


## Minireview

# Post-translational modifications drive secondary metabolite biosynthesis in *Aspergillus*: a review

Kunlong Yang <sup>1,2</sup>, Jun Tian<sup>1</sup> and Nancy P. Keller <sup>2\*</sup>

<sup>1</sup>School of Life Science, Jiangsu Normal University, Xuzhou, 221116, Jiangsu Province, People's Republic of China.

<sup>2</sup>Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin 53705.

## Summary

**Post-translational modifications (PTMs) are important for protein function and regulate multiple cellular processes and secondary metabolites (SMs) in fungi. *Aspergillus* species belong to a genus renown for an abundance of bioactive secondary metabolites, many important as toxins, pharmaceuticals and in industrial production. The genes required for secondary metabolites are typically co-localized in biosynthetic gene clusters (BGCs), which often localize in heterochromatic regions of genome and are ‘turned off’ under laboratory condition. Efforts have been made to ‘turn on’ these BGCs by genetic manipulation of histone modifications, which could convert the heterochromatic structure to euchromatin. Additionally, non-histone PTMs also play critical roles in the regulation of secondary metabolism. In this review, we collate the known roles of epigenetic and PTMs on *Aspergillus* SM production. We also summarize the proteomics approaches and bioinformatics tools for PTM identification and prediction and provide future perspectives on the emerging roles of PTM on regulation of SM biosynthesis in *Aspergillus* and other fungi.**

## Introduction

The genus *Aspergillus* is among the most common fungal taxon, which consists of a few hundred species

(Samson *et al.*, 2014). These saprophytic filamentous fungi are typically found in soil or organic debris and are adaptive and resilient to fluctuating environment conditions, growing under a wide range of temperature and at relatively low humidity. Most *Aspergillus* spp. produce abundant secondary metabolites (SMs) and fermentative enzymes, leading to a close relationship with human health, daily life and industry production. On the detrimental side are harmful mycotoxins produced by *A. flavus*, *A. parasiticus*, *A. nidulans* (Keller *et al.*, 2005) and the human pathogens – primarily *A. fumigatus* and *A. flavus* – causing invasive aspergillosis (Patterson *et al.*, 2000). On the other hand, several industrial spp. such as *A. oryzae* (Abe *et al.*, 2006) and *A. niger* (Baker, 2006) produce useful enzymes and/or fermented foods. *A. cvjetkovicii*, dominated at asymptomatic rice phyllosphere microenvironment, produces such SMs as 2 (3H)-benzofuranone and azulene to suppress the invasion of the rice blast *Magnaporthe oryzae* during different growth stages (Fan *et al.*, 2019). Dozens of *Aspergillus* genomes have been sequenced and functionally annotated, which shows a large number of biosynthetic gene clusters (BGCs) in these fungi (Kjaerbolling *et al.*, 2020; Drott *et al.*, 2021), which are responsible for the biosynthesis of secondary metabolites, including mycotoxins. Secondary metabolites play critical roles in fungal development and combating other organisms and can serve as virulence factors when invading plant or animal hosts.

The great impact of SMs produced by *Aspergillus* on human activities has driven intense focus on characterizing the *Aspergillus* secondary metabolome. The production of fungal SMs is limited to very specific ecological conditions (Collemare and Seidl, 2019) and most of the BGCs are silent in laboratory conditions. Epigenetic and post-translational modifications (PTMs) have been suggested to regulate transcription of genes and are considered to be involved not only in secondary metabolism but general environmental responses in most eukaryotes (Shwab *et al.*, 2007; Cichewicz, 2010; Yan *et al.*, 2015). In this review, we first make a brief introduction of secondary metabolite cluster architecture and regulation in

Received 8 January, 2022; revised 23 April, 2022; accepted 26 April, 2022. \*For correspondence. E-mail: [npkeller@wisc.edu](mailto:npkeller@wisc.edu) Tel. (608) 262 9795; Fax (608) 262-8418.

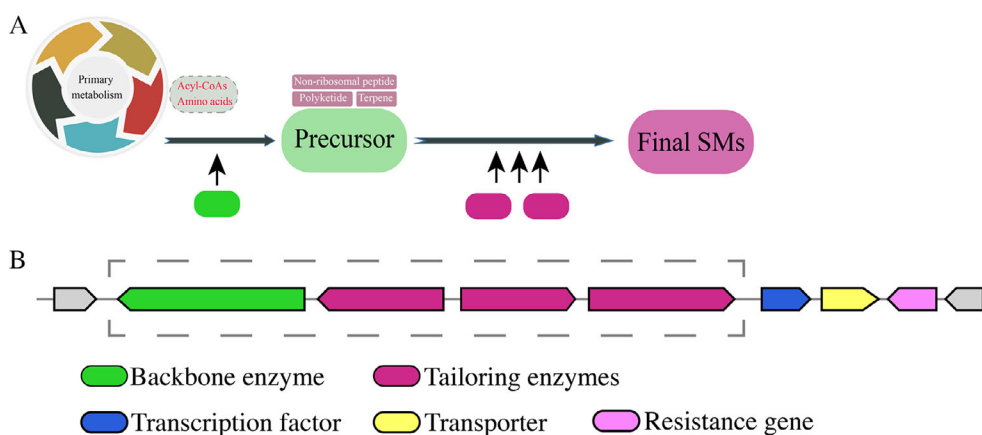
*Aspergillus* and then focus on post-translational regulation on the *Aspergillus* secondary metabolism. Finally, we also explore the connection between different PTMs on SM biosynthesis and highlight some questions that still remain to be answered.

### Overview of secondary metabolite cluster organization and regulation in *Aspergillus*

In *Aspergillus* spp. or other filamentous fungi, the synthesis of secondary metabolites is preliminarily generated from precursors catalysed by the backbone (also called core) enzymes (e.g. polyketides, non-ribosomal peptides, terpenes, PKS-NRPS hybrids and indole alkaloids) that use primary metabolites (acyl-CoA, amino acids) to generate a SM carbon backbone (Lim and Keller, 2014; Keller, 2018) (Fig. 1A). Further modification of the carbon backbone is modulated by the tailoring enzymes to form the final BGC metabolite(s). The gene number of BGCs can range from two (such as the valactamide gene cluster) (Keller, 2018) to more than 20 (such as the aflatoxin gene cluster, Fig. 6B). Genes encoding both backbone enzymes and tailoring enzymes are necessary for all canonical BGCs. Additionally, the BGCs may contain genes encoding transporters that are found to transfer the SM or its precursor, a cluster-specific transcription factor that positively regulates the other genes within the BGC (Wang *et al.*, 2021b), and some genes encoding hypothetical or protective functions (Keller, 2018) (Fig. 1B).

### Secondary metabolite in *Aspergillus*

Bioinformatics algorithms, such as antiSMASH and SMURF, are based on the presence of typical conserved backbone genes (e.g. PKSs and NRPSs) in SMs cluster, have been utilized to identify BGCs in *Aspergillus* and other filamentous fungi. However, these algorithms are merely predictive and may underestimate or overestimate inclusion of genes in a BGC (Keller, 2018). To identify more SMs or BGCs in fungi, a range of research approaches including molecular, biochemical or bioinformatics tools have been developed. The genomic sequence of five genetic model *Aspergillus* spp. (*A. fumigatus*, *A. nidulans*, *A. niger*, *A. flavus* and *A. oryzae*) has been functionally annotated, and their SMs have been identified. BGCs of *A. nidulans* and *A. fumigatus* in particular have been systematically studied with recent reviews presenting a full picture of their BGCs and transcription factor regulation of these BGCs (Caesar *et al.*, 2020; Wang *et al.*, 2021b). In antiSMASH online programmes, the number of individual PKS, NRPS, DMAT/Terpenes and other backbone genes are shown to vary considerably from an estimated 37 in *A. fumigatus* to 89 in *A. niger* (Table 1). A recent study examining 94 sequenced isolates of *A. flavus* showed a median of 82 BGCs with a range from 78 and 85 BGCs thus demonstrating variation within a species (Drott *et al.*, 2021). To date, up to 50 specific SM gene clusters have been experimentally isolated in the *Aspergilli* (de Vries *et al.*, 2017). The fluctuation of BGC number with genome size may indicate a dependence on the lifestyle




**Fig. 1.** The general schematization of secondary metabolites and their biosynthetic gene cluster.

A. Most secondary metabolites (SMs) are generated from precursors catalysed by backbone enzymes (e.g. polyketide synthase, non-ribosomal synthetase and terpene synthase and/or cyclase) that use primary metabolites (e.g. acyl-CoA, amino acids, isoprenoid) to synthesize the SM precursor (carbon backbones) (Lim and Keller, 2014). These carbon backbones are further modified by the tailoring enzymes to form the final active SMs.

B. Fungal biosynthetic gene cluster basically consists of a backbone enzyme and tailoring enzymes (e.g. methyltransferases and p450 monooxygenases) (indicated with dotted lines) (Keller, 2018; Collemare and Seidl, 2019). The transporters are also found in most of the BGCs to secrete/move pathway precursors or finished product. Additionally, BGCs may contain a cluster-specific transcription factor that activates the other genes within the BGC, resistance genes to protect the producing fungus from its own BGC product (e.g. many are antifungal) and often genes with hypothetical functions (indicated with grey).

**Table 1.** Numbers of backbone genes and SM clusters in the selected *Aspergillus* species using antiSMASH online programmes (Blin *et al.*, 2017).



Category	<i>A. flavus</i>	<i>A. oryzae</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. nidulans</i>
PKS (type 1 and 3)	20	23	10	24	17
NRPS	13	14	8	13	9
DMAT/terpenes	11	10	6	18	9
NRPS-like	12	11	6	12	10
Indole	4	5	1	2	3
Fungal-RiPP	1	1	-	-	-
Bacteriocin	-	-	-	1	-
Betalactone	1	1	1	1	3
Siderophore	1	1	-	1	-
PKS-NRPS hybrid	4	2	1	11	3
PKS-NRPS_like hybrid	2	2	-	5	2
NRPS-terpene hybrid	2	1	-	1	-
PKS-terpene hybrid	-	2	1	-	1
PKS-indole hybrid	-	1	1	-	1
NRPS-Indole hybrid	1	1	2	-	1
Number of predicted clusters	72	75	37	89	59
Genome size (Mb)	36.92	37.91	29.42	33.98	36.52

In the antiSMASH program, DMAT is categorized as a terpenes. The phylogenetic relationships were constructed with the conserved single-copy protein sequences using a python script (EasySpeciesTree).

and the specific requirements of each *Aspergillus spp.* (de Vries *et al.*, 2017).

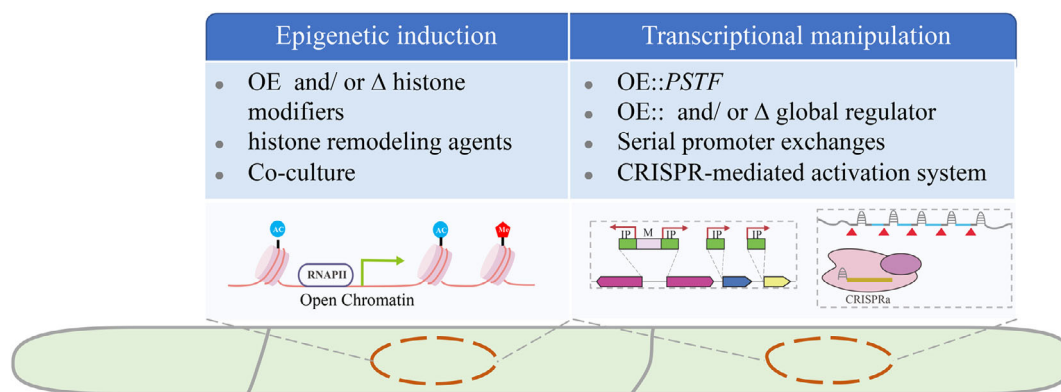
#### Initial studies connecting secondary metabolite in *Aspergillus* to epigenetic processes

The biosynthesis of fungal secondary metabolism is a complicated process that is subjected to a complex system of multi-tier regulation, such as signal transduction pathways (e.g. cAMP and MAPK signalling), environmental factors, and regulation at transcriptional, translational and epigenetic levels. A pioneering study in 2007 was the first to demonstrate a role for histone modifications in regulating fungal SMs where deletion of a HdaA, histone deacetylase (HDAC), led to induced expression of two telomere-proximal BGCs in *A. nidulans* (Shwab *et al.*, 2007). This study further went on to show that treatment of fungal cultures HDAC inhibitors led to the overexpression of several metabolites in other fungal genera. Additional seminal studies involved demonstrations that LaeA, a global regulator of SM in all filamentous fungi studied to date (Perrin *et al.*, 2007; Zhang *et al.*, 2016b) was involved in chromatin level activation of BGCs (Bok *et al.*, 2006), in part by counteracting the establishment of heterochromatic marks histone H3 lysine 9 trimethylation (Reyes-Dominguez *et al.*, 2010). Later, additional studies further established a role of LaeA in a histone modification manner (Bok and Keller, 2004; Bok *et al.*, 2006; Lind *et al.*, 2018) that is

important for histone H3 trimethyl K4. A recent study also showed that LaeA controls citric acid metabolism by regulating the expression of the citrate exporter-encoding *cexA* gene via methylation levels of the histones H3K4 and H3K9 (Kadooka *et al.*, 2020). These initial studies set the stage for a huge expansion of BGC product mining through PTM strategies.

#### Strategies to activate fungal BGCs

Although thousands of BGCs have been predicted in *Aspergillus* genomes, the majority have not been characterized and are often not expressed during fungus growth on laboratory conditions. A major challenge for researchers is to develop strategies to activate the expression of BGCs and allow isolation of their associated products. One strategy is the use of genetic manipulation at BGC transcriptional level, inducing the expression of pathway specific transcription factor (PSTF) by replacing its native promoter with a constitutive promoter (Baccile *et al.*, 2016) (e.g. *gpdA* promoter) or an inducible promoter (Yeh *et al.*, 2016) (e.g. alcohol dehydrogenase promoter *alcAp* or nitrate inducible promoter). Serial promoter exchanges strategy (Yeh *et al.*, 2016) and CRISPR-mediated activation system (CRISPRa) (Roux *et al.*, 2020) are both promising approaches for activating cryptic BGCs (Fig. 2), for those fail to express the whole cluster by single promoter replacement of PSTF. Roux *et al.* used CRISPR/



**Fig. 2.** Strategies to mine fungal secondary metabolites. The epigenetic induction methods include overexpression, deletion of the histone modifiers, treatment with histone remodelling agents, and co-cultures. The transcriptional manipulation, including overexpression of the pathway specific transcription factor (PSTF), global regulators, or deletion of negative regulators, serial promoter exchanges of the BGC genes promoters with inducible promoter, and CRISPR-mediated activation system (CRISPRa) can be used for activation of the cryptic BGCs. IP, inducible promoter; M, selective marker.

dLbCas12a-based activation system, by concurrently expressing multiple gRNAs (guide RNAs), to activate three *mic* cluster genes, leading to the discovery of the final product, dehydromicroperfuraneone (Roux *et al.*, 2020).

Another strategy that has significantly increased SM output in many fungal species is the use of epigenetic strategies that convert heterochromatic regions of the genome to euchromatin (reviewed in the study by Pfannenstiel and Keller, 2019), by which overexpression or knock-down of genes encoding global regulatory complexes (e.g. OE::*laeA*) or histone modifiers (e.g.  $\Delta$ HDAC) helps to turn on the biosynthetic clusters in fungal species with accessible genetic systems (Fig. 2). Alternatively, histone remodelling agents, such as DNA methyltransferases inhibitor 5-azacytidine, and HDAC inhibitors (Pfannenstiel and Keller, 2019) (e.g. trichostatin A, sodium valproate, vorinostat) could be utilized to identify novel metabolites by activation of silent BGCs in fungi recalcitrant to genetic manipulation.

Below we will cover the current literature that describes epigenetic and other PTMs that have been used to express BGC products in *Aspergilli*.

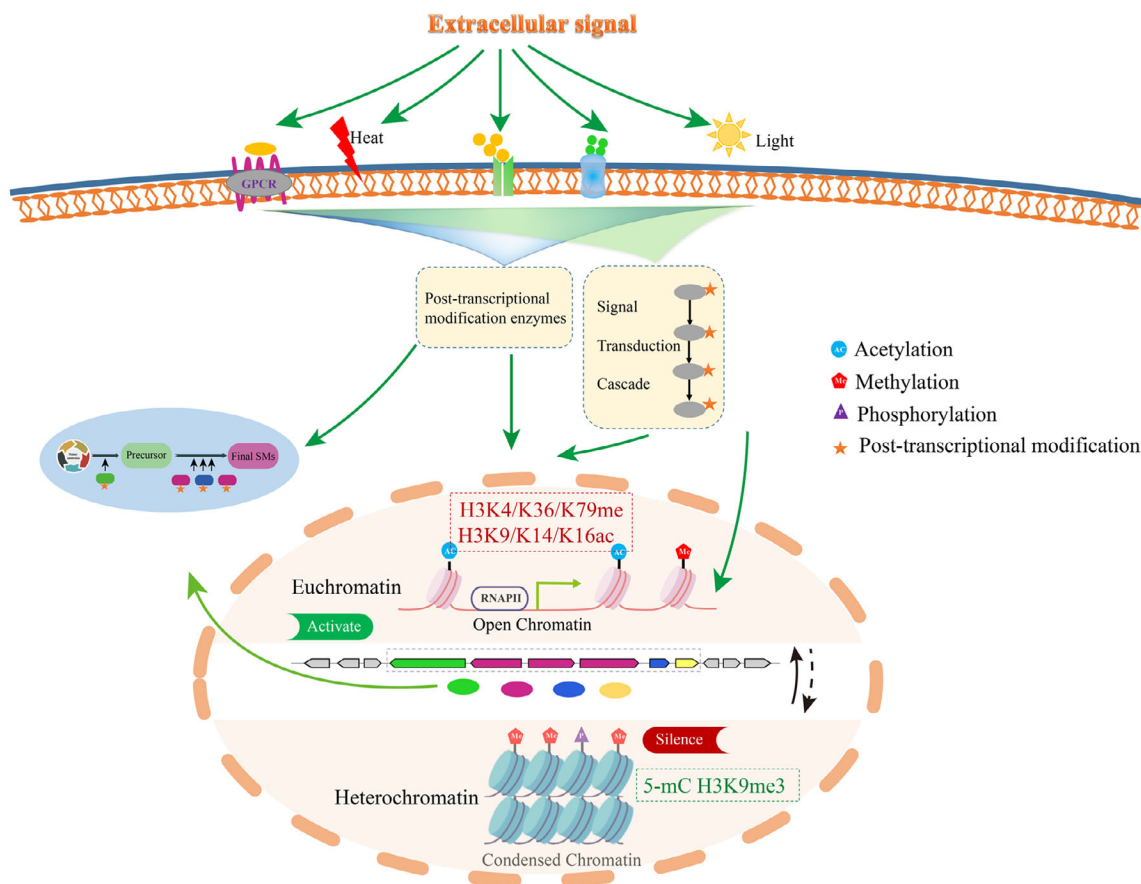
### Chromatin-dependent regulation of secondary metabolite biosynthesis

PTMs target all histone proteins (H2A, H2B, H3 and H4) that dominate the chromatin-dependent regulation of BGCs and non-histone proteins that can also impact expression or activity of BGCs and proteins encoded by these clusters. The chromatin is organized by a complex of DNA and histone proteins, where DNA wraps around histones to form the chromatin basic unit, the nucleosome (Armeev *et al.*, 2019). A transition from

heterochromatin to euchromatin could lead to loosely packed and open nucleosomes, which allows RNA polymerase to bind to DNA and activate gene expression (Fig. 3). Efforts to identify global PTMs have revealed the particular roles of non-histone proteins in fungal development and SM (Ren *et al.*, 2018; Li *et al.*, 2019; Yang *et al.*, 2019). Several PTMs occurring both in histone and non-histone proteins that are the focus of recent work interest in *Aspergillus* are listed in Table 2.

#### Chromatin reader protein

The chromatin readers are important for epigenetic modification that could direct the PTM writers and erasers to the correct chromatin region (Yun *et al.*, 2011). One of the most studied chromatin reader is SntB in *Aspergillus*, which was first found as a regulator of ST biosynthesis from a forward genetic screen in *A. nidulans* (Pfannenstiel *et al.*, 2017). The *A. nidulans* SntB is a homologue of yeast E3 ubiquitin ligase, SNT2, which has five conserved domains for recognizing specific histone PTMs, including one bromo-adjacent homology (BAH) domain, one SANT domain, and three PHD (plant homeodomain) fingers. Two mutations were found in the BAH and led to the dysfunction of SntB in *A. nidulans* (Pfannenstiel *et al.*, 2017). The deletion of *A. nidulans* *sntB* led to the decrease (e.g. cichorine, alternariol, microperfuraneone) or increased (such as F-9775A/B) production of several SMs. The function of SntB was conserved in *A. flavus* (AFLA\_029990), where the  $\Delta$ *sntB* mutant fails to produce many SMs (AFB1, aflavarin, asparosone A, aflatrem and kojic acid) but shows increase synthesis of other SMs such as leporine B and the cryptic BGC products aspergillicin A and aspergillicin F (Pfannenstiel *et al.*, 2018; Greco *et al.*, 2019). As a



**Fig. 3.** Illustration of PTMs in regulation of secondary metabolite clusters. External signals such as temperature, light, carbon/nitrogen, and so on are detected by membrane or internal receptors and passed to signal transduction cascades to regulate the activity of epigenetic or/and PTM enzymes. Then, modifications of DNA or histones affect the specific chromatin region, which can switch from heterochromatin to euchromatin leading to the initiation of SM biosynthesis, or vice versa.

chromatin reader, SntB was involved in global histone modifications in the *A. flavus*, where the *sntB* deficient mutant was enhanced in both H3 acetylation and H3K4me3 (Pfannenstiel *et al.*, 2018), indicating the hybrid roles of SntB in recognizing these two epigenetic PTMs in *Aspergillus*. A recent study of *Penicillium expansum* also revealed the conserved function of SntB (PEX2\_110610) in filamentous fungi (Tannous *et al.*, 2020), where deletion of *PesntB* led to several phenotype defects and decreased production of both patulin and citrinin. *P. expansum* SntB was found to positively regulate activation of three global regulators of SM and virulence (LaeA, CreA, and PacC), which could potentially explain the phenotype changes in the  $\Delta sntB$  mutant.

Another two readers, heterochromatin protein 1 (HP1) and SppA are also reported in *Aspergillus* and other fungi. In *A. nidulans*, the deletion of the HP1 homologue (HepA) led to decreased levels of H3K9me3 in several BGCs (ST, isopenicillin A and terraquinone A), and, as a result, upregulated the expression levels of these BGC

genes (Reyes-Dominguez *et al.*, 2010). SppA, as a member of the COMPASS complex, is also a chromatin reader, and deletion of SppA (AO090003001570) in *A. oryzae* induced astellolide production by affecting the trimethylation status of H3K4 (Shinohara *et al.*, 2016).

#### DNA methylation

DNA methylation at selected cytosine (5-mC) is an important epigenetic process that has been extensively studied in animals, plants and several fungi (Suzuki and Bird, 2008; Jeon *et al.*, 2015). In animals and plants, DNA methylation is associated with the formation of heterochromatin and plays an important role in gene transcription regulation, transposable element silencing, genome imprinting, X-chromosome inactivation and development (Law and Jacobsen, 2010). DNA methylation has been reported in several model fungi, such as *Neurospora crassa* (Hosseini *et al.*, 2020), *Candida albicans* (Russell *et al.*, 1987), *M. oryzae* (Jeon *et al.*, 2015) and *Ucinocarpus reesii* (Zemach

*et al.*, 2010), but it is absent in two model yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Antequera *et al.*, 1984; Proffitt *et al.*, 1984).

In *N. crassa*, genome-wide DNA methylation is regulated by a DNA methyltransferase (DMT) called Dim-2 (defective in methylation) (Kouzminova and Selker, 2001), which belongs to the Dnmt1 family (Zemach and Zilberman, 2010). A single DMT, DmtA, had been characterized in *A. flavus* and *A. nidulans* genomes (Lee *et al.*, 2008; Yang *et al.*, 2016b). However, although the *A. flavus* DmtA was reported to be involved in asexual development and AF production (Yang *et al.*, 2016b; Zhi *et al.*, 2017), DNA methylation levels are low or negligible in *A. flavus* and other *Aspergillus* spp. (Montiel *et al.*, 2006; Liu *et al.*, 2012). Studies using DNA methyltransferase inhibitors like 5-azacytidine (5 AC) reveal that the treatment with this chemical alters the transcriptional profile and biosynthesis of SM in several *Aspergillus* species (Williams *et al.*, 2008; Fisch *et al.*, 2009; Yang *et al.*, 2015). However, concerning the overall low levels of DNA methylation in *Aspergillus*, its role in regulation of SM biosynthesis remains unclear.

#### Histone methylation

In addition to the selected cytosine in DNA, the addition of a methyl group can also occur at the arginine, or lysine residues of both histone and nonhistone proteins. Histone methylation with three levels (mono-, di-, and tri-) has been shown to connect with regulation of secondary metabolism in *Aspergillus* (Bok and Keller, 2004; Gacek-Matthews and Berger, 2016). Methylation at H3K4, K36, or K79 is reported to coordinate with chromatin activation, whereas methylation at H3K9, K27, or H4K20 is typically related with chromatin repression (Martin and Zhang, 2005; Pfannenstiel and Keller, 2019). Several of methyltransferases and demethylases that have been identified and characterized as SM regulators are shown in Fig. 4.

#### H3K4 methylation

Methylation at Histone 3 lysine 4 (H3K4), especially for H3K4me2/3, is usually associated with transcriptional activation of BGCs (Gacek-Matthews and Berger, 2016). Di- and tri-methylation of H3K4 is catalysed by the methyltransferase Set1, which is a highly conserved component of COMPASS (the complex of proteins associated with Set1) complex. COMPASS has been shown to be associated with transcriptional silencing near telomeres (Schneider *et al.*, 2005), which could be considered as a manipulation target to activate the SM production because many BGCs are located in sub-telomeric regions in *A. flavus* (Fig. 5) and other *Aspergillus* spp. (Bok *et al.*, 2009). In *A. flavus*, AflSet1, which writes di-

and tri-methylation of H3K4, is required for AFB1 biosynthesis (Liu *et al.*, 2020). Disruption of another COMPASS component Bre2 (CclA) in *A. nidulans* activated several silent BGCs (e.g. F9775A/F9775B) and showed a significant decrease of H3K4me2/3 (Bok *et al.*, 2009). Deletion of *cclA* led to activation of the gliotoxin BGC and subsequent product in *A. fumigatus* (Palmer *et al.*, 2013). Similar deletions also affected SM synthesis in other fungal genera (Studt *et al.*, 2016a; Dallery *et al.*, 2019).

#### H3K9 methylation

Intriguingly, the methylation status of H3K4 at SM gene clusters has a close relation with H3K9me3. In *A. flavus*, the H3K4 methyltransferase AflSet1 also affects di-methylation of H3K9 (Liu *et al.*, 2020). In *A. fumigatus*, the TF BrlA, required for asexual sporulation, was found to regulate production of developmental and vegetative SMs in a concordant fashion to LaeA (Lind *et al.*, 2018). The deletion of *laeA* leads to a decrease of a modification related with euchromatin (H3K4me3) on the *brlA* promoter, while the heterochromatic mark H3K9me3 is greatly enriched (Lind *et al.*, 2018). As mentioned earlier, LaeA activity has also been associated with decreased levels of H3K9me3 (Reyes-Dominguez *et al.*, 2010). In general, H3K9 methylation can be considered an epigenetic mark for SM gene repression production in *Aspergillus* spp. and other fungi (Fig. 3).

#### H3K27 methylation

H3K27 methylation is another repressive mark for gene expression, which is catalysed by KMT6, a part of the polycomb repressive complex 2 (PRC2) in fungi (Erlendson *et al.*, 2017). In *Fusarium graminearum*, about 30% of all expressed genes in the genome are covered by H3K27me3, many of them located in SM gene clusters and are turned on or upregulated when FgKMT6 is absent (Connolly *et al.*, 2013). The deletion of *kmt6* is lethal and causes metabolic dysfunction in *Fusarium fujikuroi* (Studt *et al.*, 2016b). Although the repressive role of H3K27me3 in gene expression is established in many fungi, no H3K27 Methyltransferases or Kmt6 homologues or even the PRC2 complex have been identified in the genus *Aspergillus*.

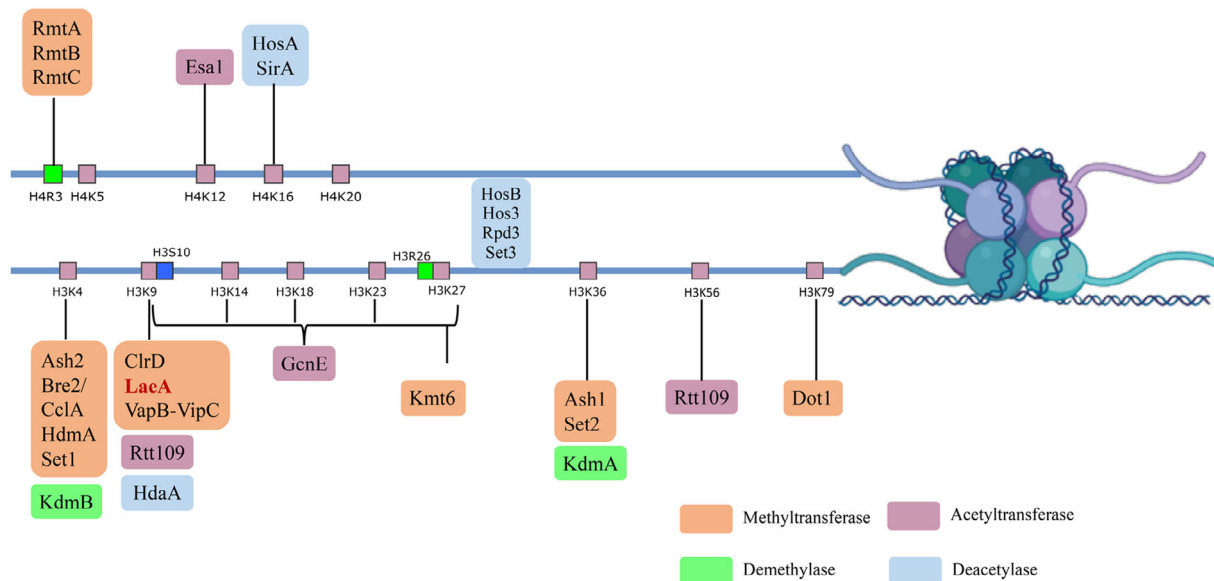
#### H3K36 methylation

H3K36 methylation is most likely involved in transcriptional activation. Two methyltransferases Set2 and Ash1 in *F. fujikuroi* have been reported to be responsible for H3K36 methylation, each functioning specifically at euchromatic and subtelomeric regions of the genome, respectively (Janevska *et al.*, 2018). Deletions of *set2* and *ash1* resulted in decreased production of gibberellins and increased production of fusarins and fusaric acid (Janevska *et al.*, 2018).

**Table 2.** Overview of the PTMs identified in fungi.

	PTMs (abbreviation)	PTM sites	Key enzymes identified in <i>Aspergillus</i>	Function in transcription	References
Histone modification	Methylation (me)	Lys	HMTs/HDMs	Activation/repression	Strauss and Reyes-Dominguez (2011)
	Acetylation (ac)	Lys	HATs/HDACs	Activation	Strauss and Reyes-Dominguez (2011)
Phosphorylation (ph)	Phosphorylation	Ser/Thr/Tyr	Kinase/phosphatase	Repression	Nathan <i>et al.</i> (2006), Ren <i>et al.</i> (2016), and Mattos <i>et al.</i> (2020)
	cAMP-protein kinase	Ser/Thr	PkaA	Activation/repression	Shimizu <i>et al.</i> (2003) and Yang <i>et al.</i> (2017)
	MAPK	Ser/Thr	MpkA, MpkB, HogA	Activation	Frawley <i>et al.</i> (2018)
	Histidine kinase	His/Asp	Sln1	Activation	Zhao <i>et al.</i> (2021)
Succinylation	TOR kinase	Ser/Thr	AGC kinase Sch9	Activation	Alves de Castro <i>et al.</i> (2016)
	Succinylation (suc)	Lys	unknown/HDACs	Activation	Xie <i>et al.</i> (2012) and Ren <i>et al.</i> (2018)
	Ubiquitination and ubiquitin-like	Lys	UBI4 (ubiquitin), UbcK (E2), Skp1-Cul1-F-box-protein (SCF) E3 ligase complex	Repression	Meister <i>et al.</i> (2019)
Ubiquitination and ubiquitin-like	Sumoylation (su)	Lys	SumO, AosA/UbaB (E1), UbcN (E2), SizA/SizB/MmsU (E3), UlpA/UlpB (Isopeptidases)	Repression	Nathan <i>et al.</i> (2006) and Harting <i>et al.</i> (2013)
	Neddylation	Lys	RubA (NEDD8), UbaC (E1), UbcL(E2), DenA/CSN (E3)	Repression	
Lipidation	Palmitoylation	Cys	AkrA (PATs)	Activation	Zhang <i>et al.</i> (2016c)

HMTs, histone methyltransferases; HDMs, histone demethyltransferases; HATs, histone acetyltransferases; HDACs, histone deacetyltransferases; MAPK, mitogen-activated protein kinase; TOR, target of rapamycin; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin protein ligase; PATs, Palmitoyl transferases.

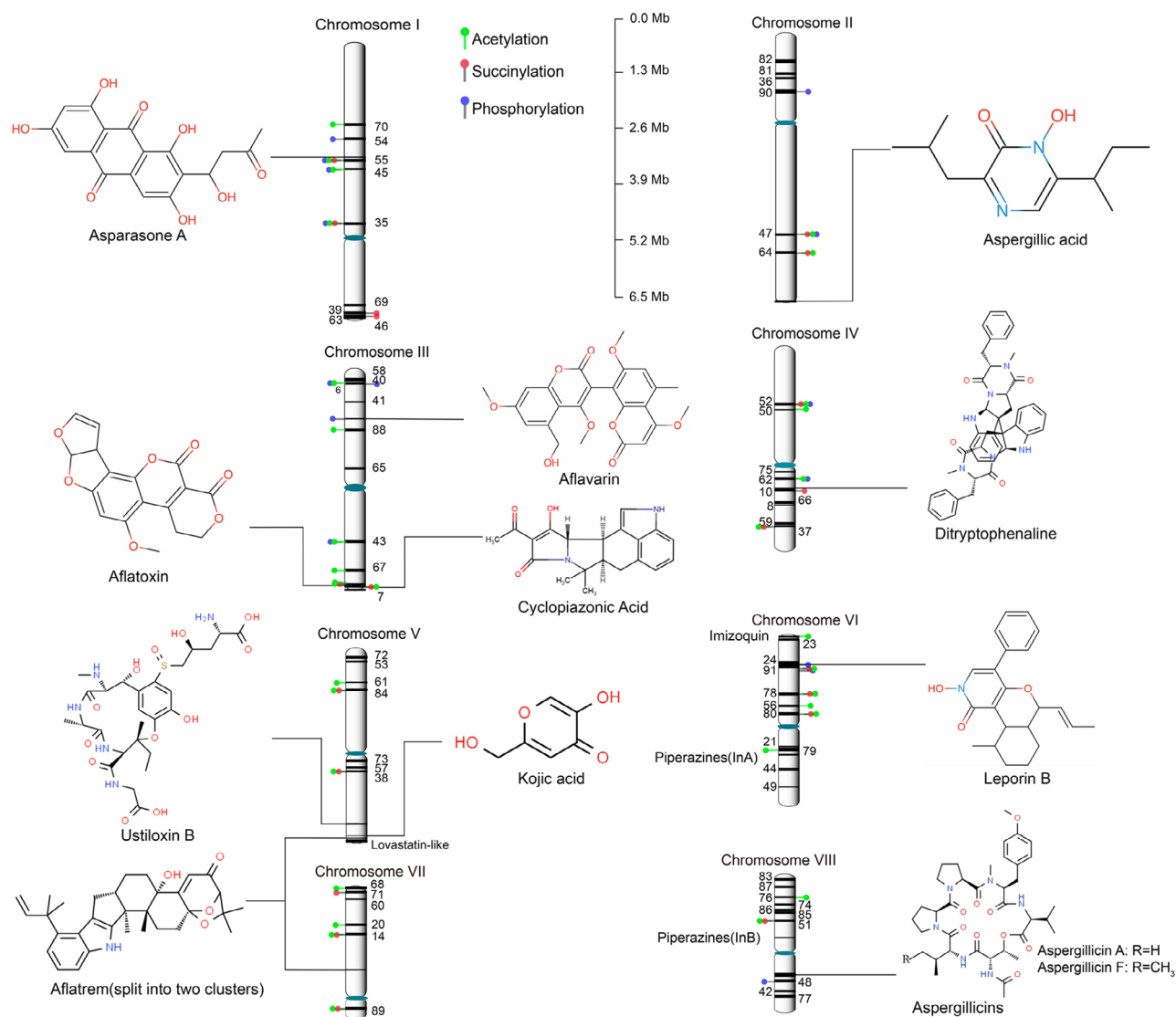


**Fig. 4.** The PTMs on histones H3 and H4 are associated with fungal development and secondary metabolism. The specific site targets of histone methyltransferases, demethylases, and histone acetyltransferases and histone deacetylases are indicated.

Although the roles of H3K36 methyltransferases have not been studied in *Aspergillus*, H3K36me3 was found to accumulate at multiple active SM gene clusters in *A. nidulans* (Gacek-Matthews *et al.*, 2015).

#### H3K79 methylation

Dot1 encodes a H3K79 methyltransferase in yeast, and its function is not clear in filamentous fungi yet. A *dot1* homologue (AFLA\_093140) in *A. flavus* functions as a



**Fig. 5.** Chromosomal position of *A. flavus* biosynthetic gene clusters (BGCs) and their known or predicted products. The PTMs that exist in a BGC pathway – where known – are indicated on the chromosome where red dot = succinylation, green dot = acetylation, blue dot = phosphorylation (Ren *et al.*, 2016; Ren *et al.*, 2018; Yang *et al.*, 2019). Chromosomal position of BGCs was plotted with TBtools (Chen *et al.*, 2020). The chromosomal position of the BGCs was visualized by referring to the newly released *A. flavus* genome (Drott *et al.*, 2020). The characterized SM BGCs including: asparasone A (Cary *et al.*, 2014), aspergillilic acid (Lebar *et al.*, 2018), aflavarin (Cary *et al.*, 2015b), aflatoxin (AF) (Cary *et al.*, 1999), cyclopiiazonic acid (Chang *et al.*, 2009), dityryptophenaline (Saruwatari *et al.*, 2014), kojic acid (Terabayashi *et al.*, 2010), ustiloxin B (Umemura *et al.*, 2014) BGCs, aflatrem BGCs (split into two clusters) (Nicholson *et al.*, 2009), imizoquin (Khalid *et al.*, 2018), leporin B (Cary *et al.*, 2015a), aspergillins (Greco *et al.*, 2019), and two piperazine BGCs (InA and InB) (Forseth *et al.*, 2013) and the other predicted BGCs reported by Drott *et al.* (2021) are indicated on the *A. flavus* chromosomes.

regulator of pathogenicity and aflatoxin (AF) synthesis in *A. flavus* (Liang *et al.*, 2017). However, it remains to be shown if Afdot1 possesses H3K79 methyltransferase activity, and how it regulates the AF BGC in *A. flavus*.

#### H4R3 methylation

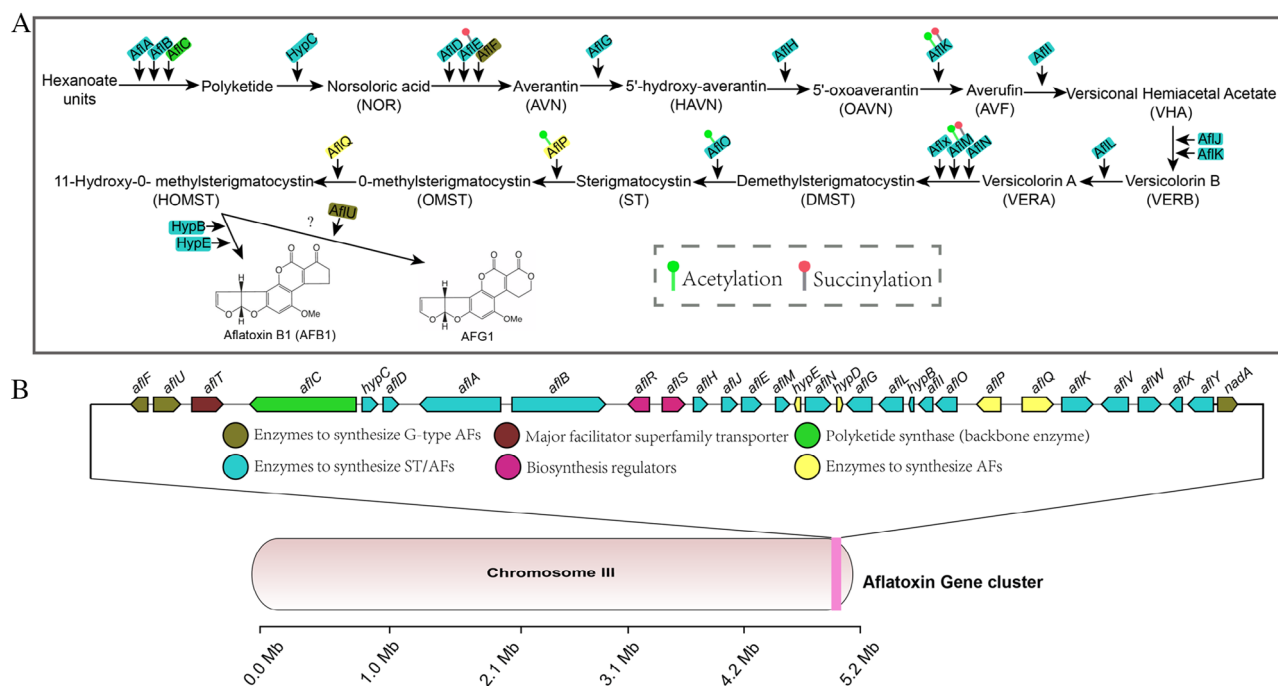
Arginine residues are alternative targets for methylation, which can lead to gene activation or repression. Three histone 4 arginine 3 methyltransferases have been

identified and defined as RmtA, RmtB, and RmtC in *A. nidulans* (Trojer *et al.*, 2004; Bauer *et al.*, 2010). The homologue of RmtA in *A. flavus* was found to play a positive role in AF biosynthesis and sclerotia formation (Satterlee *et al.*, 2016; Li *et al.*, 2017).

#### Histone demethylase

Histone demethylases are responsible for removing the reversible methylation mark on histone tail lysines. In *A.*





**Fig. 6.** Schematization of the AF biosynthetic pathway and its gene cluster.

A. Schematization of the main intermediates produced during the AF biosynthetic pathway. The PTM enzymes within the AF biosynthetic pathway are indicated.

B. AF gene cluster in *A. flavus*.

*nidulans*, two Jumonji histone H3 demethylases KdmA (homologue of yeast Rph1) and KdmB (homologue of yeast Jhd2) have been identified, which de-methylate H3K36me3 and H3K4me3 (Gacek-Matthews *et al.*, 2015; Gacek-Matthews and Berger, 2016), respectively. KdmA is a transcriptional regulator for primary and secondary metabolism genes that regulates methylation at specific loci in the genome. In *kdmA* mutants, levels of H3K36me3 were found to accumulate at regions in the penicillin and sterigmatocystin (ST) BGCs (Gacek-Matthews *et al.*, 2015). Unlike KdmA, KdmB plays a global role in regulation of SM gene expression. The deletion of *kdmB* in *A. nidulans* led to the dysregulation of 50% of known SM genes, that most of which showed decreasing expression levels (Gacek-Matthews and Berger, 2016). These findings suggest that KdmB is necessary for the normal activation of the majority of SM gene clusters (e.g. ST, emericellamides, and orsellinic acid) in *A. nidulans* (Gacek-Matthews and Berger, 2016). A study in *A. flavus* showed that the KdmB homologue, Rum1 (AFLA\_006240) is required for AF biosynthesis (Hu *et al.*, 2018).

### Histone acetylation

Histone acetylation was first characterized in *A. nidulans* as playing an important role in regulating activation of BGCs (Shwab *et al.*, 2007). In *A. parasiticus*, the transcriptional activation of AF biosynthetic pathway genes is

positively correlated with the initiation and spread of histone H4 acetylation (Roze *et al.*, 2007). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) add or remove acetyl groups on histones respectively, often on lysine residues (Fig. 4). Both activities are involved in gene activation/repression and are involved in multiple processes in filamentous fungi (Dubey and Jeon, 2017).

### Histone acetyltransferases

GcnE is one of the most well-known histone acetyltransferases that acetylates many residues including H3K14, H3K18 and H3K27 in fungi (Kong *et al.*, 2018) and belongs to the GCN5-related *N*-acetyltransferase (GNAT)-type family. Interestingly, several studies have shown that *Streptomyces* species can impact fungal growth and secondary metabolism via activating (GcnE in *A. nidulans*) or degrading (Gcn5 in *Fusarium*) this acetyltransferase (Nutzmann *et al.*, 2011; Nützmänn *et al.*, 2013; Wang *et al.*, 2021a). The GcnE homologue (AFLA\_051420) in *A. flavus* is also required for the global acetylation of histone 3 and H3K14 acetylation (Lan *et al.*, 2016). Deletion of *gcnE* leads to the loss of production AF by repressing the transcriptional activation of AF cluster genes (Lan *et al.*, 2016).

The MYST-type (named after the founding members MOZ, YBF2/SAS3, SAS2, and Tip60) acetyltransferase

family is another important HAT in fungi. Esa1 belongs to the MYST-type HAT and is a component of the NuA4 (nucleosomal acetyltransferase of histone H4) complex, that acetylates several H4 lysine residues, including H4K5, H4K8, and H4K12 (Suka *et al.*, 2001). In *A. nidulans*, overexpression of the *esaA* increases the production of ST, penicillin, terrequinone and orsellinic acid, coupled with enhanced acetylation of H4K12 at these BGCs loci (Soukup *et al.*, 2012).

Rtt109 is a member of the P300/CBP acetyltransferase family, which is responsible for the acetylation of histone 3 lysine 56 (H3K56) (Han *et al.*, 2007). In many plant-pathogenic fungi, Rtt109 is reported to play vital roles in DNA damage repair, fungal development and pathogenicity by acetylating H3K56 (Han *et al.*, 2007; Cai *et al.*, 2018). A Rtt109 homologue (AFLA\_098450) has been identified in *A. flavus* and shown to be required for AF biosynthesis (Sun *et al.*, 2021). The deletion of *rtt109* in *A. flavus* shows a significant reduction in total acetylation of histone 3 and H3K56 acetylation (Sun *et al.*, 2021). Intriguingly, H3K9 is also an acetylated target of Rtt109.

#### Histone deacetylases

Histone acetylation is generally involved in the activation of BGCs, but the loss of acetylation does not necessarily result in silencing of BGCs and in fact can also activate some BGCs (Albright *et al.*, 2015). Deacetylation is conferred by HDACs, which can be grouped into three classes in fungi (Pfannenstiel and Keller, 2019).

**Class I-Rpd3 HDACs.** Shwab *et al.* was the first study to show HDAC inhibitors (trichostatin A, TSA) can induce production of fungal natural products (Shwab *et al.*, 2007). Since then, several studies have used TSA to increase production of fungal SMs (Zutz *et al.*, 2013; Albright *et al.*, 2015; Aldholmi *et al.*, 2020). However, in addition to activating some BGCs, HDAC inhibitors and even HDAC gene suppression can inhibit expression of other BGCs (Albright *et al.*, 2015). Class I-Rpd3 HDACs are TSA targets and are well conserved in eukaryotes. The yeast Rpd3 homologue in *A. nidulans* (*rpda*, AN4493) is essential for growth and development (Tribus *et al.*, 2010). The metabolome of an *A. nidulans* *rpda* knockdown mutant showed great similarities with the treatment by the HDAC chemical inhibitor suberoylanilide hydroxamic acid (SAHA) where some SMs were upregulated and some down regulated (Albright *et al.*, 2015; Henke *et al.*, 2016). Three Class I HDACs, RpdA (AFLA\_092360), Rpd3 (RpdA-like, AFLA\_025120) and HosA (AFLA\_087410) have been characterized in *A. flavus* where HosA plays a crucial role in AFB1 biosynthesis (Lan *et al.*, 2019). An *in vitro* analysis revealed

that HDAC activity of HosA was considerably inhibited by TSA in *A. flavus* (Lan *et al.*, 2019). The deletion of *hosA* leads to the significant increase in acetylation levels of H4 and, particularly, H4K16. The homologue of HosA in *A. oryzae*, HdaD/AoHos2, is important for fungal development and kojic acid production in this species (Kawauchi *et al.*, 2013; Kawauchi and Iwashita, 2014).

**Class II-Hda1 HDACs.** HdaA belongs to the HDA1-Class II HDAC family, which contributes to the majority of HDAC activity in *A. nidulans* (Tribus *et al.*, 2005). The inactivation of *hdaA* led to the accumulation of the production of some metabolites (ST, penicillin) and the upregulation of BGC expression in *A. nidulans* (Shwab *et al.*, 2007). The homologue of HdaA (Afu5g01980) in *A. fumigatus* is also involved in regulation of several secondary metabolites including gliotoxin and some other unknown SMs (Lee *et al.*, 2009). Although HdaA is required for the majority of HDAC activity in *A. nidulans*, the global acetylation of histone 3 did not show a difference when *hdaA* was deleted in *A. fumigatus*. The function of HdaA (AFLA\_006160) was also studied in *A. flavus*, which, like many other histone modifying enzymes was found to play a positive role in AF production and sclerotia formation (Lan *et al.*, 2019). The function of the other Class II HDACs, HosB (AFLA\_114920) was also studied in *A. flavus*, which indicates that HosB is dispensable for fungal development and AF production in this fungus (Lan *et al.*, 2019). It seems that HosB plays little role in HDAC activity in fungi (Studt *et al.*, 2013).

**Class III-Sirtuin HDACs.** Sirtuins are the last class of HDACs (or called Class III HDACs) and are NAD<sup>+</sup> dependent. HstA (AN11067) was the first NAD<sup>+</sup>-dependent sirtuin studied in *A. nidulans*. Deletion of the gene had minimal effects on the production of norsolorinic acid and penicillin (Shwab *et al.*, 2007). *A. nidulans* SirA (AN10449) is the homologue of *S. cerevisiae* sirtuin Hst1 and uses NAD<sup>+</sup> as a cosubstrate to deacetylate H4K16 in the promoter regions of several BGCs (e.g. penicillin and ST). Deletion of *sirA* increases the production of these metabolites (Shimizu *et al.*, 2012). SirE (AN1226) is another sirtuin HDAC that has been studied in *A. nidulans*, which functions in regulation of both primary and secondary metabolism by deacetylating the H3K9ac, H3K18ac and H3K56ac during different growth phases (Itoh *et al.*, 2017). The function of the *A. nidulans* SirE homologue in *A. oryzae*, HstD (AO090038000370), was found to regulate development and SM biosynthesis via the regulation of the global regulator of secondary metabolism LaeA (Kawauchi *et al.*, 2013).

## Post-translational regulation of secondary metabolite biosynthesis beyond histone modification

### Acetylation of nonhistone proteins

Lysine acetylation is also widely distributed in many non-histone proteins in both prokaryotes and eukaryotes where acetylation can impact transcriptional regulation, enzymatic activity and cellular metabolism (Zhang *et al.*, 2013; Li *et al.*, 2019). Recently, a global comparative acetylome was studied in three human fungal pathogens, *A. fumigatus*, *Cryptococcus neoformans* and *C. albicans*, of which 2312 lysine acetylated (Kac) proteins were identified in *A. fumigatus* (Li *et al.*, 2019). The conservative acetylated orthologues in these three fungal pathogens were found enriched in core biological processes (e.g. TCA, ribosome and histone modifications), while the distribution of acetylation on the secondary metabolites pathways was not mentioned in this study. In *A. flavus*, the acetylome has been performed in two studies (Lv, 2017; Yang *et al.*, 2019). More than 1300 lysine acetylation sites were identified in hundreds of *A. flavus* proteins. Lv (2017) reported the acetylation of AF biosynthetic pathway enzymes AfIA (AFLA\_139380) and AfIB (AFLA\_139370) in *A. flavus* (Lv, 2017), while Yang *et al.* found that AfIK (AFLA\_139190), AfIM (AFLA\_139300), AfIO (AFLA\_139220, formerly OmtB) and AfIP (AFLA\_139210) were also acetylated in the AF biosynthetic pathway (Yang *et al.*, 2019) (Fig. 6). To discern a role for Kac of AfIO (a methyltransferase involved in conversion of demethylsterigmatocystin to sterigmatocystin (Yu *et al.*, 2000), a *afIO*<sup>K384Q</sup> mutant that mimics the non-acetylated state failed to produce AF, indicating that acetylation of AfIO is important for AF biosynthesis. Additionally, 42 Kac proteins from 30 different BGCs were identified including those involved in synthesis of cyclopiazonic acid and imizoquin (Fig. 5). These data suggest the potential global regulatory role of non-histone acetylation regulation on secondary metabolism.

### Protein phosphorylation

Phosphorylation is one of the most studied PTMs that usually occurs at serine (S), threonine (T), and tyrosine (Y) residues. The reversible protein phosphorylation is catalyzed by a kinase and a phosphatase. The two best known phosphorylation pathways are the cyclic adenosine monophosphate-dependent protein kinase A (cAMP-PKA) signalling pathway and mitogen-activated protein kinase (MAPK) cascades, both of which have been reported to be involved in multiple cellular processes and secondary metabolism in *Aspergillus*.

#### cAMP-protein kinase A signalling pathway

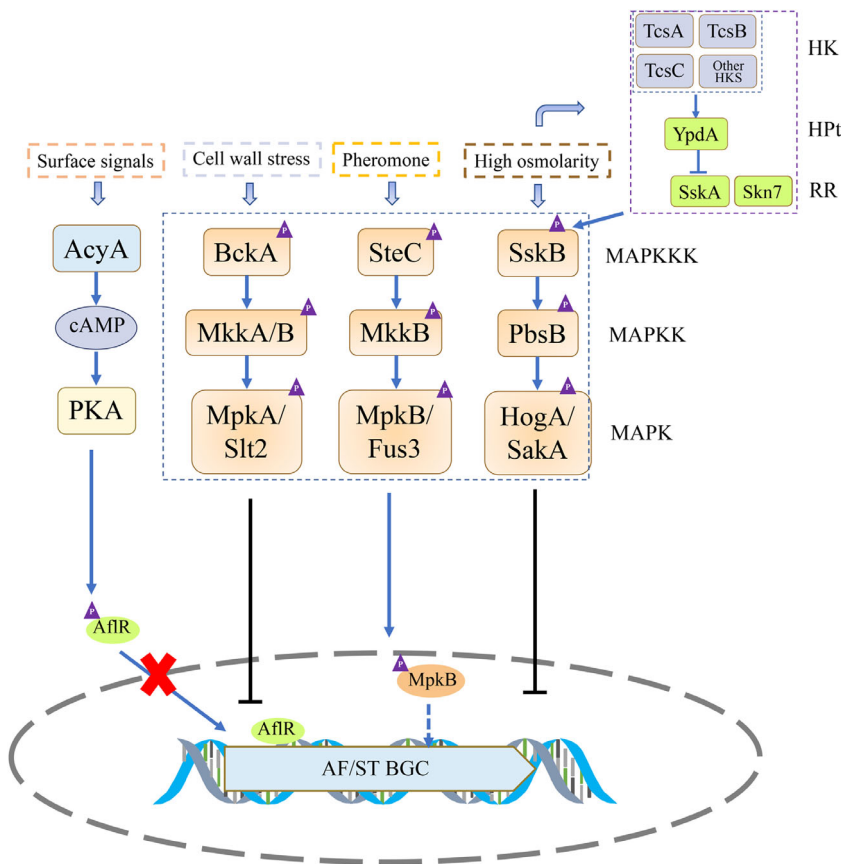
The cAMP-PKA pathway is a conserved signalling cascade in eukaryotic organisms, which utilizes cAMP as a second

messenger to regulate a wide variety of biological processes. In *Aspergillus*, the cAMP-PKA pathway controls the responses to multi extracellular stimuli (e.g. oxidative agents, osmotic stress, pH changes and thermal stress), fungal development and infection (Liebmann *et al.*, 2003; Grosse *et al.*, 2008; Yang *et al.*, 2016a). This pathway is also important for the regulation of mycotoxin biosynthesis in *Aspergillus*, which negatively regulates ST/AF biosynthesis in *A. parasiticus* and *A. nidulans* (Shimizu and Keller, 2001; Roze *et al.*, 2004) (Fig. 7). In the ST BGC gene cluster, the activity of AfIR is inhibited by PkaA via phosphorylation in *A. nidulans* (Shimizu *et al.*, 2003). We also found that the deletion of genes involved in cAMP/PKA pathway, such as the G protein alpha-subunit-encoding gene *fadA* (Yang *et al.*, 2022) or adenylate cyclase encoding gene *acyA* (Yang *et al.*, 2016a), leads to an alteration in AF production by regulating cAMP synthesis and PKA activity in *A. flavus*. There are 15 putative GPCRs identified in *A. flavus*, among which the *gprA* and *gprP* deletion mutants produce much more AF compared to the wild type (Affeldt *et al.*, 2014). In *A. nidulans*, GprH, GprM and GprI are found to regulate fungal development and mycotoxin production by functioning upstream of cAMP-PKA signalling (Dos Reis *et al.*, 2019). In *A. fumigatus*, the deletion of a regulator of G protein signalling (RGS) domain protein, RgsC, exhibits defective gliotoxin production and decreased virulence in *Galleria mellonella* (Kim *et al.*, 2017), while  $\Delta$ *rgsA* and  $\Delta$ *rgsD* mutants are increased in gliotoxin production, both of which show elevated PKA catalytic activity in this fungus (Kim *et al.*, 2019; Lwin *et al.*, 2019).

The cAMP-PKA pathway is also reported to be involved in regulation of epigenetic processes in fungi (Kang *et al.*, 2015; Bao *et al.*, 2022). The sirtuin HDAC Sir2 is phosphorylated through cAMP-PKA signalling and casein kinase 2, downstream of cAMP-PKA signalling, leading to loss of activity of Sir2 and increased acetylation of H4K16 in the promoter regions of its target genes (Kang *et al.*, 2015). A recent study in yeast demonstrated that mutations in genes of cAMP signalling inhibit the heterochromatin defects linked to overexpression of Epe1, an enzyme that removes histone modifications associated with heterochromatin (Bao *et al.*, 2022). In *M. oryzae*, the addition of cAMP could restore the defects of infection-related morphogenesis in the deletion mutant of H3K4 methyltransferase Mose1 (Pham *et al.*, 2015). In *A. flavus*, the cAMP signalling-induced AFB1 biosynthesis is found to have a closed relationship with the HDAC, HosA (Lan *et al.*, 2019).

#### MAPK cascades

MAPK pathways are composed of three conserved kinases: MAP kinase kinase kinase (MAPKKK), MAP



**Fig. 7.** The phosphoregulation of protein kinases on ST/AF biosynthesis in *Aspergillus*. The expression of AF/ST BGC is specifically activated by transcription activator AfIR. cAMP-PKA negatively regulates AF/ST biosynthesis, where AfIR is phosphorylated by the cAMP dependent kinase PKA, and the phosphorylated AfIR fails to translocate into the nucleus, leading to the inactivation of AF/ST BGC (Shimizu *et al.*, 2003). AF/ST production is also negatively regulated with an unknown mechanism of regulation by the Slt2-MAPK (Zhang *et al.*, 2020a,b) and Hog-MAPK (Tumukunde *et al.*, 2019) cascade that involves in cell wall stress and high osmolarity response, respectively. The MpkA (Fus3)-MAPKE cascade, that mediates pheromone response, consists of the three kinases SteC, MkkB and MpkB. In *A. nidulans*, phosphorylation of MpkB leads to its activation and translocation into the nucleus, then associates with transcription factors, for example SteA and VeA to regulate AF/ST biosynthesis or other SMs (Frawley *et al.*, 2018; Frawley and Bayram, 2020b). 'P' indicates phosphate groups.

kinase kinase (MAPKK) and MAP kinase (MAPK) that function in environmental signal transduction in eukaryotes. The three main MAPK-mediated signalling pathways, known as Fus3, Hog1 and Slt2-MAPK in yeast, have been identified and characterized in filamentous fungi as well, among which Slt2-MAPK regulates cell wall integrity, Fus3-MAPK involves in the pheromone response and Hog1-MAPK regulates the high osmolarity response (Turrà *et al.*, 2014). These MAPK signal transduction pathways, Slt2, Fus3 and Hog1, have been well studied in *Aspergillus*, orthologous to MpkA, MpkB and HogA, respectively. Intriguingly, the MAPK protein of Hog-MAPK cascade is duplicated in *A. fumigatus* and *A. nidulans*, both of which possess HogA/SakA and MpkC, and triplicated in *A. oryzae* and *A. niger*, that all have HogA/SakA, MpkC, and MpkD (Hagiwara *et al.*, 2016). In *A. fumigatus*, the disruption of pheromone module-MpkB pathway led to decreased levels of several secondary metabolites (e.g. gliotoxin, pseurotin A, pseurotin D, pyripyropene A) (Frawley *et al.*, 2020). The MpkB pathway is also shown to negatively regulate the activation of secondary metabolite gene clusters, including ST genes in *A. nidulans*, and the state of MpkB phosphorylation is positively regulated by HamE, a ham (hyphal anastomosis) protein characterized as a scaffold

for the MAK-2 cascade (Frawley *et al.*, 2018). An early study of *A. nidulans* showed that AnFus3/MpkB is involved in the regulation of secondary metabolite production by phosphorylating VeA (Bayram *et al.*, 2012), a member of the conserved fungal velvet complex linking secondary metabolism and fungal development with light. Four phosphorylation sites are identified in *A. nidulans* VeA that affect the interaction between VeA and VelB or FphA (Rauscher *et al.*, 2016). In turn, mutation of these sites impacted ST synthesis. The studies in *A. flavus* demonstrated that MpkA and HogA pathways act as negative regulators of AF biosynthesis. The *A. flavus sakA/hogA* mutant produces more AF than WT (Tumukunde *et al.*, 2019). The deletion of the MAPK kinase, Slt2/MpkA (AFLA\_052570), or loss of phosphorylation of Slt2 lead to a severe growth defect, but a significant increase in AF production (Zhang *et al.*, 2020b) (Fig. 7). However, it remains unclear how these MAPK pathways regulate AF biosynthesis. One of the explanations for the regulation of Slt2 on AF production is that the Slt2 protein might regulate the activity of a sirtuin HDAC such as Sir2 by phosphorylation (Ray *et al.*, 2003; Zhang *et al.*, 2020b) (see 'cAMP-protein kinase A signalling pathway' section). The MpkB/Fus3 pathway is also required for AF production (Qin *et al.*, 2020; Yang *et al.*, 2020).

Studies in yeast show that MAPK pathways are related to chromatin remodelling as well (Mas *et al.*, 2009). An early study reported by De Nadal *et al.* (2004) showed that MAPK Hog1 recruits histone deacetylase Rpd3 to activate osmo-responsive genes (De Nadal *et al.*, 2004). Mas *et al.* showed that MAPK Hog1 directs recruitment of a chromatin-remodelling complex to the ORF of osmo-responsive genes during stress in yeast (Mas *et al.*, 2009). Hog1 phosphorylates the nucleolar protein Net1, altering its affinity for the phosphatase Cdc14, whose activity is essential for mitotic exit and completion of the cell cycle (Tognetti *et al.*, 2020). A genetic analysis in yeast reveals that phosphorylation at histone residues H4-S47 and H4-T30, catalysed by the kinases Cla4 and Ste20, PAK2, and the Ste11 MAPK are important for response to osmotic and heat stress, respectively (Viéitez *et al.*, 2020).

#### Two-component systems

The TCS (also known as His-Asp phosphorelay signalling) system is also a common signal transduction cascade in both prokaryotes and eukaryotes, which consists of two components in the former, a histidine kinase (HK) and a response regulator (RR) in bacteria, while a histidine-containing phosphor-transmitter (HPt) is required additionally in eukaryotes (Fig. 7). The His-Asp phosphorelay systems are mainly involved in regulation of fungicide susceptibility, conidial development and virulence in fungal pathogens (Viaud *et al.*, 2006; Rispaill and Di Pietro, 2010). A total of 15 HK genes are identified both in *A. nidulans* and *A. oryzae* by genomic analyses (Hagiwara *et al.*, 2016), indicating that the TCS regulation networks are more complex in *Aspergillus* or other filamentous fungi. In yeast, the TCS system functions in the upstream of HOG MAPK cascade. A study of histidine kinases in *A. nidulans* showed that the HK gene *fphA* is required for the phosphorylation of SakA/HogA during heat sensing (Yu *et al.*, 2019). A recently study in *A. nidulans* demonstrated that a putative sensor HK VadJ coordinated the balance between sexual and asexual development and negatively regulated ST production (Zhao *et al.*, 2021). In *A. flavus*, the response regulators Skn7 are also showed to play roles in regulating oxidative stress response and AF biosynthesis (Zhang *et al.*, 2016a). The deletion of a phytochrome that functions as a hybrid histidine kinase in the *Monascus purpureus* led to the decrease of the *Monascus* pigments, monacolin K and citrinin production (Xiong *et al.*, 2021).

#### Target of rapamycin kinase

The serine–threonine kinase is the target of rapamycin (TOR) and regulates cell growth and stress signalling in

eukaryotes. A single TOR kinase TorA was characterized in *A. nidulans*, which contained four phosphosites during growth and three of them were dephosphorylated under oxidative stress (Carrasco-Navarro and Aguirre, 2021). The TOR signalling pathway regulates phosphorylation of the Ser/Thr kinase Sch9, a substrate of TOR, to control fungal development and mediate multiple stresses responses as well as mycotoxin synthesis in some fungal pathogens (Gu *et al.*, 2015; Alves de Castro *et al.*, 2016). An early study in *Fusarium fujikuroi* demonstrated that the TOR kinase was involved in nitrogen regulation of secondary metabolism such as gibberellins and bikaverin (Teichert *et al.*, 2006). The TOR pathway can also cooperate with cAMP/PKA signalling and the MAPK cascades and function in fungal growth and virulence of *A. fumigatus*. The deletion of *scha* in *A. fumigatus* increase the phosphorylation of SakA that responses to hyperosmotic stress (Alves de Castro *et al.*, 2016). In *A. flavus*, the inhibition of TOR kinase by rapamycin suppresses the glutamine inducing effect on AFs production (Wang *et al.*, 2017). However, it remains unclear whether TOR could regulate AF biosynthesis directly or cooperates with other signalling cascades like cAMP/PKA signalling and the MAPK cascades in *Aspergillus*.

#### Protein phosphorylation involved in metabolic pathways

Reversible protein phosphorylation also regulates many important metabolic pathways. A phosphoproteome study of the *A. fumigatus* wild-type strain and *mpkA* and *sakA* deletion mutants showed a dynamic changes of phosphopeptides when exposed to the antifungal drug caspofungin, which are mainly enriched in transcription factors, protein kinases, and cytoskeleton proteins (Mattos *et al.*, 2020). Proteomic analysis of these two MAPK mutants suggested proteins involved in secondary metabolism (e.g. AFUA\_1G10320, AFUA\_1G10330, AFUA\_1G10350 and AFUA\_1G10360 in the nidulanin-like BGC, and AFUA\_8G00410 in the fumagillin/pseurotin BGC) were also impacted. Another global phosphoproteomic analysis was also studied in *A. flavus*, of which 283 phosphoproteins were identified in this fungus (Ren *et al.*, 2016). The identified phosphoproteins were found involved in signal transduction and metabolic pathways known to impact AF biosynthesis. The enzymes that are involved in primary metabolites, e.g. glycolysis/gluconeogenesis and pentose phosphate pathway were phosphorylated. These pathways are important for the synthesis of acetyl-CoA that is the primary metabolite used for AF biosynthesis. The peroxisome protein Pex19 was also found phosphorylated in *A. flavus*. The first few precursors of AF and ST appear to be synthesized in the peroxisome (Kistler and Broz, 2015). These results indicate the association between the reversible phosphorylation and mycotoxin biosynthesis. Although the

phosphopeptides were not identified among the AF synthetic enzymes, 16 phosphorylated proteins were distributed in 14 different BGCs, including some known SMs such as leporin B and the sclerotia related-secondary metabolite, aflavarin (Fig. 5).

In *A. nidulans*, over 1964 proteins were characterized phosphorylated, among which the reversible phosphorylated state of 131 proteins were influenced by the oxidative stress agent hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Carrasco-Navarro and Aguirre, 2021). Additionally, the phosphorylation of critical components of MAPK, and TOR signalling as well as the proteins that involved in the regulation of primary and secondary metabolism were found affected by H<sub>2</sub>O<sub>2</sub> in *A. nidulans*. A system-wide characterization of protein phosphatases (PPs) in *Trichoderma reesei* showed that some of these PPs were involved in regulation of specific cellulase activity and protease production (Rodríguez-Iglesias and Schmoll, 2019). The differences in the secreted SM patterns between light and darkness were observed in the  $\Delta pp$  mutants. However, it remains unknown how PPs regulate SM biosynthesis or other metabolites production.

### Succinylation

Protein succinylation (su) is the addition of a succinyl group to a specific lysine residue of a protein occurring both in prokaryotes and eukaryotes. Succinylation has been found in both non-histone and histone proteins, which is involved in controlling various cellular processes (Xie *et al.*, 2012; Weinert *et al.*, 2013). Recently, a global analysis of lysine succinylome was studied in *A. flavus*, and a total number of 349 succinylated proteins were identified in this fungus, which were found involved in diverse biological processes, especially for the AF biosynthesis-related process (Ren *et al.*, 2018). In the enzymatic cascade of AF synthesis, AfIE (AFLA\_139310), AfIK (AFLA\_139190), and AfIM (AFLA\_139300) were found succinylated (Fig. 6). The role of the lysine succinylation at the norsolorinic acid reductase NorA (AfIE) was analysed and showed that succinylation at K370 is important for the activity of AfIE. From the published data, another 24 Ksu proteins were distributed in 20 different BGCs, such as cyclopiazonic acid (Fig. 5). Accumulating evidence suggests that succinylation on lysine overlaps with other PTMs, such as acetylation and malonylation, to function in metabolic pathways and other cellular processes (Xie *et al.*, 2012; Weinert *et al.*, 2013). Intriguingly, 20 of the 26 identified succinylated proteins involved in BGCs synthesis pathway are all acetylated (Fig. 5), and they do share the same modification site at lysine. In the model medicinal mushroom *Ganoderma lucidum*, lysine succinylation is also related to many secondary metabolites, such as

pharmacologically bioactive compounds (Wang *et al.*, 2019). A total of 47 succinylated enzymes were found to be involved in biosynthesis of triterpenoids and polysaccharides in *G. lucidum*. These data suggest that PTMs at the SM synthetic enzymes could alternatively regulate the SMs biosynthesis when fungi response to different environmental stimulus.

### Ubiquitin and ubiquitin-like modification

#### Ubiquitin

Ubiquitination is known for its role in proteasome-dependent protein degradation. The process involves three enzymes including an activating enzyme (E1), a conjugating enzyme (E2), and a ligase (E3). An ubiquitinated protein is the target of ubiquitin, a small conserved protein with 76 amino acids that attaches to the lysine residues of target proteins. In addition to degradation, ubiquitination is also found involved in the regulation of protein activity and subcellular localization. The function of a HECT (homologous to E6AP C terminus) ubiquitin ligase called Hula has been studied in several *Aspergilli*. In *A. oryzae*, Hula regulates the endocytosis of a maltose permease, MalP, which is important for alpha-amylase production (Hiramoto *et al.*, 2015). The cullin-RING ligases (CRLs) comprise the largest family of E3 ligases, among which, the SCF (Skp1/Cullin-1/F-box) ligases are the best-studied examples (Frawley and Bayram, 2020a). In SCF complex, Skp1 (S-phase kinase-associated protein 1) functions as the adaptor between the cullin scaffold and the F-box substrate receptor. There are approximately 70 F-box protein-encoding genes predicted in *A. nidulans* genome (von Zeska Kress *et al.*, 2012). One of the F-box protein GrrA (Fbx29) is involved in meiosis and sexual spore formation of ascospores in *A. nidulans* (Krappmann *et al.*, 2006). Other F-box proteins like Fbx15 and Fbx23 are found required for sexual development in this fungus (Krappmann *et al.*, 2006).

The reversible ubiquitination also regulates carbon catabolite repression (CCR) of *Aspergillus* and other filamentous fungi. In CCR, the activity of the transcription factor CreA is regulated by ubiquitination modification to some extent, which is ubiquitinated by CreD-Hula ubiquitination ligase complex, and deubiquitinated by the CreB-CreC deubiquitination (DUB) complex, leading to its activation (Alam *et al.*, 2017; Nie *et al.*, 2018). In *A. nidulans*, 22 putative deubiquitinating enzymes were identified, and a defect of one of the DUB members, UspA, leads to the delayed formation of developmental structures and altered secondary metabolism (Meister *et al.*, 2019). The presence of UspA, together with Fbx23, leads to degradation of VeA, which is a key regulator of

fungal development and SMs in *Aspergillus*. To identify some new SMs in *A. nidulans*, the disruption mutant of a gene that encodes the small ubiquitin-like protein SUMO was generated and led to the characterization of asperthecin (Szewczyk *et al.*, 2008).

#### Ubiquitin-like modification

In addition to ubiquitination, several ubiquitin-like modifications are found as well, which share similar mechanism to attach to the lysine residue of target proteins. These ubiquitin-like modifications, which include sumoylation and neddylation, regulate various cellular processes, including normal growth, signal transduction, and secondary metabolism.

**Sumoylation.** Sumoylation is the attachment of the small ubiquitin-like modifier protein SUMO to target proteins, which regulates protein interactions and activity and involves in many key cellular processes. As mentioned in ‘Ubiquitin’ section, the  $\Delta$ *sumO* mutant in *A. nidulans* dramatically increased in the production of asperthecin, it was also shown to be important for the expression of the austinol/dehydroaustinol and sterigmatocystin BGCs (Szewczyk *et al.*, 2008). Recently, a SUMOylome study of *A. nidulans* showed that a total number of 149 SUMOylated proteins were identified in this fungus, which are predicted to be involved in transcription, RNA processing and DNA replication (Horio *et al.*, 2019). In *Aspergillus*, a key sumoylation pathway is catalysed subsequently by three enzymes, E1 (activating enzyme; UbaB/AosA), E2 (conjugation enzyme; UbcN), and E3 (ligases; SizA, SizB, and MmsU), which are indicated in Table 3. The SumO isopeptidases UlpA/UlpB are responsible for removing SumO from the sumoylated proteins. In *A. nidulans*, The E1 enzymes AoaA and UbaB and the sole E2 enzyme UbcN are required for multicellular development (Harting *et al.*, 2013). The *mmsU* deletion mutant exhibited defects of growth and conidiation, while the other two E3 ligases SizA and SizB are dispensable in this fungus (Horio *et al.*, 2019). The SumO

isopeptidase UlpA and SumO processing enzyme UlpB are involved in asexual and sexual development as well in *A. nidulans*. The function of sumoylation is also reported in *A. flavus*. The deletion of *AfsumO* leads to defects of growth and conidiation and decreased production of AF (Nie *et al.*, 2016).

**Neddylation.** Neddylation is another ubiquitin-like modification that attaches the NEDD8 (neural precursor cell expressed, developmentally down-regulated 8) polypeptide to lysine residues of target proteins. Neddylation is important for fungal growth and secondary metabolism (Köhler *et al.*, 2019) and has a closed relationship with ubiquitination that could regulate the activity of the SCF E3 ligase complex by neddylation of the cullin-1 protein at a lysine residue (Deshaies *et al.*, 2010). The neddylation of cullin proteins can be deneddylation by the COP9 signalosome (CSN), a conserved complex with eight subunits. The deneddylation of cullin proteins are then blocked by Cand1 (Cullin-associated-Nedd8-dissociated protein 1) that attaches to the Nedd8 binding site with the N-terminal domain. The Can1 homologue CandA was a trimeric complex in *A. nidulans* and a dimeric complex in *A. fumigatus*. In  $\Delta$ *candA-C1* mutant, *A. nidulans* accumulated more production of many SMs such as austinol, dehydroaustinol, asperthecin and emericellin (Köhler *et al.*, 2019). The deletion mutants of the genes encoding CSN subunits CsnA, CsnB, CsnD, or CsnE in *A. nidulans* produce aberrant red pigment and can initiate the sexual cycle and develop primordia but are blocked in maturation to sexual fruit bodies (Busch *et al.*, 2003; Busch *et al.*, 2007). The CSN complex of *A. nidulans* also plays a dual role during development, which functions early for protection against oxidative stress and hormone regulation and is critical for regulation of secondary metabolism in the later growth stage (Nahlik *et al.*, 2010). CSN can interact with the deneddylation A/1 (DenA/Den1) in fungi and humans, which targets DEN1/DenA for protein degradation (Christmann *et al.*, 2013). The activity of DenA in *A. nidulans* is required for asexual development and reduces sexual fruiting body formation in light;

**Table 3.** SUMO and SUMO pathway genes in *A. nidulans* and *A. flavus*.

Pathway component	<i>A. nidulans</i>	<i>A. flavus</i>	Gene name
SUMO	AN1191	AFLA_068730	<i>sumO</i>
SumO activating enzyme E1	AN2450	AFLA_106510	<i>ubaB</i>
	AN2298	AFLA_048570	<i>aosA</i>
SumO conjugating enzyme E2	AN4399	AFLA_113060	<i>ubcN</i>
SumO E3 ligases	AN10822	AFLA_054730	<i>sizA</i>
	AN4497	AFLA_092420	<i>sizB</i>
	AN10240	AFLA_036350	<i>mmsU</i>
SumO Isopeptidases	AN2689	AFLA_095900	<i>ulpA</i>
	AN8192	AFLA_005920	<i>ulpB</i>

however, the deletion of both deneddylases (DenA and CSN) impairs multicellular fungal development (Christmann *et al.*, 2013).

### Palmitoylation

Palmitoylation is a reversible lipid modification on the side chain of cysteine residues of target proteins by protein S-acetyltransferases (PATs). Palmitoylation plays important roles in various cell functions, physiology and pathophysiology (Zhang *et al.*, 2016c). In *A. nidulans*, the function of a palmitoyl transferase AkrA was studied, which regulates Ca<sup>2+</sup> homeostasis by palmitoylating its protein substrates (Zhang *et al.*, 2016c). A total amount of 234 palmitoylated proteins are identified in *A. fumigatus* conidia, which includes several G-protein subunits and Rho GTPases that are found palmitoylated in other organisms. In this fungus, four enzymes involved in early melanin synthesis were found strongly palmitoylated during asexual development, and this lipid modification links with the specific subcellular compartments of these palmitoylated melanin enzymes (Upadhyay *et al.*, 2016).

### Other PTMs

Besides the modifications mentioned above, there are still some other PTMs, including protein glycosylation, farnesylation, prenylation, S-nitrosylation and some other ubiquitin-like modifications (e.g. urmylation, and pupylation), and N-myristoylation, that have been identified and shown to play roles in membrane targeting, fungal growth, development, cell wall synthesis and virulence in *Aspergillus* and other fungi (Fang *et al.*, 2015; Liu *et al.*, 2021). S-nitrosylation has been shown to regulate styrylpyrone biosynthesis in *Inonotus obliquus* (Zhao *et al.*, 2016) and prenylated secondary metabolites are found in many fungi (Yu *et al.*, 2011; Genovese *et al.*, 2015).

### Crosstalk between different PTMs on SM expression

Fungal secondary metabolism is, not surprisingly, regulated by combinations and interactions (e.g. crosstalk) of different modifications, especially between histone modifications. An interplay between H3K4 methylation and H3K9 methylation in *A. nidulans*, both of which are all decreased in the deletion mutant of deletion of the COMPASS component Bre2/CclA, leads to the activation of cryptic secondary metabolite clusters in this fungus (Bok *et al.*, 2009). In *A. flavus*, the acetylation of H3K56 and H3K9 is found co-regulated by a member of the P300/CBP acetyltransferase family, Rtt109, which is

required for AF biosynthesis (Sun *et al.*, 2021). The crosstalk between methylation and acetylation at H3K4 and H3K36 for transcriptional activation (Martin *et al.*, 2006; Ginsburg *et al.*, 2014) is another example to provide the valuable information of the interaction between different histone modification. A recent study in *F. graminearum* showed that TOR inactivation promotes the degradation of Gcn5 by ubiquitination, which reduces acetylation level of Atg8, leading to the translocation of deacetylated Atg8 into the cytoplasm and subsequent induction of fungal autophagy (Wang *et al.*, 2021a).

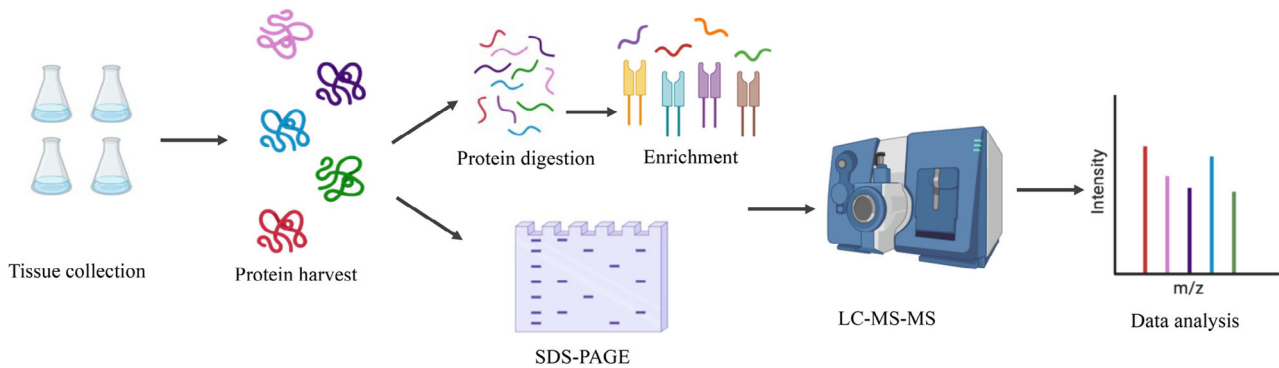
There is also interplay between different PTMs at non-histone proteins. For example, as mentioned earlier, acetylation and malonylation or succinylation of the same lysine residues is involved in the regulation of metabolic pathways and other cellular processes (Xie *et al.*, 2012; Weinert *et al.*, 2013). In *A. flavus*, we found that 20 of the 26 identified succinylated proteins associated with SMs biosynthesis are all acetylated (Fig. 5). In the AF biosynthetic pathway, AfIK and AfIM are both succinylated and acetylated (Fig. 6A). The modification on the specific lysine residues on these AF related enzymes is important for their activities and subsequent AF synthesis (Ren *et al.*, 2018; Yang *et al.*, 2019). Although it remains unclear whether these overlapping PTMs on the enzymes could affect each other, the dynamic changes of different modifications at these sites could alternatively regulate the SMs biosynthesis when fungi response to different environmental stimulus.

### Identification and prediction of PTM

To identify the specific PTM sites, many experimental methods have been performed, among which immunoaffinity enrichment combined with MS-based proteomics approach is often used to measure the PTM-modified proteins (Liu *et al.*, 2021). By combining use of high-sensitivity mass spectrometry analysis with the gel-based or gel-free approaches, like chemical labeling or immunoaffinity enrichment approaches, the current catalogue of observed PTMs is expanding in fungi (Fig. 8). These PTM detection approaches have been successfully used for identifying sites of phosphorylation, acetylation, and succinylation of many fungi. For instance, high accuracy nano-LC-MS/MS allowed for analysis of global lysine succinylation, contributing to the knowledge of regulation of various metabolic processes including AF biosynthesis in *A. flavus* (Ren *et al.*, 2018).

Since the motif sequence of the PTM site is conserved in most PTMs, many algorithms have been applied to predict the PTM sites. Based on the published phosphorylation proteomics of several fungi including *A. flavus*,





**Fig. 8.** Workflow for identifying specific PTMs analysis in fungi. Fungal tissues are collected to harvest total proteins, which can be enriched by different method (e.g. immuno-affinity enrichment) or subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel separation. The enriched or separated peptides/proteins are subjected to mass spectrometry analysis and data analysis.

*A. nidulans*, *F. graminearum*, Bai *et al.* established the Fungi Phosphorylation Database (FPD) that can serve as a comprehensive protein phosphorylation data resource for further fungal studies (Bai *et al.*, 2017). Some other bioinformatics programmes, such as NetPhos, GPS and Scansite, are also used to predict phosphorylation sites (Table 4). Methylated sites can be predicted by GPS-MSP, acetylation sites and proteins can be predicted by NetAcet and GPS-PAIL, succinylation sites can be predicted by HybridSucc, sumoylated sites can be predicted by GPS-SUMO and SUMOplot (Table 4). Although the bioinformatics prediction is useful for identification of specific PTM sites, it is not enough for understanding their global roles in biological mechanisms. Other approaches, such as the information of protein–

protein interaction (PPI) and protein structure, could help to identify PTM site and their roles in biological processes.

### Future prospects

It has been more than 10 years since the first published research on epigenetic (histone acetylation) regulation of secondary metabolism in fungi (Shwab *et al.*, 2007). Rapid progress in mining new fungal SMs has been made with the applications of PTMs on chromatin remodelling, which provides powerful tools to activate silent BGCs in fungal genome. Thus far, the research of regulation fungal SMs is mainly focused on transcriptional regulation and chromatin modifications (histone

**Table 4.** The available tools for specific PTM and site prediction.

PTM type	Tool	Website	Function
Methylation	GPS-MSP	<a href="http://msp.biocuckoo.org/online.php">http://msp.biocuckoo.org/online.php</a>	Prediction of general or type-specific methyl and methylarginine residues in proteins
Acetylation	NetAcet-1.0	<a href="https://services.healthtech.dtu.dk/service.php?NetAcet-1.0">https://services.healthtech.dtu.dk/service.php?NetAcet-1.0</a>	Prediction of N-terminal acetylation in eukaryotic proteins
	GPS-PAIL	<a href="http://pail.biocuckoo.org">http://pail.biocuckoo.org</a>	Prediction of acetylation substrates and sites of HATs
Phosphorylation	FPD	<a href="http://bis.zju.edu.cn/FPD/">http://bis.zju.edu.cn/FPD/</a>	Fungi phosphorylation database
	NetPhos-3.1	<a href="https://services.healthtech.dtu.dk/service.php?NetPhos-3.1">https://services.healthtech.dtu.dk/service.php?NetPhos-3.1</a>	Prediction of Generic phosphorylation sites in eukaryotic proteins
	GPS	<a href="http://gps.biocuckoo.cn">http://gps.biocuckoo.cn</a>	Prediction of kinase-specific phosphorylation sites
	Scansite	<a href="http://scansite.mit.edu">http://scansite.mit.edu</a>	Prediction of phosphorylation sites
Succinylation	HybridSucc	<a href="http://hybridsucc.biocuckoo.org">http://hybridsucc.biocuckoo.org</a>	Prediction of lysine succinylation site
Ubiquitination	iUbiq-Lys	<a href="http://www.jci-bioinfo.cn/iUbiq-Lys">http://www.jci-bioinfo.cn/iUbiq-Lys</a>	Prediction of lysine ubiquitination sites in proteins
	UbiNet	<a href="http://140.138.144.145/~ubinet/index.php">http://140.138.144.145/~ubinet/index.php</a>	Prediction of E3 ligase-mediated ubiquitination networks
Sumoylation	GPS-SUMO	<a href="http://sumosp.biocuckoo.org/online.php">http://sumosp.biocuckoo.org/online.php</a>	Prediction of sumoylated sites
	SUMOplot	<a href="https://www.abcepta.com/sumonlot">https://www.abcepta.com/sumonlot</a>	Prediction of sumoylated sites
Palmitoylation	CSS-Palm	<a href="http://csspalm.biocuckoo.org/online.php">http://csspalm.biocuckoo.org/online.php</a>	Prediction of S-palmitoylation proteins
	Terminator	<a href="https://bioweb.i2bc.paris-saclay.fr/terminator3/">https://bioweb.i2bc.paris-saclay.fr/terminator3/</a>	Prediction of N-terminal acetylation and S-palmitoylation of proteins
S-nitrosylation	SwissPalm	<a href="http://swisspalm.epfl.ch">http://swisspalm.epfl.ch</a>	Prediction of S-palmitoylation proteins
	SeqPalm	<a href="http://lishuyan.lzu.edu.cn/seqpalm/index">http://lishuyan.lzu.edu.cn/seqpalm/index</a>	Prediction of S-palmitoylation proteins
	GPS-SNO	<a href="http://sno.biocuckoo.org">http://sno.biocuckoo.org</a>	Prediction of S-nitrosylation site

methylation and histone acetylation). However, recent studies on non-histone protein modifications have indicated the potential roles of PTMs on regulation of fungal secondary metabolism. Understanding the important roles of the non-histone PTMs, such as succinylation, ubiquitin-like modifications, palmitoylation, and others, need more extensive studies in *Aspergillus* and other fungi.

Additionally, some other PTMs, such as S-nitrosylation, glycosylation, or lysine lactylation are found involved in biological process including SMS synthesis, but they are largely unknown in the context of regulation of SM or other metabolism processes and need further investigation. Efforts on the dynamic balance between different PTMs and the regulatory networks mediated by these PTMs will help to characterize new SMs or antibiotics in *Aspergillus* and other fungi. Also some SMs produced by fungi are toxic or act as virulence factors. Because many PTMs are important for regulating activities of the enzymes involved in SM biosynthesis, the application of chemical inhibitors of PTM enzymes may be useful in controlling synthesis of harmful secondary metabolites.

### Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (31900036, 31972171), the Natural Science Foundation of Jiangsu Province (BK20190994), the Program of Natural Science Foundation of the Jiangsu Higher Education Institutions of China (19KJB180016), Natural Science Foundation by Xuzhou City (KC21295), the China Scholarship Council, and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

### References

- Abe, K., Gomi, K., Hasegawa, F., and Machida, M. (2006) Impact of *Aspergillus oryzae* genomics on industrial production of metabolites. *Mycopathologia* **162**: 143–153.
- Affeldt, K., Carrig, J., Amare, M., and Keller, N. (2014) Global survey of canonical *Aspergillus flavus* G protein-coupled receptors. *MBio* **5**: e01501–e01514.
- Alam, M.A., Kamlangdee, N., and Kelly, J.M. (2017) The CreB deubiquitinating enzyme does not directly target the CreA repressor protein in *Aspergillus nidulans*. *Curr Genet* **63**: 647–667.
- Albright, J.C., Henke, M.T., Soukup, A.A., McClure, R.A., Thomson, R.J., Keller, N.P., and Kelleher, N.L. (2015) Large-scale metabolomics reveals a complex response of *Aspergillus nidulans* to epigenetic perturbation. *ACS Chem Biol* **10**: 1535–1541.
- Aldholmi, M., Wilkinson, B., and Ganesan, A. (2020) Epigenetic modulation of secondary metabolite profiles in *Aspergillus calidoustus* and *Aspergillus westerdijkiae* through histone deacetylase (HDAC) inhibition by vorinostat. *J Antibiot (Tokyo)* **73**: 410–413.
- Alves de Castro, P., Dos Reis, T.F., Dolan, S.K., Oliveira Manfioli, A., Brown, N.A., Jones, G.W., et al. (2016) The *Aspergillus fumigatus* SchA<sup>SCH9</sup> kinase modulates SakA<sup>HOG1</sup> MAP kinase activity and it is essential for virulence. *Mol Microbiol* **102**: 642–671.
- Antequera, F., Tamame, M., Villanueva, J.R., and Santos, T. (1984) DNA methylation in the fungi. *J Biol Chem* **259**: 8033–8036.
- Armeev, G.A., Gribkova, A.K., Pospelova, I., Komarova, G. A., and Shaytan, A.K. (2019) Linking chromatin composition and structural dynamics at the nucleosome level. *Curr Opin Struct Biol* **56**: 46–55.
- Baccile, J.A., Spraker, J.E., Le, H.H., Brandenburger, E., Gomez, C., Bok, J.W., et al. (2016) Plant-like biosynthesis of isoquinoline alkaloids in *Aspergillus fumigatus*. *Nat Chem Biol* **12**: 419–424.
- Bai, Y., Chen, B., Li, M., Zhou, Y., Ren, S., Xu, Q., et al. (2017) FPD: a comprehensive phosphorylation database in fungi. *Fungal Biol* **121**: 869–875.
- Baker, S.E. (2006) *Aspergillus niger* genomics: past, present and into the future. *Med Mycol* **44**: S17–S21.
- Bao, K., Shan, C.M., Chen, X., Raiymbek, G., Monroe, J.G., Fang, Y., et al. (2022) The cAMP signaling pathway regulates Epe1 protein levels and heterochromatin assembly. *PLoS Genet* **18**: e1010049.
- Bauer, I., Graessle, S., Loidl, P., Hohenstein, K., and Brosch, G. (2010) Novel insights into the functional role of three protein arginine methyltransferases in *Aspergillus nidulans*. *Fungal Genet Biol* **47**: 551–561.
- Bayram, Ö., Bayram, Ö., Ahmed, Y.L., Maruyama, J., Valerius, O., Rizzoli, S.O., et al. (2012) The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. *PLoS Genet* **8**: e1002816.
- Blin, K., Wolf, T., Chevrette, M.G., Lu, X., Schwalen, C.J., Kautsar, S.A., et al. (2017) antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res* **45**: W36–W41.
- Bok, J.W., Chiang, Y.M., Szewczyk, E., Reyes-Dominguez, Y., Davidson, A.D., Sanchez, J.F., et al. (2009) Chromatin-level regulation of biosynthetic gene clusters. *Nat Chem Biol* **5**: 462–464.
- Bok, J.W., and Keller, N.P. (2004) LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryot Cell* **3**: 527–535.
- Bok, J.W., Noordermeer, D., Kale, S.P., and Keller, N.P. (2006) Secondary metabolic gene cluster silencing in *Aspergillus nidulans*. *Mol Microbiol* **61**: 1636–1645.
- Busch, S., Eckert, S.E., Krappmann, S., and Braus, G.H. (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. *Mol Microbiol* **49**: 717–730.
- Busch, S., Schwier, E.U., Nahlik, K., Bayram, Ö., Helmstaedt, K., Draht, O.W., et al. (2007) An eight-subunit COP9 signalosome with an intact JAMM motif is required for fungal fruit body formation. *Proc Natl Acad Sci* **104**: 8089–8094.
- Caesar, L.K., Kelleher, N.L., and Keller, N.P. (2020) In the fungus where it happens: history and future propelling *Aspergillus nidulans* as the archetype of natural products research. *Fungal Genet Biol* **144**: 103477.

- Cai, Q., Wang, J.J., Shao, W., Ying, S.H., and Feng, M.G. (2018) Rtt109-dependent histone H3 K56 acetylation and gene activity are essential for the biological control potential of *Beauveria bassiana*. *Pest Manag Sci* **74**: 2626–2635.
- Carrasco-Navarro, U., and Aguirre, J. (2021) H<sub>2</sub>O<sub>2</sub> induces major phosphorylation changes in critical regulators of signal transduction, gene expression, metabolism and developmental networks in *Aspergillus nidulans*. *J Fungi (Basel)* **7**: 624.
- Cary, J.W., Han, Z., Yin, Y., Lohmar, J.M., Shantappa, S., Harris-Coward, P.Y., et al. (2015b) Transcriptome analysis of *Aspergillus flavus* reveals veA-dependent regulation of secondary metabolite gene clusters, including the novel Aflavarin cluster. *Eukaryot Cell* **14**: 983–997.
- Cary, J.W., Harris-Coward, P.Y., Ehrlich, K.C., Di Mavungu, J.D., Malysheva, S.V., De Saeger, S., et al. (2014) Functional characterization of a veA-dependent polyketide synthase gene in *Aspergillus flavus* necessary for the synthesis of asparosone, a sclerotium-specific pigment. *Fungal Genet Biol* **64**: 25–35.
- Cary, J.W., Linz, J.E., and Bhatnagar, D. (1999) *Microbial Foodborne Diseases: Mechanisms of Pathogenesis and Toxin Synthesis*. Boca Raton: CRC Press.
- Cary, J.W., Uka, V., Han, Z., Buyst, D., Harris-Coward, P.Y., Ehrlich, K.C., et al. (2015a) An *Aspergillus flavus* secondary metabolic gene cluster containing a hybrid PKS-NRPS is necessary for synthesis of the 2-pyrindones, leporins. *Fungal Genet Biol* **81**: 88–97.
- Chang, P.K., Horn, B.W., and Dorner, J.W. (2009) Clustered genes involved in cyclopiazonic acid production are next to the aflatoxin biosynthesis gene cluster in *Aspergillus flavus*. *Fungal Genet Biol* **46**: 176–182.
- Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., and Xia, R. (2020) TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant* **13**: 1194–1202.
- Christmann, M., Schmalzer, T., Gordon, C., Huang, X., Bayram, O., Schinke, J., et al. (2013) Control of multicellular development by the physically interacting deneddylases DEN1/DenA and COP9 signalosome. *PLoS Genet* **9**: e1003275.
- Cichewicz, R.H. (2010) Epigenome manipulation as a pathway to new natural product scaffolds and their congeners. *Nat Prod Rep* **27**: 11–22.
- Collemare, J., and Seidl, M.F. (2019) Chromatin-dependent regulation of secondary metabolite biosynthesis in fungi: is the picture complete? *FEMS Microbiol Rev* **43**: 591–607.
- Connolly, L.R., Smith, K.M., and Freitag, M. (2013) The *Fusarium graminearum* histone H3 K27 methyltransferase KMT6 regulates development and expression of secondary metabolite gene clusters. *PLoS Genet* **9**: e1003916.
- Dallery, J.F., Le Goff, G., Adelin, E., Iorga, B.I., Pigné, S., O'Connell, R.J., and Ouazzani, J. (2019) Deleting a chromatin remodeling gene increases the diversity of secondary metabolites produced by *Colletotrichum higginsianum*. *J Nat Prod* **82**: 813–822.
- De Nadal, E., Zapater, M., Alepuz, P.M., Sumoy, L., Mas, G., and Posas, F. (2004) The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. *Nature* **427**: 370–374.
- de Vries, R.P., Riley, R., Wiebenga, A., Aguilar-Osorio, G., Amillis, S., Uchima, C.A., et al. (2017) Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus *Aspergillus*. *Genome Biol* **18**: 28.
- Deshaies, R.J., Emberley, E.D., and Saha, A. (2010) Control of cullin-ring ubiquitin ligase activity by nedd8. *Subcell Biochem* **54**: 41–56.
- Dos Reis, T.F., Mellado, L., Lohmar, J.M., Silva, L.P., Zhou, J.J., Calvo, A.M., et al. (2019) GPCR-mediated glucose sensing system regulates light-dependent fungal development and mycotoxin production. *PLoS Genet* **15**: e1008419.
- Drott, M.T., Rush, T.A., Satterlee, T.R., Giannone, R.J., Abraham, P.E., Greco, C., et al. (2021) Microevolution in the pansecondary metabolome of *Aspergillus flavus* and its potential macroevolutionary implications for filamentous fungi. *Proc Natl Acad Sci* **118**: e2021683118.
- Drott, M.T., Satterlee, T.R., Skerker, J.M., Pfannenstiel, B.T., Glass, N.L., Keller, N.P., and Milgroom, M.G. (2020) The frequency of sex: population genomics reveals differences in recombination and population structure of the aflatoxin-producing fungus *Aspergillus flavus*. *mBio* **11**: e00963–e00920.
- Dubey, A., and Jeon, J. (2017) Epigenetic regulation of development and pathogenesis in fungal plant pathogens. *Mol Plant Pathol* **18**: 887–898.
- Erlendson, A.A., Friedman, S., and Freitag, M. (2017) A matter of scale and dimensions: chromatin of chromosome landmarks in the fungi. *Microbiol Spectr* **5**: 10.
- Fan, X., Matsumoto, H., Wang, Y., Hu, Y., Liu, Y., Fang, H., et al. (2019) Microenvironmental interplay predominated by beneficial *Aspergillus* abates fungal pathogen incidence in Paddy environment. *Environ Sci Technol* **53**: 13042–13052.
- Fang, W., Robinson, D.A., Raimi, O.G., Blair, D.E., Harrison, J.R., Lockhart, D.E., et al. (2015) N-myristoyltransferase is a cell wall target in *Aspergillus fumigatus*. *ACS Chem Biol* **10**: 1425–1434.
- Fisch, K.M., Gillaspay, A.F., Gipson, M., Henrikson, J.C., Hoover, A.R., Jackson, L., et al. (2009) Chemical induction of silent biosynthetic pathway transcription in *Aspergillus Niger*. *J Ind Microbiol Biotechnol* **36**: 1199–1213.
- Forseth, R.R., Amaike, S., Schwenk, D., Affeldt, K.J., Hoffmeister, D., Schroeder, F.C., and Keller, N.P. (2013) Homologous NRPS-like gene clusters mediate redundant small-molecule biosynthesis in *Aspergillus flavus*. *Angew Chem Int Ed Engl* **52**: 1590–1594.
- Frawley, D., and Bayram, Ö. (2020a) Identification of SkpA-CulA-F-box E3 ligase complexes in pathogenic *Aspergilli*. *Fungal Genet Biol* **140**: 103396.
- Frawley, D., and Bayram, Ö. (2020b) The pheromone response module, a mitogen-activated protein kinase pathway implicated in the regulation of fungal development, secondary metabolism and pathogenicity. *Fungal Genet Biol* **144**: 103469.
- Frawley, D., Karahoda, B., Sarikaya Bayram, Ö., and Bayram, Ö. (2018) The HamE scaffold positively regulates MpkB phosphorylation to promote development and secondary metabolism in *Aspergillus nidulans*. *Sci Rep* **8**: 16588.

- Frawley, D., Stroe, M.C., Oakley, B.R., Heinekamp, T., Straßburger, M., Fleming, A.B., *et al.* (2020) The pheromone module SteC-MkkB-MpkB-SteD-HamE regulates development stress responses and secondary metabolism in *Aspergillus fumigatus*. *Front Microbiol* **11**: 811.
- Gacek-Matthews, A., and Berger, H. (2016) KdmB, a Jumonji histone H3 demethylase, regulates genome-wide H3K4 trimethylation and is required for normal induction of secondary metabolism in *Aspergillus nidulans*. **12**: e1006222.
- Gacek-Matthews, A., Noble, L.M., Gruber, C., Berger, H., Sulyok, M., Marcos, A.T., *et al.* (2015) KdmA, a histone H3 demethylase with bipartite function, differentially regulates primary and secondary metabolism in *Aspergillus nidulans*. *Mol Microbiol* **96**: 839–860.
- Genovese, S., Fiorito, S., Epifano, F., and Taddeo, V.A. (2015) A novel class of emerging anticancer compounds: oxyprenylated secondary metabolites from plants and fungi. *Curr Med Chem* **22**: 3426–3433.
- Ginsburg, D.S., Anlembom, T.E., Wang, J., Patel, S.R., Li, B., and Hinnebusch, A.G. (2014) NuA4 links methylation of histone H3 lysines 4 and 36 to acetylation of histones H4 and H3. *J Biol Chem* **289**: 32656–32670.
- Greco, C., Pfannenstiel, B.T., Liu, J.C., and Keller, N.P. (2019) Depsipeptide Aspergillins revealed by chromatin reader protein deletion. *ACS Chem Biol* **14**: 1121–1128.
- Grosse, C., Heinekamp, T., Kniemeyer, O., Gehrke, A., and Brakhage, A.A. (2008) Protein kinase a regulates growth, sporulation, and pigment formation in *Aspergillus fumigatus*. *Appl Environ Microbiol* **74**: 4923–4933.
- Gu, Q., Zhang, C., Yu, F., Yin, Y., Shim, W.B., and Ma, Z. (2015) Protein kinase FgSch9 serves as a mediator of the target of rapamycin and high osmolarity glycerol pathways and regulates multiple stress responses and secondary metabolism in *Fusarium graminearum*. *Environ Microbiol* **17**: 2661–2676.
- Hagiwara, D., Sakamoto, K., Abe, K., and Gomi, K. (2016) Signaling pathways for stress responses and adaptation in *Aspergillus* species: stress biology in the post-genomic era. *Biosci Biotechnol Biochem* **80**: 1667–1680.
- Han, J., Zhou, H., Horazdovsky, B., Zhang, K., Xu, R.M., and Zhang, Z. (2007) Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* **315**: 653–655.
- Harting, R., Bayram, Ö., Laubinger, K., Valerius, O., and Baus, G.H. (2013) Interplay of the fungal sumoylation network for control of multicellular development. *Mol Microbiol* **90**: 1125–1145.
- Henke, M.T., Soukup, A.A., Goering, A.W., McClure, R.A., Thomson, R.J., Keller, N.P., and Kelleher, N.L. (2016) New aspercryptins, lipopeptide natural products, revealed by HDAC inhibition in *Aspergillus nidulans*. *ACS Chem Biol* **11**: 2117–2123.
- Hiramoto, T., Tanaka, M., Ichikawa, T., Matsuura, Y., Hasegawa-Shiro, S., Shintani, T., and Gomi, K. (2015) Endocytosis of a maltose permease is induced when amyolytic enzyme production is repressed in *Aspergillus oryzae*. *Fungal Genet Biol* **82**: 136–144.
- Horio, T., Szweczyk, E., Oakley, C.E., Osmani, A.H., Osmani, S.A., and Oakley, B.R. (2019) SUMOlock reveals a more complete *Aspergillus nidulans* SUMOylome. *Fungal Genet Biol* **127**: 50–59.
- Hosseini, S., Meunier, C., Nguyen, D., Reimegård, J., and Johannesson, H. (2020) Comparative analysis of genome-wide DNA methylation in *Neurospora*. *Epigenetics* **15**: 972–987.
- Hu, Y., Yang, G., Zhang, D., Liu, Y., Li, Y., Lin, G., *et al.* (2018) The PHD transcription factor Rum1 regulates morphogenesis and aflatoxin biosynthesis in *Aspergillus flavus*. *Toxins (Basel)* **10**: 301.
- Itoh, E., Odakura, R., Oinuma, K.-I., Shimizu, M., Masuo, S., and Takaya, N. (2017) Sirtuin E is a fungal global transcriptional regulator that determines the transition from the primary growth to the stationary phase. *J Biol Chem* **292**: 11043–11054.
- Janevska, S., Baumann, L., Sieber, C.M.K., Münsterkötter, M., Ulrich, J., Kämper, J., *et al.* (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**: 153–171.
- Jeon, J., Choi, J., Lee, G.W., Park, S.Y., Huh, A., Dean, R. A., and Lee, Y.H. (2015) Genome-wide profiling of DNA methylation provides insights into epigenetic regulation of fungal development in a plant pathogenic fungus, *Magnaporthe oryzae*. *Sci Rep* **5**: 8567.
- Kadooka, C., Nakamura, E., Mori, K., Okutsu, K., Yoshizaki, Y., Takamine, K., *et al.* (2020) LaeA controls citric acid production through regulation of the citrate exporter-encoding *cexA* gene in *Aspergillus luchuensis* *mut. kawachii*. *Appl Environ Microbiol* **86**: e01950–e01919.
- Kang, W.K., Kim, Y.H., Kang, H.A., Kwon, K.S., and Kim, J. Y. (2015) Sir2 phosphorylation through cAMP-PKA and CK2 signaling inhibits the lifespan extension activity of Sir2 in yeast. *Elife* **4**: e09709.
- Kawauchi, M., and Iwashita, K. (2014) Functional analysis of histone deacetylase and its role in stress response, drug resistance and solid-state cultivation in *Aspergillus oryzae*. *J Biosci Bioeng* **118**: 172–176.
- Kawauchi, M., Nishiura, M., and Iwashita, K. (2013) Fungus-specific sirtuin HstD coordinates secondary metabolism and development through control of LaeA. *Eukaryot Cell* **12**: 1087–1096.
- Keller, N.P. (2018) Fungal secondary metabolism: regulation, function and drug discovery. *Nat Rev Microbiol* **17**: 167–180.
- Keller, N.P., Turner, G., and Bennett, J.W. (2005) Fungal secondary metabolism - from biochemistry to genomics. *Nat Rev Microbiol* **3**: 937–947.
- Khalid, S., Baccile, J.A., Spraker, J.E., Tannous, J., Imran, M., Schroeder, F.C., and Keller, N.P. (2018) NRPS-derived isoquinolines and lipopeptides mediate antagonism between plant pathogenic fungi and bacteria. *ACS Chem Biol* **13**: 171–179.
- Kim, Y., Heo, I.B., Yu, J.H., and Shin, K.S. (2017) Characteristics of a regulator of G-protein signaling (RGS) *rgsC* in *Aspergillus fumigatus*. *Front Microbiol* **8**: 2058.
- Kim, Y., Lee, M.W., Jun, S.C., Choi, Y.H., Yu, J.H., and Shin, K.S. (2019) RgsD negatively controls development,

- toxigenesis, stress response, and virulence in *Aspergillus fumigatus*. *Sci Rep* **9**: 811.
- Kistler, H.C., and Broz, K. (2015) Cellular compartmentalization of secondary metabolism. *Front Microbiol* **6**: 68.
- Kjaerbolting, I., Vesth, T., Frisvad, J.C., Nybo, J.L., Theobald, S., Kildgaard, S., et al. (2020) A comparative genomics study of 23 *Aspergillus* species from section Flavi. *Nat Commun* **11**: 1106.
- Köhler, A.M., Harting, R., Langeneckert, A.E., Valerius, O., Gerke, J., Meister, C., et al. (2019) Integration of fungus-specific CandA-C1 into a trimeric CandA complex allowed splitting of the gene for the conserved receptor exchange factor of CullinA E3 ubiquitin ligases in *Aspergilli*. *MBio* **10**: e01094–e01019.
- Kong, X., van Diepeningen, A.D., van der Lee, T.A.J., Waalwijk, C., Xu, J., Xu, J., et al. (2018) The *Fusarium graminearum* histone acetyltransferases are important for morphogenesis, DON biosynthesis, and pathogenicity. *Front Microbiol* **9**: 654.
- Kouzminova, E., and Selker, E.U. (2001) Dim-2 encodes a DNA methyltransferase responsible for all known cytosine methylation in *Neurospora*. *EMBO J* **20**: 4309–4323.
- Krappmann, S., Jung, N., Medic, B., Busch, S., Prade, R.A., and Braus, G.H. (2006) The *Aspergillus nidulans* F-box protein GrrA links SCF activity to meiosis. *Mol Microbiol* **61**: 76–88.
- Lan, H., Sun, R., Fan, K., Yang, K., Zhang, F., Nie, X.Y., et al. (2016) The *Aspergillus flavus* histone acetyltransferase AflGcnE regulates morphogenesis, aflatoxin biosynthesis, and pathogenicity. *Front Microbiol* **7**: 1324.
- Lan, H., Wu, L., Sun, R., Keller, N.P., Yang, K., Ye, L., et al. (2019) The HosA histone deacetylase regulates aflatoxin biosynthesis through direct regulation of aflatoxin cluster genes. *Mol Plant Microbe Interact* **32**: 1210–1228.
- Law, J.A., and Jacobsen, S.E. (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet* **11**: 204–220.
- Lebar, M.D., Cary, J.W., Majumdar, R., Carter-Wientjes, C. H., Mack, B.M., Wei, Q., et al. (2018) Identification and functional analysis of the aspergillid acid gene cluster in *Aspergillus flavus*. *Fungal Genet Biol* **116**: 14–23.
- Lee, D.W., Freitag, M., Selker, E.U., and Aramayo, R. (2008) A cytosine methyltransferase homologue is essential for sexual development in *Aspergillus nidulans*. *PLoS One* **3**: e2531.
- Lee, I., Oh, J.-H., Keats Shwab, E., Dagenais, T.R.T., Andes, D., and Keller, N.P. (2009) HdaA, a class 2 histone deacetylase of *Aspergillus fumigatus*, affects germination and secondary metabolite production. *Fungal Genet Biol* **46**: 782–790.
- Li, Y., He, Y., Li, X., Fasoyin, O.E., Hu, Y., Liu, Y., et al. (2017) Histone methyltransferase aflmtA gene is involved in the morphogenesis, mycotoxin biosynthesis, and pathogenicity of *Aspergillus flavus*. *Toxicon* **127**: 112–121.
- Li, Y., Li, H., Sui, M., Li, M., Wang, J., Meng, Y., et al. (2019) Fungal acetylome comparative analysis identifies an essential role of acetylation in human fungal pathogen virulence. *Commun Biol* **2**: 154.
- Liang, L., Liu, Y., Yang, K., Lin, G., Xu, Z., Lan, H., et al. (2017) The putative histone methyltransferase DOT1 regulates aflatoxin and pathogenicity attributes in *Aspergillus flavus*. *Toxins (Basel)* **9**: 232.
- Liebmann, B., Gattung, S., Jahn, B., and Brakhage, A.A. (2003) cAMP signaling in *Aspergillus fumigatus* is involved in the regulation of the virulence gene pksP and in defense against killing by macrophages. *Mol Genet Genomics* **269**: 420–435.
- Lim, F.Y., and Keller, N.P. (2014) Spatial and temporal control of fungal natural product synthesis. *Nat Prod Rep* **31**: 1277–1286.
- Lind, A.L., Lim, F.Y., Soukup, A.A., Keller, N.P., and Rokas, A. (2018) An LaeA- and BrIA-dependent cellular network governs tissue-specific secondary metabolism in the human pathogen *Aspergillus fumigatus*. *mSphere* **3**: e00050–e00018.
- Liu, S.Y., Lin, J.Q., Wu, H.L., Wang, C.C., Huang, S.J., Luo, Y.F., et al. (2012) Bisulfite sequencing reveals that *Aspergillus flavus* holds a hollow in DNA methylation. *PLoS One* **7**: e30349.
- Liu, W., Triplett, L., and Chen, X.L. (2021) Emerging roles of posttranslational modifications in plant-pathogenic fungi and bacteria. *Annu Rev Phytopathol* **59**: 99–124.
- Liu, Y., Zhang, M., Xie, R., Zhang, F., Wang, S., Pan, X., et al. (2020) The methyltransferase AflSet1 is involved in fungal morphogenesis, AFB1 biosynthesis, and virulence of *Aspergillus flavus*. *Front Microbiol* **11**: 234.
- Lv, Y. (2017) Proteome-wide profiling of protein lysine acetylation in *Aspergillus flavus*. *PLoS One* **12**: e0178603.
- Lwin, H.P., Choi, Y.H., Lee, M.W., Yu, J.H., and Shin, K.S. (2019) RgsA attenuates the PKA signaling, stress response, and virulence in the human opportunistic pathogen *Aspergillus fumigatus*. *Int J Mol Sci* **20**: 5628.
- Martin, C., and Zhang, Y. (2005) The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol* **6**: 838–849.
- Martin, D.G., Grimes, D.E., Baetz, K., and Howe, L. (2006) Methylation of histone H3 mediates the association of the NuA3 histone acetyltransferase with chromatin. *Mol Cell Biol* **26**: 3018–3028.
- Mas, G., de Nadal, E., Dechant, R., Rodríguez de la Concepción, M.L., Logie, C., Jimeno-González, S., et al. (2009) Recruitment of a chromatin remodelling complex by the Hog1 MAP kinase to stress genes. *EMBO J* **28**: 326–336.
- Mattos, E.C., Palmisano, G., and Goldman, G.H. (2020) Phosphoproteomics of *Aspergillus fumigatus* exposed to the antifungal drug Caspofungin. *mSphere* **5**: e00365–e00320.
- Meister, T., Khler, S., and Braus, G.H. (2019) COP9 signalosome interaction with UspA/Usp15 Deubiquitinase controls VeA-mediated fungal multicellular development. *Biomolecules* **9**: 238.
- Montiel, M.D., Lee, H.A., and Archer, D.B. (2006) Evidence of RIP (repeat-induced point mutation) in transposase sequences of *Aspergillus oryzae*. *Fungal Genet Biol* **43**: 439–445.
- Nahlik, K., Dumkow, M., Bayram, Ö., Helmstaedt, K., Busch, S., Valerius, O., et al. (2010) The COP9 signalosome mediates transcriptional and metabolic response to hormones, oxidative stress protection and cell wall rearrangement during fungal development. *Mol Microbiol* **78**: 964–979.

- Nathan, D., Ingvarsdottir, K., Sterner, D.E., Bylebyl, G.R., Dokmanovic, M., Dorsey, J.A., *et al.* (2006) Histone sumoylation is a negative regulator in *Saccharomyces cerevisiae* and shows dynamic interplay with positive-acting histone modifications. *Genes Dev* **20**: 966–976.
- Nicholson, M.J., Koulman, A., Monahan, B.J., Pritchard, B. L., Payne, G.A., and Scott, B. (2009) Identification of two aflatoxin biosynthesis gene loci in *Aspergillus flavus* and metabolic engineering of *Penicillium paxilli* to elucidate their function. *Appl Environ Microbiol* **75**: 7469–7481.
- Nie, X., Li, B., and Wang, S. (2018) Epigenetic and post-translational modifications in regulating the biology of *Aspergillus* species. *Adv Appl Microbiol* **105**: 191–226.
- Nie, X., Yu, S., Qiu, M., Wang, X., Wang, Y., Bai, Y., *et al.* (2016) *Aspergillus flavus* SUMO contributes to fungal virulence and toxin attributes. *J Agric Food Chem* **64**: 6772–6782.
- Nützmann, H.W., Fischer, J., Scherlach, K., Hertweck, C., and Brakhage, A.A. (2013) Distinct amino acids of histone H3 control secondary metabolism in *Aspergillus nidulans*. *Appl Environ Microbiol* **79**: 6102–6109.
- Nützmann, H.W., Reyes-Dominguez, Y., Scherlach, K., Schroeckh, V., Horn, F., Gacek, A., *et al.* (2011) Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation. *Proc Natl Acad Sci* **108**: 14282–14287.
- Palmer, J.M., Bok, J.W., Lee, S., Dagenais, T.R.T., Andes, D.R., Kontoyiannis, D.P., and Keller, N.P. (2013) Loss of CclA, required for histone 3 lysine 4 methylation, decreases growth but increases secondary metabolite production in *Aspergillus fumigatus*. *PeerJ* **1**: e4.
- Patterson, T.F., Kirkpatrick, W.R., White, M., Hiemenz, J.W., Wingard, J.R., Dupont, B., *et al.* (2000) Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 *Aspergillus* study group. *Medicine (Baltimore)* **79**: 250–260.
- Perrin, R.M., Fedorova, N.D., Bok, J.W., Cramer, R.A., Wortman, J.R., Kim, H.S., *et al.* (2007) Transcriptional regulation of chemical diversity in *Aspergillus fumigatus* by LaeA. *PLoS Pathog* **3**: e50.
- Pfannenstiel, B.T., Greco, C., Sukowaty, A.T., and Keller, N. P. (2018) The epigenetic reader SntB regulates secondary metabolism, development and global histone modifications in *Aspergillus flavus*. *Fungal Genet Biol* **120**: 9–18.
- Pfannenstiel, B.T., and Keller, N.P. (2019) On top of biosynthetic gene clusters: how epigenetic machinery influences secondary metabolism in fungi. *Biotechnol Adv* **37**: 107345.
- Pfannenstiel, B.T., Zhao, X., Wortman, J., and Wiemann, P. (2017) Revitalization of a forward genetic screen identifies three new regulators of fungal secondary metabolism in the genus *Aspergillus*. *MBio* **8**: e01246–e01217.
- Pham, K.T., Inoue, Y., Vu, B.V., Nguyen, H.H., Nakayashiki, T., Ikeda, K., and Nakayashiki, H. (2015) MoSET1 (histone H3K4 methyltransferase in *Magnaporthe oryzae*) regulates global gene expression during infection-related morphogenesis. *PLoS Genet* **11**: e1005385.
- Proffitt, J.H., Davie, J.R., Swinton, D., and Hattman, S. (1984) 5-Methylcytosine is not detectable in *Saccharomyces cerevisiae* DNA. *Mol Cell Biol* **4**: 985–988.
- Qin, L., Li, D., Zhao, J., Yang, G., Wang, Y., Yang, K., *et al.* (2020) The membrane mucin Msb2 regulates aflatoxin biosynthesis and pathogenicity in fungus *Aspergillus flavus*. *J Microbial Biotechnol* **14**: 628–642.
- Rauscher, S., Pacher, S., Hedtke, M., Kniemeyer, O., and Fischer, R. (2016) A phosphorylation code of the *Aspergillus nidulans* global regulator VelvetA (VeA) determines specific functions. *Mol Microbiol* **99**: 909–924.
- Ray, A., Hector, R.E., Roy, N., Song, J.H., Berkner, K.L., and Runge, K.W. (2003) Sir3p phosphorylation by the Slt2p pathway effects redistribution of silencing function and shortened lifespan. *Nat Genet* **33**: 522–526.
- Ren, S., Yang, M., Li, Y., Zhang, F., Chen, Z., Zhang, J., *et al.* (2016) Global Phosphoproteomic analysis reveals the involvement of phosphorylation in aflatoxins biosynthesis in the pathogenic fungus *Aspergillus flavus*. *Sci Rep* **6**: 34078.
- Ren, S., Yang, M., Yue, Y., Ge, F., Li, Y., Guo, X., *et al.* (2018) Lysine Succinylation contributes to aflatoxin production and pathogenicity in *Aspergillus flavus*. *Mol Cell Proteomics* **17**: 457–471.
- Reyes-Dominguez, Y., Bok, J.W., Berger, H., Shwab, E.K., Basheer, A., Gallmetzer, A., *et al.* (2010) Heterochromatic marks are associated with the repression of secondary metabolism clusters in *Aspergillus nidulans*. *Mol Microbiol* **76**: 1376–1386.
- Rispail, N., and Di Pietro, A. (2010) The two-component histidine kinase Fhk1 controls stress adaptation and virulence of *Fusarium oxysporum*. *Mol Plant Pathol* **11**: 395–407.
- Rodriguez-Iglesias, A., and Schmolli, M. (2019) Protein phosphatases regulate growth, development, cellulases and secondary metabolism in *Trichoderma reesei*. *Sci Rep* **9**: 10995.
- Roux, I., Woodcraft, C., Hu, J., Wolters, R., Gilchrist, C.L.M., and Chooi, Y.H. (2020) CRISPR-mediated activation of biosynthetic gene clusters for bioactive molecule discovery in filamentous fungi. *ACS Synth Biol* **9**: 1843–1854.
- Roze, L.V., Arthur, A.E., Hong, S.Y., Chanda, A., and Linz, J.E. (2007) The initiation and pattern of spread of histone H4 acetylation parallel the order of transcriptional activation of genes in the aflatoxin cluster. *Mol Microbiol* **66**: 713–726.
- Roze, L.V., Beaudry, R.M., Keller, N.P., and Linz, J.E. (2004) Regulation of aflatoxin synthesis by FadA/cAMP/protein kinase a signaling in *Aspergillus parasiticus*. *Mycopathologia* **158**: 219–232.
- Russell, P.J., Welsch, J.A., Rachlin, E.M., and McCloskey, J. A. (1987) Different levels of DNA methylation in yeast and mycelial forms of *Candida albicans*. *J Bacteriol* **169**: 4393–4395.
- Samson, R.A., Visagie, C.M., Houbbraken, J., Hong, S.B., Hubka, V., Klaassen, C.H., *et al.* (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Stud Mycol* **78**: 141–173.
- Saruwatari, T., Yagishita, F., Mino, T., Noguchi, H., Hotta, K., and Watanabe, K. (2014) Cytochrome P450 as dimerization catalyst in diketopiperazine alkaloid biosynthesis. *Chembiochem* **15**: 656–659.
- Satterlee, T., Cary, J.W., and Calvo, A.M. (2016) RmtA, a putative arginine methyltransferase, regulates secondary

- metabolism and development in *Aspergillus flavus*. *PLoS One* **11**: e0155575.
- Schneider, J., Wood, A., Lee, J.S., Schuster, R., Dueker, J., Maguire, C., *et al.* (2005) Molecular regulation of histone H3 trimethylation by COMPASS and the regulation of gene expression. *Mol Cell* **19**: 849–856.
- Shimizu, K., Hicks, J.K., Huang, T.P., and Keller, N.P. (2003) Pka, Ras and RGS protein interactions regulate activity of AfIR, a Zn(II)2Cys6 transcription factor in *Aspergillus nidulans*. *Genetics* **165**: 1095–1104.
- Shimizu, K., and Keller, N.P. (2001) Genetic involvement of a cAMP-dependent protein kinase in a G protein signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans*. *Genetics* **157**: 591–600.
- Shimizu, M., Masuo, S., Fujita, T., Doi, Y., Kamimura, Y., and Takaya, N. (2012) Hydrolase controls cellular NAD, Sirtuin, and secondary metabolites. *Mol Cell Biol* **32**: 3743–3755.
- Shinohara, Y., Kawatani, M., Futamura, Y., Osada, H., and Koyama, Y. (2016) An overproduction of astellolides induced by genetic disruption of chromatin-remodeling factors in *Aspergillus oryzae*. *J Antibiot (Tokyo)* **69**: 4–8.
- Shwab, E.K., Bok, J.W., Tribus, M., Galehr, J., Graessle, S., and Keller, N.P. (2007) Histone deacetylase activity regulates chemical diversity in *Aspergillus*. *Eukaryot Cell* **6**: 1656–1664.
- Soukup, A.A., Chiang, Y.M., Bok, J.W., Reyes-Dominguez, Y., Oakley, B.R., Wang, C.C., *et al.* (2012) Overexpression of the *Aspergillus nidulans* histone 4 acetyltransferase EsaA increases activation of secondary metabolite production. *Mol Microbiol* **86**: 314–330.
- Strauss, J., and Reyes-Dominguez, Y. (2011) Regulation of secondary metabolism by chromatin structure and epigenetic codes. *Fungal Genet Biol* **48**: 62–69.
- Studt, L., Janevska, S., Arndt, B., Boedi, S., Sulyok, M., Humpf, H.U., *et al.* (2016a) Lack of the COMPASS component Ccl1 reduces H3K4 Trimethylation levels and affects transcription of secondary metabolite genes in two plant-pathogenic *Fusarium* species. *Front Microbiol* **7**: 2144.
- Studt, L., Rösler, S.M., Burkhardt, I., Arndt, B., Freitag, M., Humpf, H.U., *et al.* (2016b) Knock-down of the methyltransferase Kmt6 relieves H3K27me3 and results in induction of cryptic and otherwise silent secondary metabolite gene clusters in *Fusarium fujikuroi*. *Environ Microbiol* **18**: 4037–4054.
- Studt, L., Schmidt, F.J., Jahn, L., Sieber, C.M.K., Connolly, L.R., Niehaus, E.-M., *et al.* (2013) Two histone deacetylases, FfHda1 and FfHda2, are important for *Fusarium fujikuroi* secondary metabolism and virulence. *Appl Environ Microbiol* **79**: 7719–7734.
- Suka, N., Suka, Y., Carmen, A.A., Wu, J., and Grunstein, M. (2001) Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and Euchromatin. *Mol Cell* **8**: 473–479.
- Sun, R., Wen, M., Wu, L., Lan, H., Yuan, J., and Wang, S. (2021) The fungi-specific histone acetyltransferase Rtt109 mediates morphogenesis, aflatoxin synthesis and pathogenicity in *Aspergillus flavus* by acetylating H3K9. *IMA Fungus* **12**: 9.
- Suzuki, M.M., and Bird, A. (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* **9**: 465–476.
- Szewczyk, E., Chiang, Y.M., Oakley, C.E., Davidson, A. D., Wang, C.C., and Oakley, B.R. (2008) Identification and characterization of the asperthecin gene cluster of *Aspergillus nidulans*. *Appl Environ Microbiol* **74**: 7607–7612.
- Tannous, J., Barda, O., Luciano-Rosario, D., Prusky, D.B., Sionov, E., and Keller, N.P. (2020) New insight into pathogenicity and secondary metabolism of the plant pathogen *Penicillium expansum* through deletion of the epigenetic reader SntB. *Front Microbiol* **11**: 610.
- Teichert, S., Wottawa, M., Schönig, B., and Tudzynski, B. (2006) Role of the *Fusarium fujikuroi* TOR kinase in nitrogen regulation and secondary metabolism. *Eukaryot Cell* **5**: 1807–1819.
- Terabayashi, Y., Sano, M., Yamane, N., Marui, J., Tamano, K., Sagara, J., *et al.* (2010) Identification and characterization of genes responsible for biosynthesis of kojic acid, an industrially important compound from *Aspergillus oryzae*. *Fungal Genet Biol* **47**: 953–961.
- Tognetti, S., Jiménez, J., Viganò, M., Duch, A., Queralt, E., de Nadal, E., and Posas, F. (2020) Hog1 activation delays mitotic exit via phosphorylation of Net1. *Proc Natl Acad Sci* **117**: 8924–8933.
- Tribus, M., Bauer, I., Galehr, J., Rieser, G., Trojer, P., Brosch, G., *et al.* (2010) A novel motif in fungal class 1 histone deacetylases is essential for growth and development of *Aspergillus*. *Mol Biol Cell* **21**: 345–353.
- Tribus, M., Galehr, J., Trojer, P., Brosch, G., Loidl, P., Marx, F., *et al.* (2005) HdaA, a major class 2 histone deacetylase of *Aspergillus nidulans*, affects growth under conditions of oxidative stress. *Eukaryot Cell* **4**: 1736–1745.
- Trojer, P., Dangl, M., Bauer, I., Graessle, S., Loidl, P., and Brosch, G. (2004) Histone methyltransferases in *Aspergillus nidulans*: evidence for a novel enzyme with a unique substrate specificity. *Biochemistry* **43**: 10834–10843.
- Tumukunde, E., Li, D., Qin, L., Li, Y., Shen, J., Wang, S., and Yuan, J. (2019) Osmotic-adaptation response of sakA/hogA gene to aflatoxin biosynthesis, morphology development and pathogenicity in *Aspergillus flavus*. *Toxins (Basel)* **11**: 41.
- Turrà, D., Segorbe, D., and Di Pietro, A. (2014) Protein kinases in plant-pathogenic fungi: conserved regulators of infection. *Annu Rev Phytopathol* **52**: 267–288.
- Umemura, M., Nagano, N., Koike, H., Kawano, J., Ishii, T., Miyamura, Y., *et al.* (2014) Characterization of the biosynthetic gene cluster for the ribosomally synthesized cyclic peptide ustiloxin B in *Aspergillus flavus*. *Fungal Genet Biol* **68**: 23–30.
- Upadhyay, S., Xu, X., and Lin, X. (2016) Interactions between melanin enzymes and their atypical recruitment to the secretory pathway by palmitoylation. *mBio* **7**: e01925–e01916.
- Viaud, M., Fillinger, S., Liu, W., Polepalli, J.S., Le Pêcheur, P., Kunduru, A.R., *et al.* (2006) A class III histidine kinase acts as a novel virulence factor in *Botrytis cinerea*. *Mol Plant Microbe Interact* **19**: 1042–1050.

- Viéitez, C., Martínez-Cebrián, G., Solé, C., Böttcher, R., Potel, C.M., Savitski, M.M., *et al.* (2020) A genetic analysis reveals novel histone residues required for transcriptional reprogramming upon stress. *Nucleic Acids Res* **48**: 3455–3475.
- von Zeska Kress, M.R., Harting, R., Bayram, Ö., Christmann, M., Irmer, H., Valerius, O., *et al.* (2012) The COP9 signalosome counteracts the accumulation of cullin SCF ubiquitin E3 RING ligases during fungal development. *Mol Microbiol* **83**: 1162–1177.
- Wang, B., Han, X., Bai, Y., Lin, Z., Qiu, M., Nie, X., *et al.* (2017) Effects of nitrogen metabolism on growth and aflatoxin biosynthesis in *Aspergillus flavus*. *J Hazard Mater* **324**: 691–700.
- Wang, G., Xu, L., Yu, H., Gao, J., and Guo, L. (2019) Systematic analysis of the lysine succinylome in the model medicinal mushroom *Ganoderma lucidum*. *BMC Genomics* **20**: 585.
- Wang, J., Xu, C., Sun, Q., Xu, J., Chai, Y., Berg, G., *et al.* (2021a) Post-translational regulation of autophagy is involved in intra-microbiome suppression of fungal pathogens. *Microbiome* **9**: 131.
- Wang, W., Yu, Y., Keller, N.P., and Wang, P. (2021b) Presence, mode of action, and application of pathway specific transcription factors in *Aspergillus* biosynthetic gene clusters. *Int J Mol Sci* **22**: 8709.
- Weinert, B.T., Schölz, C., Wagner, S.A., Iesmantavicius, V., Su, D., Daniel, J.A., and Choudhary, C. (2013) Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation. *Cell Rep* **4**: 842–851.
- Williams, R.B., Henrikson, J.C., Hoover, A.R., Lee, A.E., and Cichewicz, R.H. (2008) Epigenetic remodeling of the fungal secondary metabolome. *Org Biomol Chem* **6**: 1895–1897.
- Xie, Z., Dai, J., Dai, L., Tan, M., Cheng, Z., Wu, Y., *et al.* (2012) Lysine succinylation and lysine malonylation in histones. *Mol Cell Proteomics* **11**: 100–107.
- Xiong, X., Liu, Y., Zhang, J., Wang, S., Li, L., and Gao, M. (2021) Mutational analysis of MpPhy reveals magnetoreception and photosensitivity involvement in secondary metabolites biosynthesis in *Monascus purpureus*. *J Photochem Photobiol B* **217**: 112164.
- Yan, M., Nie, X., Wang, H., Gao, N., Liu, H., and Chen, J. (2015) SUMOylation of Wor1 by a novel SUMO E3 ligase controls cell fate in *Candida albicans*. *Mol Microbiol* **98**: 69–89.
- Yang, G., Cao, X., Ma, G., Qin, L., Wu, Y., Lin, J., *et al.* (2020) MAPK pathway-related tyrosine phosphatases regulate development, secondary metabolism and pathogenicity in fungus *Aspergillus flavus*. *Environ Microbiol* **22**: 5232–5247.
- Yang, G., Yue, Y., Ren, S., Yang, M., Zhang, Y., Cao, X., *et al.* (2019) Lysine acetylation contributes to development, aflatoxin biosynthesis and pathogenicity in *Aspergillus flavus*. *Environ Microbiol* **21**: 4792–4807.
- Yang, K., Geng, Q., Luo, Y., Xie, R., Sun, T., Wang, Z., *et al.* (2022) Dysfunction of FadA-cAMP signalling decreases *Aspergillus flavus* resistance to antimicrobial natural preservative Perillaldehyde and AFB1 biosynthesis. *Environ Microbiol* **24**: 1590–1607.
- Yang, K., Liang, L., Ran, F., Liu, Y., Li, Z., Lan, H., *et al.* (2016b) The DmtA methyltransferase contributes to *Aspergillus flavus* conidiation, sclerotial production, aflatoxin biosynthesis and virulence. *Sci Rep* **6**: 23259.
- Yang, K., Liu, Y., Liang, L., Li, Z., Qin, Q., Nie, X., and Wang, S. (2017) The high-affinity phosphodiesterase PdeH regulates development and aflatoxin biosynthesis in *Aspergillus flavus*. *Fungal Genet Biol* **101**: 7–19.
- Yang, K., Qin, Q., Liu, Y., Zhang, L., Liang, L., Lan, H., *et al.* (2016a) Adenylate cyclase AcyA regulates development, aflatoxin biosynthesis and fungal virulence in *Aspergillus flavus*. *Front Cell Infect Microbiol* **6**: 190.
- Yang, K., Zhuang, Z., Zhang, F., Song, F., Zhong, H., Ran, F., *et al.* (2015) Inhibition of aflatoxin metabolism and growth of *Aspergillus flavus* in liquid culture by a DNA methylation inhibitor. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* **32**: 554–563.
- Yeh, H.H., Ahuja, M., Chiang, Y.M., Oakley, C.E., Moore, S., Yoon, O., *et al.* (2016) Resistance gene-guided genome mining: serial promoter exchanges in *Aspergillus nidulans* reveal the biosynthetic pathway for Fellutamide B, a proteasome inhibitor. *ACS Chem Biol* **11**: 2275–2284.
- Yu, J., Woloshuk, C.P., Bhatnagar, D., and Cleveland, T.E. (2000) Cloning and characterization of avfA and omtB genes involved in aflatoxin biosynthesis in three *Aspergillus* species. *Gene* **248**: 157–167.
- Yu, X., Xie, X., and Li, S.M. (2011) Substrate promiscuity of secondary metabolite enzymes: prenylation of hydroxynaphthalenes by fungal indole prenyltransferases. *Appl Microbiol Biotechnol* **92**: 737–748.
- Yu, Z., Ali, A., Igbalajobi, O.A., Streng, C., Leister, K., Krauß, N., *et al.* (2019) Two hybrid histidine kinases, TcsB and the phytochrome FphA, are involved in temperature sensing in *Aspergillus nidulans*. *Mol Microbiol* **112**: 1814–1830.
- Yun, M., Wu, J., Workman, J.L., and Li, B. (2011) Readers of histone modifications. *Cell Res* **21**: 564–578.
- Zemach, A., McDaniel, I.E., Silva, P., and Zilberman, D. (2010) Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* **328**: 916–919.
- Zemach, A., and Zilberman, D. (2010) Evolution of eukaryotic DNA methylation and the pursuit of safer sex. *Curr Biol* **20**: R780–R785.
- Zhang, F., Geng, L., Deng, J., Huang, L., Zhong, H., Xin, S., *et al.* (2020b) The MAP kinase AflStt2 modulates aflatoxin biosynthesis and peanut infection in the fungus *Aspergillus flavus*. *Int J Food Microbiol* **322**: 108576.
- Zhang, F., Huang, L., Deng, J., Tan, C., Geng, L., Liao, Y., *et al.* (2020a) A Cell Wall integrity-related MAP kinase kinase AflBck1 is required for growth and virulence in fungus *Aspergillus flavus*. *Mol Plant Microbe Interact* **33**: 680–692.
- Zhang, F., Xu, G., Geng, L., Lu, X., Yang, K., Yuan, J., *et al.* (2016a) The stress response regulator AflSk7 influences morphological development, stress response, and pathogenicity in the fungus *Aspergillus flavus*. *Toxins (Basel)* **8** (7): 202.
- Zhang, K., Zheng, S., Yang, J.S., Chen, Y., and Cheng, Z. (2013) Comprehensive profiling of protein lysine acetylation in *Escherichia coli*. *J Proteome Res* **12**: 844–851.



- Zhang, X., Zhu, Y., Bao, L., Gao, L., Yao, G., Li, Y., *et al.* (2016b) Putative methyltransferase LaeA and transcription factor CreA are necessary for proper asexual development and controlling secondary metabolic gene cluster expression. *Fungal Genet Biol* **94**: 32–46.
- Zhang, Y., Zheng, Q., Sun, C., Song, J., Gao, L., Zhang, S., *et al.* (2016c) Palmitoylation of the cysteine residue in the DHHC motif of a Palmitoyl transferase mediates Ca<sup>2+</sup> homeostasis in *Aspergillus*. *PLoS Genet* **12**: e1005977.
- Zhao, Y., He, M., Xi, Q., Ding, J., Hao, B., Keller, N.P., and Zheng, W. (2016) Reversible S-nitrosylation limits over synthesis of fungal styrylpyrone upon nitric oxide burst. *Appl Microbiol Biotechnol* **100**: 4123–4134.
- Zhao, Y., Lee, M.K., Lim, J., Moon, H., Park, H.S., Zheng, W., and Yu, J.H. (2021) The putative sensor histidine kinase VadJ coordinates development and sterigmatocystin production in *Aspergillus nidulans*. *J Microbiol* **59**: 746–752.
- Zhi, Q.Q., Li, J.Y., Liu, Q.Y., and He, Z.M. (2017) A cytosine methyltransferase ortholog dmtA is involved in the sensitivity of *Aspergillus flavus* to environmental stresses. *Fungal Biol* **121**: 501–514.
- Zutz, C., Gacek, A., Sulyok, M., Wagner, M., Strauss, J., and Rychli, K. (2013) Small chemical chromatin effectors alter secondary metabolite production in *Aspergillus clavatus*. *Toxins (Basel)* **5**: 1723–1741.