, Lisa D, Thuc Le, and Guoping Fan. 2013. “DNA Methylation and Its Basic Function.” *Neuropsychopharmacology* 38 (1): 23–38. https://doi.org/10.1038/npp.2012.112.

More, T.T., S. Yan, R.D. Tyagi, and R.Y. Surampalli. 2010. “Potential Use of Filamentous Fungi for Wastewater Sludge Treatment.” *Bioresource Technology* 101 (20): 7691–7700. https://doi.org/10.1016/j.biortech.2010.05.033.

Moretti, Antonio. 2009. “Taxonomy of Fusarium Genus: A Continuous Fight between Lumpers and Splitters.” *Zbornik Matice Srpske Za Prirodne Nauke*, no. 117: 7–13. https://doi.org/10.2298/ZMSPN0917007M.

Morillo-Huesca, Macarena, Marta Clemente-Ruiz, Eloísa Andújar, and Félix Prado. 2010. “The SWR1 Histone Replacement Complex Causes Genetic Instability and Genome-Wide Transcription Misregulation in the Absence of H2A.Z.” *PLoS ONE* 5 (8). https://doi.org/10.1371/journal.pone.0012143.

Moye-Rowley, W. Scott. 2003. “Regulation of the Transcriptional Response to Oxidative Stress in Fungi: Similarities and Differences.” *Eukaryotic Cell* 2 (3): 381–89. https://doi.org/10.1128/ec.2.3.381-389.2003.

Myung, Kyung, Shaojie Li, Robert Butchko, Mark Busman, Robert H Proctor, Hamed Abbas, and Ana M Calvo. 2009. “FvVE1 Regulates Biosynthesis of the Mycotoxins Fumonisins and Fusarins in Fusarium Verticillioides.” *Journal of Agricultural and Food Chemistry* 57 (May): 5089–94. https://doi.org/10.1021/jf900783u.

Nahas, Ely, Hector F. Terenzi, and Antonio Rossi. 1982. “Effect of Carbon Source and PH on the Production and Secretion of Acid Phosphatase (EC 3.1.3.2) and Alkaline Phosphatase (EC 3.1.3.1) in Neurospora Crassa.” *Microbiology-Sgm* 128 (September): 2017–21. https://doi.org/10.1099/00221287-128-9-2017.

Nelson, Paul E, M Cecilia Dignani, and Elias J Anaissie. 1994. “Taxonomy, Biology, and Clinical Aspects of Fusarium Speciest.” *CLIN. MICROBIOL. REV.* 7: 26.

Nelson, Paul E., T. A. Toussoun, and W. F. O. Marasas. 1983. *Fusarium Species: An Illustrated Manual for Identification*. University Park: Pennsylvania State University Press.

Nishida, Hiromi, and Choong-Soo Yun. 2011. “Extraction of Tentative Mobile Introns in Fungal Histone Genes.” *Mobile Genetic Elements* 1 (1): 78–79. https://doi.org/10.4161/mge.1.1.15431.

Nislow, C., E. Ray, and L. Pillus. 1997. “SET1, a Yeast Member of the Trithorax Family, Functions in Transcriptional Silencing and Diverse Cellular Processes.” *Molecular Biology of the Cell* 8 (12): 2421–36. https://doi.org/10.1091/mbc.8.12.2421.

Niu, Guodong. 2010. “TOXICITY OF MYCOTOXINS TO INSECTS AND UNDERLYING MOLECULAR AND BIOCHEMICAL MECHANISMS,” 145.

Nucci, Marcio, and Elias Anaissie. 2007. “Fusarium Infections in Immunocompromised Patients.” *Clinical Microbiology Reviews* 20 (4): 695–704. https://doi.org/10.1128/CMR.00014-07.

Ou, Horng D., Sébastien Phan, Thomas J. Deerinck, Andrea Thor, Mark H. Ellisman, and Clodagh C. O’Shea. 2017. “ChromEMT: Visualizing 3D Chromatin Structure and Compaction in Interphase and Mitotic Cells.” *Science* 357 (6349): eaag0025. https://doi.org/10.1126/science.aag0025.

Park, Young-Jun, Pamela N. Dyer, David J. Tremethick, and Karolin Luger. 2004. “A New Fluorescence Resonance Energy Transfer Approach Demonstrates That the Histone Variant H2AZ Stabilizes the Histone Octamer within the Nucleosome.” *The Journal of Biological Chemistry* 279 (23): 24274–82. https://doi.org/10.1074/jbc.M313152200.

Peñalva, Miguel A., and Herbert N. Arst. 2002. “Regulation of Gene Expression by Ambient PH in Filamentous Fungi and Yeasts.” *Microbiology and Molecular Biology Reviews: MMBR* 66 (3): 426–46, table of contents. https://doi.org/10.1128/mmbr.66.3.426-446.2002.

Peraica, M., B. Radić, A. Lucić, and M. Pavlović. 1999. “Toxic Effects of Mycotoxins in Humans.” *Bulletin of the World Health Organization* 77 (9): 754–66.

Persson, E. Christina, Vikash Sewram, Alison A. Evans, W. Thomas London, Yvette Volkwyn, Yen-Ju Shen, Jacobus A. Van Zyl, et al. 2012. “Fumonisin B1 and Risk of Hepatocellular Carcinoma in Two Chinese Cohorts.” *Food and Chemical Toxicology* 50 (3–4): 679–83. https://doi.org/10.1016/j.fct.2011.11.029.

Peserico, Alessia, and Cristiano Simone. 2011. “Physical and Functional HAT/HDAC Interplay Regulates Protein Acetylation Balance.” In *Journal of Biomedicine & Biotechnology*. https://doi.org/10.1155/2011/371832.

Pestka, J. J. 2008. “Mechanisms of Deoxynivalenol-Induced Gene Expression and Apoptosis.” *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment* 25 (9): 1128–40.

Piña, B., and P. Suau. 1987. “Changes in Histones H2A and H3 Variant Composition in Differentiating and Mature Rat Brain Cortical Neurons.” *Developmental Biology* 123 (1): 51–58. https://doi.org/10.1016/0012-1606(87)90426-X.

Pitt, John I., and Ailsa D. Hocking. 2009. “Primary Keys and Miscellaneous Fungi.” In *Fungi and Food Spoilage*, by John I. Pitt and Ailsa D. Hocking, 53–143. Boston, MA: Springer US. https://doi.org/10.1007/978-0-387-92207-2_5.

Ponts, Nadia, Leslie Couedelo, Laetitia Pinson-Gadais, Marie-Noëlle Verdal-Bonnin, Christian

Barreau, and Florence Richard-Forget. 2009. “Fusarium Response to Oxidative Stress by H2O2 Is Trichothecene Chemotype-Dependent.” *FEMS Microbiology Letters* 293 (2): 255–62. https://doi.org/10.1111/j.1574-6968.2009.01521.x.

Ponts, Nadia, Laetitia Pinson-Gadais, Christian Barreau, Florence Richard-Forget, and Thérèse Ouellet. 2007. “Exogenous H $_2$ O $_2$ and Catalase Treatments Interfere with *Tri* Genes Expression in Liquid Cultures of *Fusarium Graminearum*.” *FEBS Letters* 581 (3): 443–47. https://doi.org/10.1016/j.febslet.2007.01.003.

Ponts, Nadia, Laetitia Pinson-Gadais, Marie-NoÃ«lle Verdal-Bonnin, Christian Barreau, and Florence Richard-Forget. 2006. “Accumulation of Deoxynivalenol and Its 15-Acetylated Form Is Significantly Modulated by Oxidative Stress in Liquid Cultures of Fusarium Graminearum: DON-ADON Accumulation Is Modulated by Oxidative Stress.” *FEMS Microbiology Letters* 258 (1): 102–7. https://doi.org/10.1111/j.1574-6968.2006.00200.x.

Pozzi, C. R., B. Corrêa, J. G. Xavier, G. M. Direito, R. B. Orsi, and S. V. Matarazzo. 2001. “Effects of Prolonged Oral Administration of Fumonisin B1 and Aflatoxin B1 in Rats.” *Mycopathologia* 151 (1): 21–27.

Proctor, R. H., T. M. Hohn, and S. P. McCormick. 1995. “Reduced Virulence of Gibberella Zeae Caused by Disruption of a Trichothecene Toxin Biosynthetic Gene.” *Molecular Plant-Microbe Interactions: MPMI* 8 (4): 593–601.

Protection Against Trichothecene Mycotoxins. 1983. National Academies.

Prussin II, Aaron, Szanyi, Welling, Shane Ross, and David Schmale. 2014. “Estimating the Production and Release of Ascospores from a Field-Scale Source of Fusarium Graminearum Inoculum.” *Plant Disease* 98 (April): 497. https://doi.org/10.1094/PDIS-04-13-0404-RE.

Punt, P. J., and C. A. van den Hondel. 1992. “Transformation of Filamentous Fungi Based on Hygromycin B and Phleomycin Resistance Markers.” *Methods in Enzymology* 216: 447–57. https://doi.org/10.1016/0076-6879(92)16041-h.

Purschwitz, Janina, Sylvia Müller, and Reinhard Fischer. 2009. “Mapping the Interaction Sites of Aspergillus Nidulans Phytochrome FphA with the Global Regulator VeA and the White Collar Protein LreB.” *Molecular Genetics and Genomics: MGG* 281 (1): 35–42. https://doi.org/10.1007/s00438-008-0390-x.

Purschwitz, Janina, Sylvia Müller, Christian Kastner, Michelle Schöser, Hubertus Haas, Eduardo A. Espeso, Ali Atoui, Ana M. Calvo, and Reinhard Fischer. 2008. “Functional and Physical Interaction of Blue- and Red-Light Sensors in Aspergillus Nidulans.” *Current Biology: CB* 18 (4): 255–59. https://doi.org/10.1016/j.cub.2008.01.061.

“Regulations.” 2019. May 10, 2019. http://www.mycotoxins.info/en/regulations/.

Reinhard, Fischer, and Riquleme Meritxell. 2017. “Unravelling the Riddle of the Filamentous Fungi,” 2017.

Reyes-Dominguez, Yazmid, Stefan Boedi, Michael Sulyok, Gerlinde Wiesenberger, Norbert Stoppacher, Rudolf Krska, and Joseph Strauss. 2012. “Heterochromatin Influences the Secondary Metabolite Profile in the Plant Pathogen Fusarium Graminearum.” *Fungal Genetics and Biology* 49 (1–3): 39–47. https://doi.org/10.1016/j.fgb.2011.11.002.

Reyes-Dominguez, Yazmid, Jin Woo Bok, Harald Berger, E. Keats Shwab, Asjad Basheer, Andreas Gallmetzer, Claudio Scazzocchio, Nancy Keller, and Joseph Strauss. 2010. “Heterochromatic Marks Are Associated with the Repression of Secondary Metabolism Clusters in Aspergillus Nidulans.” *Molecular Microbiology* 76 (6): 1376–86. https://doi.org/10.1111/j.1365-2958.2010.07051.x.

Ries, Laure N. A., Sarah R. Beattie, Eduardo A. Espeso, Robert A. Cramer, and Gustavo H. Goldman. 2016. “Diverse Regulation of the CreA Carbon Catabolite Repressor in Aspergillus Nidulans.” *Genetics* 203 (1): 335–52. https://doi.org/10.1534/genetics.116.187872.

Robens, J, and K Cardwell. 2003. “The Costs of Mycotoxin Management to the USA: Management of Aflatoxins in the United States: Journal of Toxicology: Toxin Reviews: Vol 22, No 2-3.” 2003. https://www.tandfonline.com/doi/abs/10.1081/TXR-120024089.

Rodrigues-Pousada, Claudina, Regina A. Menezes, and Catarina Pimentel. 2010. “The Yap Family and Its Role in Stress Response.” *Yeast (Chichester, England)* 27 (5): 245–58. https://doi.org/10.1002/yea.1752.

Roguev, A. 2001. “The Saccharomyces Cerevisiae Set1 Complex Includes an Ash2 Homologue and Methylates Histone 3 Lysine 4.” *The EMBO Journal* 20 (24): 7137–48. https://doi.org/10.1093/emboj/20.24.7137.

Rojas Echenique, José I., Sergey Kryazhimskiy, Alex N. Nguyen Ba, and Michael M. Desai. 2019. “Modular Epistasis and the Compensatory Evolution of Gene Deletion Mutants.” Edited by Geraldine Butler. *PLOS Genetics* 15 (2): e1007958. https://doi.org/10.1371/journal.pgen.1007958.

Rountree, M. R., and E. U. Selker. 2010. “DNA Methylation and the Formation of Heterochromatin in *Neurospora Crassa*.” *Heredity* 105 (1): 38–44. https://doi.org/10.1038/hdy.2010.44.

Sánchez-Rangel, Diana, Eric-Edmundo Hernández-Domínguez, Claudia-Anahí Pérez-Torres, Randy Ortiz-Castro, Emanuel Villafán, Benjamín Rodríguez-Haas, Alexandro Alonso-Sánchez, et al. 2018. “Environmental PH Modulates Transcriptomic Responses in the Fungus Fusarium Sp. Associated with KSHB Euwallacea Sp. near Fornicatus.” *BMC Genomics* 19 (1). https://doi.org/10.1186/s12864-018-5083-1.

Sankaran, Sindhuja, Samir Kumar Khanal, Nagapadma Jasti, Bo Jin, Anthony L. Pometto III, and J. Hans Van Leeuwen. 2010. “Use of Filamentous Fungi for Wastewater Treatment and Production of High Value Fungal Byproducts: A Review.” *Critical Reviews in Environmental Science and Technology* 40 (5): 400–449. https://doi.org/10.1080/10643380802278943.

Santos-Rosa, Helena, Robert Schneider, Andrew J. Bannister, Julia Sherriff, Bradley E. Bernstein, N. C. Tolga Emre, Stuart L. Schreiber, Jane Mellor, and Tony Kouzarides. 2002. “Active Genes Are Tri-Methylated at K4 of Histone H3.” *Nature* 419 (6905): 407–11. https://doi.org/10.1038/nature01080.

Sarikaya Bayram, Ozlem, Ozgür Bayram, Oliver Valerius, Hee Soo Park, Stefan Irniger, Jennifer Gerke, Min Ni, Kap-Hoon Han, Jae-Hyuk Yu, and Gerhard H. Braus. 2010. “LaeA Control of Velvet Family Regulatory Proteins for Light-Dependent Development and Fungal Cell-Type Specificity.” *PLoS Genetics* 6 (12): e1001226. https://doi.org/10.1371/journal.pgen.1001226.

Sarma, Kavitha, and Danny Reinberg. 2005. “Histone Variants Meet Their Match.” *Nature Reviews Molecular Cell Biology* 6 (2): 139–49. https://doi.org/10.1038/nrm1567.

Scazzocchio, C. 2000. “The Fungal GATA Factors.” *Current Opinion in Microbiology* 3 (2): 126–31.

Schmitt, E. K., R. Kempken, and U. Kück. 2001. “Functional Analysis of Promoter Sequences of Cephalosporin C Biosynthesis Genes from Acremonium Chrysogenum: Specific DNA-Protein Interactions and Characterization of the Transcription Factor PACC.” *Molecular Genetics and Genomics: MGG* 265 (3): 508–18.

Schneider, Caroline A., Wayne S. Rasband, and Kevin W. Eliceiri. 2012. “NIH Image to ImageJ: 25 Years of Image Analysis.” *Nature Methods* 9 (7): 671–75. https://doi.org/10.1038/nmeth.2089.

Schübeler, Dirk. 2015. “Function and Information Content of DNA Methylation.” *Nature* 517 (7534): 321–26. https://doi.org/10.1038/nature14192.

Schuldiner, Maya, Sean R. Collins, Natalie J. Thompson, Vladimir Denic, Arunashree Bhamidipati, Thanuja Punna, Jan Ihmels, et al. 2005. “Exploration of the Function and Organization of the Yeast Early Secretory Pathway through an Epistatic Miniarray Profile.” *Cell* 123 (3): 507–19. https://doi.org/10.1016/j.cell.2005.08.031.

Schulmeister, Alexandra, Martina Schmid, and Eric M. Thompson. 2007. “Phosphorylation of the Histone H3.3 Variant in Mitosis and Meiosis of the Urochordate Oikopleura Dioica.” *Chromosome Research: An International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology* 15 (2): 189–201. https://doi.org/10.1007/s10577-006-1112-z.

Selker, E. U. 2004. “Genome Defense and DNA Methylation in Neurospora.” *Cold Spring*

Harbor Symposia on Quantitative Biology 69 (January): 119–24. https://doi.org/10.1101/sqb.2004.69.119.

Selker, E. U., and J. N. Stevens. 1985. “DNA Methylation at Asymmetric Sites Is Associated with Numerous Transition Mutations.” *Proceedings of the National Academy of Sciences of the United States of America* 82 (23): 8114–18. https://doi.org/10.1073/pnas.82.23.8114.

Seong, Kye-Yong, Matias Pasquali, Xiaoying Zhou, Jongwoo Song, Karen Hilburn, Susan McCormick, Yanhong Dong, Jin-Rong Xu, and H. Corby Kistler. 2009. “Global Gene Regulation by Fusarium Transcription Factors Tri6 and Tri10 Reveals Adaptations for Toxin Biosynthesis.” *Molecular Microbiology* 72 (2): 354–67. https://doi.org/10.1111/j.1365-2958.2009.06649.x.

Sevilla, Ana, and Olivier Binda. 2014. “Post-Translational Modifications of the Histone Variant H2az.” *Stem Cell Research* 12 (1): 289–95. https://doi.org/10.1016/j.scr.2013.11.004.

Seymour, Michael, Lexiang Ji, Alex M. Santos, Masayuki Kamei, Takahiko Sasaki, Evelina Y. Basenko, Robert J. Schmitz, Xiaoyu Zhang, and Zachary A. Lewis. 2016. “Histone H1 Limits DNA Methylation in Neurospora Crassa.” *G3 (Bethesda, Md.)* 6 (7): 1879–89. https://doi.org/10.1534/g3.116.028324.

Shaaban, Mona, Jonathan Palmer, Wael A. EL-Naggar, M. A. EL-Sokkary, EL-Sayed E. Habib, and Nancy P. Keller. 2010. “Involvement of Transposon-Like Elements in Penicillin Gene Cluster Regulation.” *Fungal Genetics and Biology : FG & B* 47 (5): 423–32. https://doi.org/10.1016/j.fgb.2010.02.006.

Shetty, Nandini P., Hans J. Lyngs Jørgensen, Jens Due Jensen, David B. Collinge, and H. Shekar Shetty. 2008. “Roles of Reactive Oxygen Species in Interactions between Plants and Pathogens.” *European Journal of Plant Pathology* 121 (3): 267–80. https://doi.org/10.1007/s10658-008-9302-5.

Shi, Leilei, Hong Wen, and Xiaobing Shi. 2017. “The Histone Variant H3.3 in Transcriptional Regulation and Human Disease.” *Journal of Molecular Biology* 429 (13): 1934–45. https://doi.org/10.1016/j.jmb.2016.11.019.

Shilatifard, Ali. 2012. “The COMPASS Family of Histone H3K4 Methylases: Mechanisms of Regulation in Development and Disease Pathogenesis.” *Annual Review of Biochemistry* 81: 65–95. https://doi.org/10.1146/annurev-biochem-051710-134100.

Smith, Kristina M., Pallavi A. Phatale, Christopher M. Sullivan, Kyle R. Pomraning, and Michael Freitag. 2011. “Heterochromatin Is Required for Normal Distribution of Neurospora Crassa CenH3 ▿.” *Molecular and Cellular Biology* 31 (12): 2528–42. https://doi.org/10.1128/MCB.01285-10.

Snitkin, Evan S., and Daniel Segrè. 2011. “Epistatic Interaction Maps Relative to Multiple Metabolic Phenotypes.” *PLOS Genetics* 7 (2): e1001294.

https://doi.org/10.1371/journal.pgen.1001294.

Storz, Jay F. 2018. “Compensatory Mutations and Epistasis for Protein Function.” *Current Opinion in Structural Biology* 50: 18–25. https://doi.org/10.1016/j.sbi.2017.10.009.

Studt, L., F. J. Schmidt, L. Jahn, C. M. K. Sieber, L. R. Connolly, E.-M. Niehaus, M. Freitag, H.-U. Humpf, and B. Tudzynski. 2013. “Two Histone Deacetylases, FfHda1 and FfHda2, Are Important for Fusarium Fujikuroi Secondary Metabolism and Virulence.” *Applied and Environmental Microbiology* 79 (24): 7719–34. https://doi.org/10.1128/AEM.01557-13.

Studt, Lena, Slavica Janevska, Birgit Arndt, Stefan Boedi, Michael Sulyok, Hans-Ulrich Humpf, Bettina Tudzynski, and Joseph Strauss. 2016. “Lack of the COMPASS Component Ccl1 Reduces H3K4 Trimethylation Levels and Affects Transcription of Secondary Metabolite Genes in Two Plant-Pathogenic Fusarium Species.” *Frontiers in Microbiology* 7: 2144. https://doi.org/10.3389/fmicb.2016.02144.

Studt, Lena, Sarah M. Rösler, Immo Burkhardt, Birgit Arndt, Michael Freitag, Hans-Ulrich Humpf, Jeroen S. Dickschat, and Bettina Tudzynski. 2016. “Knock-down of the Methyltransferase Kmt6 Relieves H3K27me3 and Results in Induction of Cryptic and Otherwise Silent Secondary Metabolite Gene Clusters in Fusarium Fujikuroi.” *Environmental Microbiology* 18 (11): 4037–54. https://doi.org/10.1111/1462-2920.13427.

Suárez, T., and M. A. Peñalva. 1996. “Characterization of a Penicillium Chrysogenum Gene Encoding a PacC Transcription Factor and Its Binding Sites in the Divergent PcbAB-PcbC Promoter of the Penicillin Biosynthetic Cluster.” *Molecular Microbiology* 20 (3): 529–40. https://doi.org/10.1046/j.1365-2958.1996.5421065.x.

Sun, Zhen, and Emily Bernstein. 2019. “Histone Variant MacroH2A: From Chromatin Deposition to Molecular Function.” *Essays In Biochemistry*, April, EBC20180062. https://doi.org/10.1042/EBC20180062.

Sydenham, E. W., W. F. Marasas, P. G. Thiel, G. S. Shephard, and J. J. Nieuwenhuis. 1991. “Production of Mycotoxins by Selected Fusarium Graminearum and F. Crookwellense Isolates.” *Food Additives and Contaminants* 8 (1): 31–41. https://doi.org/10.1080/02652039109373953.

Sydenham, Eric W., Gordon S. Shephard, Pieter G. Thiel, Walter F. O. Marasas, and Sonja Stockenstrom. 1991. “Fumonisin Contamination of Commercial Corn-Based Human Foodstuffs.” *Journal of Agricultural and Food Chemistry* 39 (11): 2014–18. https://doi.org/10.1021/jf00011a028.

Takami, Yasunari, Shunichi Takeda, and Tatsuo Nakayama. 1995. “Targeted Disruption of H2B-V Encoding a Particular H2B Histone Variant Causes Changes in Protein Patterns on Two-Dimensional Polyacrylamide Gel Electrophoresis in the DT40 Chicken B Cell Line.” *Journal of Biological Chemistry* 270 (51): 30664–70. https://doi.org/10.1074/jbc.270.51.30664.

Talbert, Paul B, Kami Ahmad, Geneviève Almouzni, Juan Ausió, Frederic Berger, Prem L Bhalla, William M Bonner, et al. 2012. “A Unified Phylogeny-Based Nomenclature for Histone Variants.” *Epigenetics & Chromatin* 5 (June): 7. https://doi.org/10.1186/1756-8935-5-7.

Talbert, Paul B., and Steven Henikoff. 2014. “Environmental Responses Mediated by Histone Variants.” *Trends in Cell Biology* 24 (11): 642–50. https://doi.org/10.1016/j.tcb.2014.07.006.

Tautz, D. 1992. “Redundancies, Development and the Flow of Information.” *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* 14 (4): 263–66. https://doi.org/10.1002/bies.950140410.

Teng, Xinchen, Margaret Dayhoff-Brannigan, Wen-Chih Cheng, Catherine E. Gilbert, Cierra N. Sing, Nicola L. Diny, Sarah J. Wheelan, et al. 2013. “Genome-Wide Consequences of Deleting Any Single Gene.” *Molecular Cell* 52 (4): 485–94. https://doi.org/10.1016/j.molcel.2013.09.026.

Thambirajah, Anita A., Deanna Dryhurst, Toyotaka Ishibashi, Andra Li, Allison H. Maffey, and Juan Ausió. 2006. “H2A.Z Stabilizes Chromatin in a Way That Is Dependent on Core Histone Acetylation.” *Journal of Biological Chemistry* 281 (29): 20036–44. https://doi.org/10.1074/jbc.M601975200.

Tilburn, J., S. Sarkar, D.A. Widdick, E.A. Espeso, M. Orejas, J. Mungroo, M.A. Peñalva, and H.N. Arst. 1995. “The Aspergillus PacC Zinc Finger Transcription Factor Mediates Regulation of Both Acid- and Alkaline-Expressed Genes by Ambient PH.” *The EMBO Journal* 14 (4): 779–90. https://doi.org/10.1002/j.1460-2075.1995.tb07056.x.

Tisch, Doris, and Monika Schmoll. 2010. “Light Regulation of Metabolic Pathways in Fungi.” *Applied Microbiology and Biotechnology* 85 (5): 1259–77. https://doi.org/10.1007/s00253-009-2320-1.

Tost, Jörg. 2010. “DNA Methylation: An Introduction to the Biology and the Disease-Associated Changes of a Promising Biomarker.” *Molecular Biotechnology* 44 (1): 71–81. https://doi.org/10.1007/s12033-009-9216-2.

Tupaki-Sreepurna, Ananya, and Anupma Jyoti Kindo. 2018. “Fusarium: The Versatile Pathogen.” *Indian Journal of Medical Microbiology* 36 (1): 8. https://doi.org/10.4103/ijmm.IJMM_16_24.

Turner, Nicholas W., Sreenath Subrahmanyam, and Sergey A. Piletsky. 2009. “Analytical Methods for Determination of Mycotoxins: A Review.” *Analytica Chimica Acta* 632 (2): 168–80. https://doi.org/10.1016/j.aca.2008.11.010.

Ueno, Y. 1985. “The Toxicology of Mycotoxins.” *Critical Reviews in Toxicology* 14 (2): 99–132. https://doi.org/10.3109/10408448509089851.

Volpe, Thomas A., Catherine Kidner, Ira M. Hall, Grace Teng, Shiv I. S. Grewal, and Robert A.

Martienssen. 2002. “Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi.” *Science* 297 (5588): 1833–37. https://doi.org/10.1126/science.1074973.

Voss, K. A., G. W. Smith, and W. M. Haschek. 2007. “Fumonisins: Toxicokinetics, Mechanism of Action and Toxicity.” *Animal Feed Science and Technology*, Fusarium and their toxins: Mycology, occurrence, toxicity, control and economic impact, 137 (3): 299–325. https://doi.org/10.1016/j.anifeedsci.2007.06.007.

W Peplow, Andrew, Isaac B Meek, Melinda C Wiles, Timothy Phillips, and Marian N Beremand. 2003. “Tri16 Is Required for Esterification of Position C-8 during Trichothecene Mycotoxin Production by Fusarium Sporotrichioides.” *Applied and Environmental Microbiology* 69 (November): 5935–40. https://doi.org/10.1128/AEM.69.10.5935-5940.2003.

Wang, E., W. P. Norred, C. W. Bacon, R. T. Riley, and A. H. Merrill. 1991. “Inhibition of Sphingolipid Biosynthesis by Fumonisins. Implications for Diseases Associated with Fusarium Moniliforme.” *The Journal of Biological Chemistry* 266 (22): 14486–90.

Wang, G., A. Ma, C. M. Chow, D. Horsley, N. R. Brown, I. G. Cowell, and P. B. Singh. 2000. “Conservation of Heterochromatin Protein 1 Function.” *Molecular and Cellular Biology* 20 (18): 6970–83. https://doi.org/10.1128/mcb.20.18.6970-6983.2000.

Wang, Gang G., C. David Allis, and Ping Chi. 2007. “Chromatin Remodeling and Cancer, Part II: ATP-Dependent Chromatin Remodeling.” *Trends in Molecular Medicine* 13 (9): 373–80. https://doi.org/10.1016/j.molmed.2007.07.004.

Wang, H., H. Wei, J. Ma, and X. Luo. 2000. “The Fumonisin B1 Content in Corn from North China, a High-Risk Area of Esophageal Cancer.” *Journal of Environmental Pathology, Toxicology and Oncology: Official Organ of the International Society for Environmental Toxicology and Cancer* 19 (1–2): 139–41.

Weidenbörner, Martin. 2007. *Mycotoxins in Feedstuffs*. New York: Springer-Verlag. https://www.springer.com/gp/book/9780387464114.

Wessler, S. R. 2006. “Transposable Elements and the Evolution of Eukaryotic Genomes.” *Proceedings of the National Academy of Sciences* 103 (47): 17600–601. https://doi.org/10.1073/pnas.0607612103.

Westphal, Klaus Ringsborg, Rasmus Dam Wollenberg, Florian-Alexander Herbst, Jens Laurids Sørensen, Teis Esben Sondergaard, and Reinhard Wimmer. 2018. “Enhancing the Production of the Fungal Pigment Aurofusarin in Fusarium Graminearum.” *Toxins* 10 (11). https://doi.org/10.3390/toxins10110485.

Wiemann, Philipp, Daren W. Brown, Karin Kleigrewe, Jin Woo Bok, Nancy P. Keller, Hans-

Ulrich Humpf, and Bettina Tudzynski. 2010. “FfVel1 and FfLae1, Components of a Velvet-like Complex in Fusarium Fujikuroi, Affect Differentiation, Secondary Metabolism and Virulence.” *Molecular Microbiology* 77 (4): 972–94. https://doi.org/10.1111/j.1365-2958.2010.07263.x.

Windels, C. E. 2000. “Economic and Social Impacts of Fusarium Head Blight: Changing Farms and Rural Communities in the Northern Great Plains.” *Phytopathology* 90 (1): 17–21. https://doi.org/10.1094/PHYTO.2000.90.1.17.

Wong, Koon Ho, Michael J. Hynes, and Meryl A. Davis. 2008. “Recent Advances in Nitrogen Regulation: A Comparison between Saccharomyces Cerevisiae and Filamentous Fungi.” *Eukaryotic Cell* 7 (6): 917–25. https://doi.org/10.1128/EC.00076-08.

Wu, Felicia. 2007. “Measuring the Economic Impacts of Fusarium Toxins in Animal Feeds.” *Animal Feed Science and Technology*, Fusarium and their toxins: Mycology, occurrence, toxicity, control and economic impact, 137 (3): 363–74. https://doi.org/10.1016/j.anifeedsci.2007.06.010.

Xu, Yong Zhong, Cynthia Kanagaratham, and Danuta Radzioch. 2013. “Chromatin Remodelling During Host-Bacterial Pathogen Interaction.” *Chromatin Remodelling*, April. https://doi.org/10.5772/55977.

Xue-Franzén, Yongtao, Johan Henriksson, Thomas R. Bürglin, and Anthony P. H. Wright. 2013. “Distinct Roles of the Gcn5 Histone Acetyltransferase Revealed during Transient Stress-Induced Reprogramming of the Genome.” *BMC Genomics* 14 (July): 479. https://doi.org/10.1186/1471-2164-14-479.

Yan, C, L H Lee, and L I Davis. 1998. “Crm1p Mediates Regulated Nuclear Export of a Yeast AP-1-like Transcription Factor.” *The EMBO Journal* 17 (24): 7416–29. https://doi.org/10.1093/emboj/17.24.7416.

Yang, Fen, Susanne Jacobsen, Hans J. L. Jørgensen, David B. Collinge, Birte Svensson, and Christine Finnie. 2013. “Fusarium Graminearum and Its Interactions with Cereal Heads: Studies in the Proteomics Era.” *Frontiers in Plant Science* 4: 37. https://doi.org/10.3389/fpls.2013.00037.

You, Bang-Jau, Miin-Huey Lee, and Kuang-Ren Chung. 2009. “Gene-Specific Disruption in the Filamentous Fungus Cercospora Nicotianae Using a Split-Marker Approach.” *Archives of Microbiology* 191 (7): 615–22. https://doi.org/10.1007/s00203-009-0489-4.

Zhang, Huiming, Zhaobo Lang, and Jian-Kang Zhu. 2018. “Dynamics and Function of DNA Methylation in Plants.” *Nature Reviews Molecular Cell Biology* 19 (8): 489–506. https://doi.org/10.1038/s41580-018-0016-z.

Zhang, Jianzhi. 2012. “Genetic Redundancies and Their Evolutionary Maintenance.” In *Evolutionary Systems Biology*, edited by Orkun S. Soyer, 751:279–300. New York, NY: Springer

New York. http://link.springer.com/10.1007/978-1-4614-3567-9_13.

Zhang, Xiaoli, Xinqiang Liu, Yanli Zhao, Jiasen Cheng, Jiatao Xie, Yanping Fu, Daohong Jiang, and Tao Chen. 2016. “Histone H3 Lysine 9 Methyltransferase DIM5 Is Required for the Development and Virulence of Botrytis Cinerea.” *Frontiers in Microbiology* 7 (August). https://doi.org/10.3389/fmicb.2016.01289.

Zhang, Xiaoyu, Junshi Yazaki, Ambika Sundaresan, Shawn Cokus, Simon W.-L. Chan, Huaming Chen, Ian R. Henderson, et al. 2006. “Genome-Wide High-Resolution Mapping and Functional Analysis of DNA Methylation in Arabidopsis.” *Cell* 126 (6): 1189–1201. https://doi.org/10.1016/j.cell.2006.08.003.

Zheng, Dawei, Shijie Zhang, Xiaoying Zhou, Chenfang Wang, Ping Xiang, Qian Zheng, and Jin-Rong Xu. 2012. “The FgHOG1 Pathway Regulates Hyphal Growth, Stress Responses, and Plant Infection in Fusarium Graminearum.” *PLOS ONE* 7 (11): e49495. https://doi.org/10.1371/journal.pone.0049495.

Zinedine, Abdellah, Jose Miguel Soriano, Juan Carlos Moltó, and Jordi Mañes. 2007. “Review on the Toxicity, Occurrence, Metabolism, Detoxification, Regulations and Intake of Zearalenone: An Oestrogenic Mycotoxin.” *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association* 45 (1): 1–18. https://doi.org/10.1016/j.fct.2006.07.030.

Régulation épigénétique de la production de mycotoxines chez *Fusarium graminearum*

Résumé:

La contamination des aliments par les mycotoxines représente un risque potentiel pour la santé humaine et animale. Des rapports de l'Autorité européenne de sécurité des aliments, l'EFSA, indiquent que près de la moitié des aliments dérivés des céréales et de céréales non transformées collectées entre 2007 et 2012 dans 21 pays européens étaient contaminées par des trichothécènes de type B, ou TCTB, et en particulier du déoxynivalénol, ou DON. Ces mycotoxines sont produites par des champignons phytopathogènes sur les grains en cours de remplissage, avant la récolte. Dans un contexte de changement climatique, cette situation pourrait s'aggraver. Ainsi, maitriser les contaminations en mycotoxines est une tâche urgente qui ne peut être repoussée. En Europe, le DON est principalement produit par *Fusarium graminearum*. Si les étapes de la voie de biosynthèse des TCTB sont assez bien décrites, les mécanismes moléculaires impliqués dans la régulation de cette voie restent, à ce jour, mal compris.

Des études récentes ont mis en évidence que les modifications post-traductionnelles des histones canoniques ainsi que de leurs variantes joue un rôle critique dans les régulations des biosynthèses des mycotoxines et autres métabolites secondaires chez les champignons filamenteux, en modifiant la structure de la chromatine. Chez *F. graminearum*, il a été prouvé que l'histone désacétylase HDF1 est impliquée dans l'activation de la production de DON. Par contre, la marque histone H3K27me3 réprime 14 % de son génome, y compris des gènes impliqués dans les voies métaboliques secondaires. La variante d'histone H2A.Z, trouvée de façon ubiquitaire chez les eucaryotes, participe à de nombreux processus biologiques dont la stabilité génomique, la réparation de l'ADN, la régulation de transcription et la désactivation des télomères. Toutefois, les mécanismes sous-jacents de ces fonctions restent flous. Chez certaines espèces, la fonction de H2A.Z semble essentielle. À ce jour, la seule étude ciblant directement la fonction de H2A.Z chez les champignons filamenteux a été réalisée chez *Neurospora crassa* et a identifié son rôle dans la réponse au stress oxydatif. Nous avons ici fait l'hypothèse que H2A.Z est impliquée dans des processus biologiques importants chez *F. graminearum*, y compris ceux impliqués dans la production de métabolites secondaires dont les mycotoxines. Ce projet vise ainsi à caractériser les rôles joués par H2A.Z dans le contrôle du développement, du métabolisme et de la virulence chez *F. graminearum*.

À l'aide d'une approche de génétique inverse, nous avons créé six mutants ne possédant plus le gène codant H2A.Z dans trois souches différentes de *F. graminearum.* Tous les mutants présentent un déficit en sporulation, germination, croissance radiale et production de DON. Cependant, l'intensité des effets observés dépend du fond génétique considéré. En outre, le rajout du gène sauvage codant H2A.Z ne restaure pas les phénotypes sauvages. Les séquençages des génomes complets des mutants ont montrés que, bien que H2A.Z ait été totalement éliminé du génome, des mutations compensatoires se produisent à d'autres loci, indépendamment du fond génétique, dans des gènes impliqués dans le remodelage de la chromatine. De manière frappante, une mutation supplémentaire a été détectée dans les mutants délétés pour H2AZ dans lesquels l'allèle sauvage a été réintroduit. Nous avons également construit les mutants surexprimés H2A.Z, mais aucune différence significative de phénotype entre les mutants et la souche sauvage n'a été observée. L'ensemble de ces résultats indique que H2A.Z est essentielle chez F. graminearum, l'occurrence de mutations compensatoires ayant compensé l'effet létal de la délétion infligée. Nous émettons l'hypothèse que les profondes réorganisations des réseaux génétique permettent cette plasticité, avec certaines conséquences en termes d'évolution et d'adaptation.

Mots clés:

Chromatine; H2A.Z; mycotoxines; mutation compensatoire

Epigenetic regulations of the production of mycotoxins by *Fusarium graminearum*

Abstract:

Contamination of food with mycotoxins is a significant risk to human and animal health. Reports from the European Food Safety Authority indicate that nearly half of the food derived from cereals and unprocessed grains collected between 2007 and 2012 in 21 European countries were contaminated with Type B trichothecenes, or TCTB, and especially deoxynivalenol, or DON. These mycotoxins are produced by fungal phytopathogens on growing kernels, before harvest. On account of the global climate change, this situation may become increasingly serious. Therefore, preventing the production of mycotoxins is a task which brooks no delay. In Europe, DON is predominantly produced by *Fusarium graminearum*. Even though the sequential steps of the TCTB biosynthetic pathway are fairly well described, the molecular events involved in regulating this pathway are complex and remain, so far, widely misunderstood.

Recent studies highlighted post-translational modifications of canonical histones as well as their variants as critical players in the regulation of mycotoxin and other secondary metabolite biosyntheses in filamentous fungi, by altering chromatin structure. In *F. graminearum*, it was shown that the histone deacetylase HDF1 could be involved in the activation of DON production. In contrast, H3K27me3 histone mark represses 14 % of its genome, including genes involved in secondary metabolic pathways. Histone variant H2A.Z is ubiquitous in eukaryotes and is involved in a diverse range of biological processes, including genome stability, DNA repair, transcriptional regulation and telomere silencing. However, the underlying mechanisms of these functions remain unclear. In some species, the function of H2A.Z appears to be essential. Up to now, the only one study targeted directly on the function of H2A.Z in filamentous fungi was carried out on *Neurospora crassa*, and identifying a role in oxidative stress response. Here, we hypothesized that H2A.Z may be involved in important biological processes of *F. graminearum* including those involved in the production of secondary metabolism. Therefore, this project aims to characterize the roles played by the histone variant H2A.Z in controlling development, metabolism and virulence in *F. graminearum.*

Using a reverse genetics approach, we created six H2A.Z deleted mutants in three different *F. graminearum* strains. All mutants exhibit deficiency in sporulation, germination, radial growth and DON production; however, intensities in the observed effects depend on the considered genetic background. Additionally, adding back wild-type H2A.Z could not rescue mutant phenotypes. Whole-genome sequencing showed that, although H2A.Z has been totally removed from the genome, compensatory mutations occur at other sites in each mutant regardless of the genetic background, in genes involved in chromatin remodeling. Strikingly, one extra mutation was detected in the H2A.Z add-back mutants. H2A.Z overexpressed mutants have also been constructed, but no significant difference in phenotype can be observed with wild type. Considering our results as a whole, we draw the conclusion that H2A.Z is essential in *F. graminearum.* It is the occurrence of compensatory mutations that rescued part of the lethality caused by H2A.Z deletion. We hypothesize that profound reorganizations of gene networks allow such plasticity, with certain consequences in terms of evolution and adaptation.

Keywords:

Chromatin; H2A.Z; mycotoxins; compensatory mutation

INRA – UR1264 MycSA

71 avenue Edouard Bourlaux, CS20032
33882 Villenave d'Ornon Cedex, France