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MINI-REVIEW

Secondary metabolism in *Fusarium fujikuroi*: strategies to unravel the function of biosynthetic pathways

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Abstract

The fungus *Fusarium fujikuroi* causes *bakanae* disease of rice due to its ability to produce the plant hormones, the gibberellins. The fungus is also known for producing harmful mycotoxins (e.g., fusaric acid and fusarins) and pigments (e.g., bikaverin and fusarubins). However, for a long time, most of these well-known products could not be linked to biosynthetic gene clusters. Recent genome sequencing has revealed altogether 47 putative gene clusters. Most of them were orphan clusters for which the encoded natural product(s) were unknown. In this review, we describe the current status of our research on identification and functional characterizations of novel secondary metabolite gene clusters. We present several examples where linking known metabolites to the respective biosynthetic genes has been achieved and describe recent strategies and methods to access new natural products, e.g., by genetic manipulation of pathway-specific or global transcritption factors. In addition, we demonstrate that deletion and over-expression of histone-modifying genes is a powerful tool to activate silent gene clusters and to discover their products.

Keywords *Fusarium fujikuroi* · Secondary metabolism · Gene regulation · Transcription factors · Genome mining · Global regulators · AreA · AreB · Vel1 · Lae1 · Sge1 · Csm1 · Histone acetyltransferases · Histone methyltranferases · Histone deacetylases

Introduction

The rice-pathogenic fungus *Fusarium fujikuroi* (formerly *Gibberella fujikuroi*), a member of the monophyletic but diverse *Fusarium fujikuroi* species complex (FFC), is one of the first described plant pathogens (Kvas et al. 2009). The fungus causes *bakanae* (foolish seedling) disease which led and still leads to serious crop losses in all rice-growing countries: typical symptoms are excessively elongated rice seedlings with chlorotic stems and leaves. The infected plants are infertile and therefore produce no/few edible grains (Ou 1985). The unusual early elongation of seedlings is attributed to the production of gibberellins (GAs), a family of plant hormones that are secreted by the fungus (Tudzynski et al. 2016; Bömke and Tudzynski 2009). Sometimes, the fungus causes stunting of rice shoots, probably due to the production of fumonisins and fusaric acid. The type of symptoms (stunting or hyper-elongation) and the severity of the disease depend not only on the ability of the strains to produce high levels of fumonisins and fusaric acid on the one hand, and GAs on the other hand, but also on the resistance levels of the host (Fiyaz et al. 2016; Niehaus et al. 2017a).

Today, the fungus is used worldwide for the industrial-scale production of GAs which are used to manage fruit size and quality (e.g., grapes, apples and oranges), to malt barley for beer production, to increase the size of ornamental flowers (e.g., gardenia or geranium flowers), and to increase the sugar yield in sugarcane. GAs are also extensively used to induce parthenocarpic seedless fruits (e.g., grapes and melons) which are at the same time larger and sweeter (Sponsel and Hedden 2010).

Besides GAs, the fungus is also known to produce additional secondary metabolites (SMs), such as harmful mycotoxins as well as pigments (Fig. 1a–j). For example, it produces the mycotoxins fusaric acid, moniliformin, fusarins, fumonisins, and beauvericin (Moretti et al. 1996; Desjardins et al. 1997; Desjardins and Proctor 2007; Kvas et al. 2009; Barrero et al. 1991; Bacon et al. 1996) as well as the pigments

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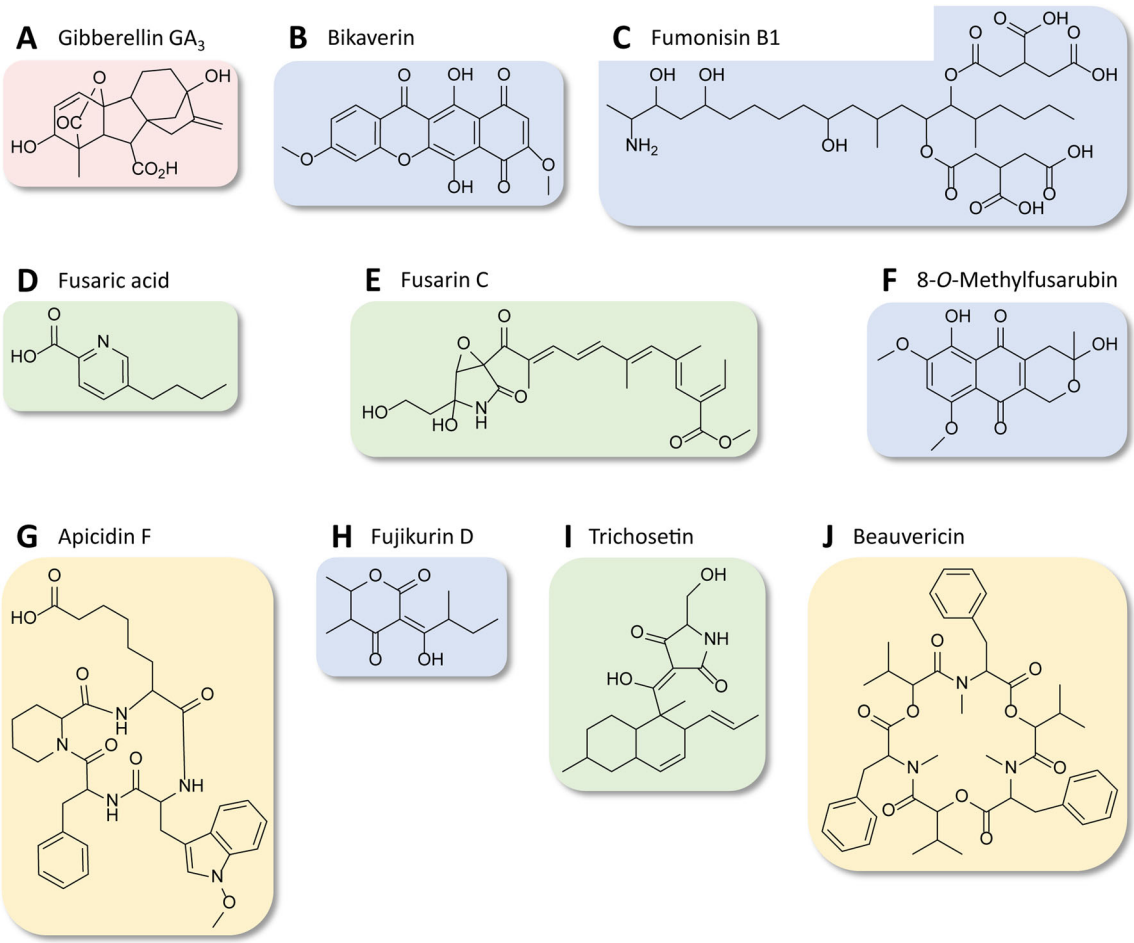

Fig. 1 Chemical structures of *F. fujikuroi* secondary metabolites. Gibberellin GA_3 is produced by a terpene cyclase (**a**), metabolites highlighted in blue (**b**, **c**, **f** and **h**) are polyketide synthase (PKS) products, those highlighted in yellow (**g**, **j**) are non-ribosomal peptide synthetase (NRPS)-derived, and metabolites shown in green (**d**, **e** and **i**) are PKS-NRPS products

neurosporaxanthin (carotenoid), bikaverin, and fusarubins (Avalos et al. 2012; Balan et al. 1970; Linnemannstöns et al. 2002b; Studt et al. 2012). However, the biosynthetic genes for the production of these SMs and the genetic capacity of the fungus to produce even more yet unknown products remained unknown for most of them. Only in 1998, the genes for the biosynthesis of the best known SMs of *F. fujikuroi*, the GAs, were cloned by differencial cDNA screenings. Subsequent chromosome walking revealed that all seven pathway genes are located adjacent to each other in a gene cluster (Tudzynski and Hölter 1998). At that time, an increasing number of identified SM biosynthetic genes provided evidence that clustering of those genes is a common feature not only for prokaryotic metabolic pathways but also for genes involved in fungal secondary metabolism (Keller and Hohn 1997).

Some years later, the key enzyme-encoding genes for bikaverin and carotenoid biosynthesis were cloned by PCR and heterologous hybridization of a genomic library, respectively, and were also shown to be organized in gene clusters (Linnemannstöns et al. 2002b; Linnemannstöns et al. 2002a; Wiemann et al. 2009). However, only the availability of the genome sequences of *F. fujikuroi* (Wiemann et al. 2013) and highly related *Fusarium* species, such as *F. verticillioides* (Ma et al. 2010), *F. circinatum* (Wingfield et al. 2012), *F. mangiferae* and *F. proliferatum* (Niehaus et al. 2016b), revealed a complete overview of the genetic capacity of the members of the FFC to produce SMs. The in silico analyses revealed not only common gene clusters but also surprising differences between the related species. These FFC genome sequences allowed the researchers to create a preliminary catalog of genes encoding polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), dimethylallyltryptophan synthases (DMATSs), and terpene cyclases (TCs), the key enzymes for SM biosynthesis (Wiemann et al. 2013; Niehaus et al. 2016b; Hansen et al. 2015).

The purpose of this review is to briefly summarize our current knowledge on secondary metabolism in *F. fujikuroi*. As most of the SM gene clusters are not expressed under standard laboratory conditions, different approaches have been developed for the activation of those silent gene clusters, e.g., optimization of culture conditions (pH, nutrient

availability, light), genetic manipulation of pathway-specific transcription factors (TFs), global regulators, and histone modifications affecting epigenetic control. We describe the approaches by which cryptic and silent gene clusters have been deciphered and new products identified.

In silico analysis of the genome of *F. fujikuroi*

To estimate the genetic potential of *F. fujikuroi* to produce SMs other than GAs, the genome of *F. fujikuroi* strain IMI58289 was searched for genes predicted to encode the four classes of core enzymes: PKSs, NRPSs, TCs, and DMATSs. The genes were identified in silico by the presence of characteristic protein domain content and by BLAST analyses (Wiemann et al. 2013). This analysis revealed 47 potential SM key genes: 17 genes that encode putative type I PKSs, one type III PKS, 15 NRPSs, 2 DMATSs, and 12 TCs. The latter enzymes include nine sesquiterpene cyclases (STC1–STC9), one diterpene cyclase (*ent*-kaurene synthase, DTC1), one triterpene cyclase (TrTC1), and one tetraterpene cyclase (phytoene synthase, TeTC1) (Niehaus et al. 2016b). Four of the PKSs contain typical domains of both PKS and NRPS and are also referred to as PKS-NRPS hybrids. Based on domain architecture, 14 of the predicted PKSs belong to the class of reducing-type PKSs (R-PKSs): they contain the characteristic ketoreductase (KR), dehydratase (DH), and enoyl reductase (ER) domains. The remaining three PKSs are non-reducing-type PKSs (NR-PKSs) because they lack the KR, DH, and ER domains (Chiang et al. 2010). NR-PKSs synthesize aromatic polyketides, e.g., the red pigment bikaverin (Wiemann et al. 2009).

At the time of genome sequencing, only some of the gene clusters and their products were known: the GA gene cluster with the bifunctional *ent*-kaurene/*ent*-copalyl diphosphate synthase (DTC1; Cps/ks; Fig. 2a), and the pigment gene clusters for bikaverin (PKS4; Bik1; Fig. 2b) and carotenoid (TeTC1) biosynthesis (Tudzynski and Hölter 1998; Wiemann et al. 2009; Studt et al. 2012; Linnemannstöns et al. 2002a) (Table 1). To unravel cryptic gene clusters, it was important to find the correct conditions for their expression. Therefore, genome-wide expression studies under high and low nitrogen, as well as acidic and alkaline pH conditions, were performed. These experiments revealed that about half of the key genes are not expressed under these standard conditions. For the others, there were clear indications for a nitrogen and/or pH-dependent gene expression (Wiemann et al. 2013). Due to the high level of genome conservation between the *Fusarium* species, especially between members of the FFC, the products of some of the newly identified putative gene clusters could be predicted by comparative genomics, e.g., the products for four NRPS genes required for synthesis of the siderophores ferricrocin, fusarinine, and ferrichrome and the insecticidal mycotoxin beauvericin (BEA), as well as the PKS genes required for synthesis of fusaric acid, equisetin, and fumonisins (Niehaus et al. 2016b) (Table 1).

Linking biosynthetic gene clusters to their metabolites via comparative genomics

Due to the genome sequencing of some members of the FFC by our and other groups, comparative genomics helped to identify several gene clusters and link them to their products (Ma et al. 2010; Niehaus et al. 2016b; Wiemann et al. 2013; Chiara et al. 2015). In *F. verticillioides*, the gene clusters for fumonisins, fusaric acid, and fusarins were already well characterized (Brown et al. 2007; Brown et al. 2012). Based on these data, highly conserved orthologous gene clusters have now been identified and functionally studied in the genome of *F. fujikuroi* (Fig. 2c–e).

After the identification of the PKS Fum1, the key enzyme of fumonisin (FUM) biosynthesis in the maize pathogen *F. verticillioides*, subsequent deletion of the adjacent genes resulted in the identification of the FUM biosynthetic cluster consisting of 17 co-expressed genes (Proctor et al. 1999; Brown et al. 2012; Brown et al. 2007). The FUM gene cluster of *F. verticillioides*, the main FUM producer among the genus *Fusarium*, shows a high homology to the predicted PKS11 gene cluster *in F. fujikuroi* (*FFUJ_09240* to *FFUJ_09254*). Exceptions are *FUM20* which is absent and *FUM17* which is non-functional due to a premature truncation (Fig. 2c) (Rösler et al. 2016a). Both genes are probably not involved in FUM biosynthesis in *F. verticillioides* (Proctor et al. 2003). However, despite the strong similarities of the FUM clusters that occur in both fungi, the gene expression and FUM production levels are much lower in *F. fujikuroi*. Only the overexpression of the cluster-specific $Zn(II)_2Cys_6$-type TF gene *FUM21* in *F. fujikuroi* led to the activation of *FUM* gene expression and an about 1000-fold elevation of toxin levels compared to the wild type (WT) (Rösler et al. 2016a). The data provide evidence that even the low-level producer *F. fujikuroi* can become a potent FUM producer, leading to potentially high contamination of rice products.

Only recently, genome sequencing of eight additional *F. fujikuroi* isolates from different rice-growing countries revealed strain-specific differences: while most of the isolates produce GAs but not FUM, one Korean isolate (B14) is able to produce FUM but not GAs and, therefore, causes stunting and early withering instead of the typical hyper-elongation of rice seedlings (Niehaus et al. 2017a).

One of the oldest known SMs of *F. fujikuroi* and some other *Fusarium* species is fusaric acid (FSA), a mycotoxin with high phytotoxic properties but low toxicity to animals and humans (Fig. 1d). The key gene for fusaric acid biosynthesis, the PKS-encoding gene *FUB1*, was first identified in *F. verticillioides*. Its deletion resulted in total loss of FSA production. Four adjacent

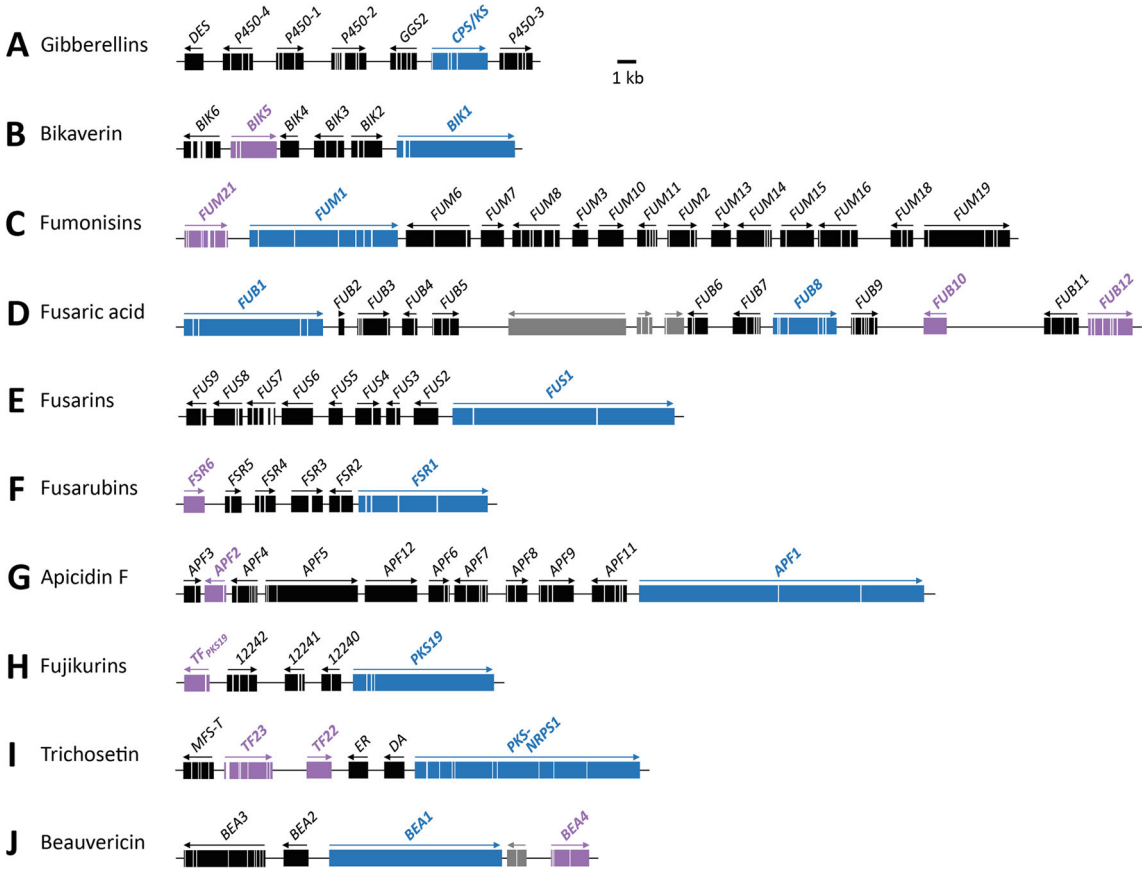

Fig. 2 Gene clusters responsible for the biosynthesis of *F. fujikuroi* secondary metabolites. The designations (**a**-**j**) correspond to the panels in Fig. 1. The GA gene cluster Key enzyme- and transcription factor-encoding genes are highlighted in blue and violet, respectively. Genes that do not belong to the clusters are gray. The direction of transcription is indicated with arrows and white bars represent introns (color figure online)

genes were shown to be co-regulated with *FUB1* suggesting that five genes belong to the putative FSA gene cluster (Brown et al. 2012). An orthologous gene cluster exists in *F. fujikuroi*, and its function was studied by targeted gene replacement of all five postulated biosynthetic genes (*FUB1-FUB5*) (Fig. 2d) and subsequent product analysis. Only two of the five cluster genes were shown to be essential for the biosynthesis of FSA (Niehaus et al. 2014b). However, the biosynthetic pathway leading to FSA and especially the origin of the nitrogen atom, which is incorporated into the FSA backbone, remained unknown. Then, in 2016, an extended *FUB* gene cluster encoding a second key enzyme (NRPS34) and two $Zn(II)_2Cys_6$-type TFs, Fub10 and Fub12, was identified independently in both fungi (Studt et al. 2016b; Brown et al. 2015) (Fig. 2d).

Besides FSA, various *Fusarium* species also produce the potent mutagen fusarin C and some drivatives (Fig. 1e). The first fusarin (FUS) biosynthetic gene, *fusS*, encoding a hybrid of a type I iterative PKS fused to an NRPS module, was found in *F. moniliforme* and *F. venenatum* (Song et al. 2004). Later, functional *fusS* homologs have been discovered also in *F. graminearum* (*GzFUS1*), *F. verticillioides* (*FUS1*), and *F. fujikuroi* (*fusA*), respectively (Brown et al. 2012; Diaz-Sanchez et al. 2012; Gaffoor et al. 2005). Although many *Fusarium* species inside and outside the FFC contain *fusS* orthologs and predicted FUS gene clusters, there are surprising differences in gene cluster organization and gene expression between closely related members of the FFC. For instance, *F. mangiferae*, belonging to the Asian clade of the FFC together with *F. fujikuroi*, has only remnants of the FUS cluster (Wiemann et al. 2013).

Besides the members of the genus *Fusarium*, a similar FUS gene cluster has been identified in the distantly related fungus *Metarhizium anisopliae* where it is responsible for the biosynthesis of 7-desmethyl analogs of fusarin C and (8*Z*)-fusarin C, named NG-391 and NG-393, respectively (Krasnoff et al. 2006).

After genome sequencing, the entire FUS gene cluster containing nine co-regulated genes (*FUS1-FUS9*) (Fig. 2e) has been functionally characterized in *F. fujikuroi*. Beside *FUS1*, encoding the PKS-NRPS key enzyme, the other genes encode a putative α/β hydrolase with a predicted peptidase domain (*FUS2*), a glutathione *S*-transferase (*FUS3*), a peptidase A1 (*FUS4*), a serine hydrolase (*FUS5*), a major facilitator superfamily (MFS) transporter (*FUS6*), an aldehyde dehydrogenase (*FUS7*), a cytochrome P450 monooxygenase (*FUS8*), and a methyltransferase (*FUS9*) (Kleigrewe et al. 2012; Niehaus

Table 1 Secondary metabolite key genes with known or predicted products before the release of the *F. fujikuroi* genome sequence (Wiemann et al. 2013)

Key gene	Gene code	Product	Reference
PKS-NRPS1	FFUJ_02219	Equisetin (p)	Hansen et al. (2015)
PKS3	FFUJ_03984	Fusarubins	Studt et al. (2012); Brown et al. (2012)
PKS4	FFUJ_06742	*Bikaverin*	Linnemannstöns et al. (2002b)
PKS6	FFUJ_02105	Fusaric acid (p)	Brown et al. (2012)
PKS-NRPS10	FFUJ_10058	Fusarins (p)	Brown et al. (2012)
PKS11	FFUJ_09241	Fumonisins (p)	Brown et al. (2012)
NRPS2	FFUJ_04614	Ferricrocin (p) (intracell. siderophore)	Tobiasen et al. (2007); Oide et al. (2015)
NRPS6	FFUJ_10736	Fusarinine (p) (extracell. siderophore)	Varga et al. (2005)
NRPS17	FFUJ_03641	Ferrichrome (p)	Varga et al. (2005)
NRPS22	FFUJ_09296	Beauvericin (p)	Xu et al. (2008); Zhang et al. (2013)
DTC1	FFUJ_14336	*Gibberellins*	Tudzynski and Hölter (1998)
TeTC	FFUJ_11802	*Carotenoids*	Linnemannstöns et al. (2002a)

Italicized letters: clusters were experimentally studied in *F. fujikuroi* before genome sequencing

p predicted due to homology to other fungi

et al. 2013). Deletion of each single gene and subsequent product analyses revealed that only four of them (*FUS1*, *FUS2*, *FUS8*, and *FUS9*) are required for FUS biosynthesis, while WT-like FUS levels were found in the culture filtrates of Δ*fus3*, Δ*fus4*, Δ*fus5*, Δ*fus6*, and Δ*fus7* (Niehaus et al. 2013). Previously, it has been shown for *F. moniliforme* (and probably for other *Fusarium* species, too) that the PKS-NRPS forms the amide linkage between the heptaketide part of the PKS and the activated L-homoserine of the NRPS part (Rees et al. 2007). Analysis of the biosynthetic pathway in *F. fujikuroi* revealed that the product of Fus1, called pre-fusarin, is released as an alcohol with an open ring structure. Pre-fusarin is then hydroxylated by the P450 monooxygenase Fus8 at carbon C-20 to 20-hydroxy-fusarin (Niehaus et al. 2013). The subsequent ring closure is most likely catalyzed by Fus2 what enables the P450 monooxygenase Fus8 to catalyze an additional hydroxylation at the C-20 atom. In a last reaction, the methyltransferase Fus9 methylates the hydroxy group at C-21 and forms fusarin C (Kleigrewe et al. 2012; Niehaus et al. 2013).

F. fujikuroi produces a second PKS-derived red pigment—fusarubin

At the time of genome sequencing, the only characterized PKS gene cluster in *F. fujikuroi* was the one for bikaverin (BIK) biosynthesis (Wiemann et al. 2009). The *BIK* genes were shown to be strongly repressed by conditions of high nitrogen and an alkaline pH (Wiemann et al. 2009). Surprisingly, the culture fluid was still deeply red pigmented in cultures with low levels of sodium nitrate, which makes the medium alkaline (pH 6.5–7.5) within the first 24 h of cultivation. High-performance liquid chromatography coupled to UV light and Fourier transformation mass spectrometry (HPLC-UV-FTMS) detection clearly demonstrated that the red pigment was indeed not BIK, but an unknown compound (Studt et al. 2012). We assumed the involvement of a second NR-PKS because all green and red naphthoquinone piments so far studied were shown to be synthesized by this group of enzymes. A BLASTp analysis with the Bik1 sequence against the just sequenced genome of *F. fujikuroi* IMI58289 led to the identification of a second NR-PKS, designated Fsr1, with 40% identity to Bik1. Phylogenetic analysis of the KS domains of known fungal NR-PKSs revealed the highest similarity of Fsr1 to the Pgl1 proteins of *F. verticillioides* and *F. graminearum*, which are both responsible for perithecial pigmentation (Gaffoor et al. 2005; Proctor et al. 2007). However, no chemical structure had been proposed for these perithecial pigments.

Deletion of *FSR1* and the five co-regulated genes (Fig. 2f) downstream revealed that *FSR4* and *FSR5* are not involved in biosynthesis of the unknown pigment, whereas deletion of *FSR1* and *FSR6*, the latter encoding a putative $Zn(II)_2Cys_6$ TF, resulted in the total loss of pigmentation (Studt et al. 2012). Expression studies confirmed that Fsr6 acts as a

pathway-specific TF. The structure of the main compounds found in the cultures of the WT and deletion mutants was extensively elucidated by MS, characteristic UV spectra, and nuclear magnetic resonance (NMR) data. By these methods, a whole set of chemically related naphthoquinones, the fusarubins (FSR), was identified (Fig. 1f). Based on these data, a model for the biosynthetic pathway of FSR was suggested (Studt et al. 2012). It is noteworthy that naphthoquinones from *Fusarium javanicum* with a similar structure have been described seven decades ago and were designated javanicin and oxyjavanicin (Arnstein and Cook 1947). Oxyjavanicin was later renamed FSR.

Genome mining by over-expression of cluster-specific transcription factor genes

Our genome-wide expression studies showed that most of the in silico identified gene clusters in *F. fujikuroi* are orphan clusters for which the products are still unknown. In most cases, the genes are not expressed or expressed at very low levels under laboratory conditions making the chemical analysis of the products difficult (Wiemann et al. 2013). To identify as many new SMs as possible, we searched for those putative gene clusters which contain typical TF-encoding genes (Table 2). In *F. fujikuroi*, 17 out of the 47 gene clusters (36%) contain one or two TFs genes. There are several successful examples demonstrating that over-expression of pathway-specific TF genes by using strong or inducible promoters is a sufficient approach to activate normally silent gene clusters and to identify new SMs (Brakhage 2013).

Two of the predicted clusters with PKS19 and NRPS31 (Fig. 2g, h) as key enzymes were of special interest because they were present only in the genome of *F. fujikuroi*, but absent from any other sequenced genomes of highly related FFC species (Wiemann et al. 2013). A gene cluster similar to the NRPS31 (FFUJ_00003) cluster has been characterized in the distantly related species *Fusarium semitectum* and shown to be responsible for the synthesis of the cyclic tetrapeptide apicidin, an inhibitor of histone deacetylases (HDACs) (Jin et al. 2010). In both species, the cluster encodes an atypical "bank" TF, which contains a basic DNA-binding domain and four ankyrin repeats. However, in contrast to typical bZIP TFs, bank TFs do not contain leucine zipper or helix-loop-helix motifs. Over-expression of this TF gene in *F. fujikuroi* led to elevated expression of all cluster genes and a 10-fold enhanced product formation under inducing high nitrogen conditions. De-regulated gene expression was observed even under repressing conditions of low nitrogen (Wiemann et al. 2013). However, instead of the expected apicidin, NMR and MS analyses of the over-expression mutant revealed the presence of a similar but distinct product designated apicidinF (APF) (Fig. 1g).

In contrast to apicidin, APF contains L-phenylalanine instead of L-isoleucine, and L-2-aminooctanedioic acid instead of L-2-amino-8-oxodecanoic acid. The other two amino acids, *N*-methoxy-L-tryptophan and D-pipecolic acid, are common in both compounds. To study the biosynthesis of APF, knock-out mutants of several cluster genes were generated and analyzed for their ability to produce APF or intermediates by HPLC coupled to high-resolution MS (Niehaus et al. 2014a). These studies showed that L-phenylalanine can be directly activated, while the three non-proteinogenic amino acids are synthesized by pathway-specific enzymes encoded by cluster genes. Besides APF, new derivatives were identified in the cultures of deletion mutants, which were designated apicidin J and K. Based on chemical analysis of the deletion mutants, a model for the APF biosynthetic pathway was suggested (Niehaus et al. 2014a).

The predicted PKS19 cluster consists of five genes encoding PKS19 (FFUJ_12239), a protein with unknown function (FFUJ_12240), a ToxD-like protein (FFUJ_12241), a putative MFS transporter (FFUJ_12242), a $Zn(II)_2Cys_6$ TF (FFUJ_12243), and a P450 monooxygenase (FFUJ_12244) (Fig. 2h) (Wiemann et al. 2013). Microarray and Northern blot analyses indicated that the predicted cluster genes are only expressed at very low levels. To activate the expression of the PKS19 cluster genes and potentially induce production of the yet unknown product(s), the cluster-specific TF-encoding gene (*FFUJ_12243*) was over-expressed resulting in enhanced expression of *PKS19* (*FFUJ_12239*) and three additional predicted cluster genes (*FFUJ_12240*, *FFUJ_12242*, and *FFUJ_12243*). However, only simultaneous double over-expression of the TF- and PKS19-encoding genes (OE::*PKS19*/OE::*TF*) led to identification of four metabolites that were not produced by the WT. These compounds have similar molecular formula and UV spectra (Wiemann et al. 2013). The structures of the four newly discovered products, named fujikurins A-D (Fig. 1h), were elucidated using a combined approach of NMR and MS. They are all cyclic lactones with 1,3-diketo elements (von Bargen et al. 2015). Recently, orthologous PKS19 clusters have been found in the genomes of two newly sequenced *F. proliferatum* strains (Niehaus et al. 2016b).

Beside the FUS key gene *FUS1*, three additional PKS-NRPS genes have been identified in the genome of *F. fujikuroi*. One of them is *PKS-NRPS1* (*FFUJ_02219*), the key gene of a predicted gene cluster with high similarity to the equisetin gene cluster from the distantly related fungus *Fusarium heterosporum* (Kakule et al. 2013). However, the *F. fujikuroi* cluster lacks the equisetin *N*-methyltransferase gene *eqxD* and, consequently, equisetin cannot be the final product. Both the *F. fujikuroi* and the *F. heterosporum* clusters harbor two genes encoding $Zn(II)_2Cys_6$ TFs (Table 2; Fig. 2i). One of them was shown to act as positive regulator of the cluster genes in *F. heterosporum* (Kakule et al. 2013).

Table 2 Secondary metabolite gene clusters regulated at least by one of the known regulators

Key gene	Gene code	Product	ps-TF	Global regulators
Non-ribosomal peptide synthetases (NRPSs)				
NRPS4*	FFUJ_08113		No	AreB, Csm1, Lae1, Kmt6, Gcn5
NRPS6	FFUJ_10736	Fusarinine	No	Csm1, Lae1
NRPS11	FFUJ_10934		1 (?)	AreA, AreB, Csm1, Lae1
NRPS13	FFUJ_02440		No	AreB,
NRPS17	FFUJ_03641	Ferrichrome	No	AreA, AreB
NRPS20*	FFUJ_06720		1 (?)	Csm1
NRPS21*	FFUJ_02022		1 (?)	AreB
NRPS22*	FFUJ_09296	Beauvericin	1 (−)	AreA, Kmt6, Hda1, Lae1
NRPS23	FFUJ_12008		1 (?)	AreB
NRPS31*	FFUJ_00003	Apicidin F	1 (+)	AreB, VeC, AreB, Sge1, Csm1, Gcn5
NRPS34*	FFUJ_02115	Fusaric acid	2 (1+)	AreB, Lae1, Csm1, Hda1/2
Polyketide synthases (PKSs)				
PKS1*	FFUJ_02219	Trichosetin	2 (1+)	AreB, Kmt6,Gcn5
PKS2*	FFUJ_00118		No	Kmt6
PKS3	FFUJ_03984	Fusarubins	1 (+)	Lae1, Csm1, Hda1,
PKS4*	FFUJ_06742	Bikaverin	1 (+)	AreB, Lae1, Csm1, Hda1/2
PKS6*	FFUJ_02105	Fusaric acid	2 (1+)	AreB, Lae1, Csm1, Hda1/2
PKS7	FFUJ_06260		1 (?)	AreB, Csm1
PKS8*	FFUJ_12090		No	Lae1, Csm1
PKS9/NRPS	FFUJ_14695		No	AreB
PKS10/NRPS	FFUJ_10058	Fusarins	No	AreA, AreB, Gcn5, Hda1/2
PKS11*	FFUJ_09241	Fumonisins	1 (+)	AreA, AreB, Lae1, Csm1, Gcn5
PKS13	FFUJ_12020	Gibepyrone	1 (no effect)	VeC,Sge1, Csm1
PKS19	FFUJ_12239	Fujikurins	1 (+)	Csm1
PKS20/NRPS	FFUJ_12707		No	AreA, AreB, Csm1,
Terpene cyclases (TCs)				
DTC1*	FFUJ_14336	Gibberellins	No	AreA, AreB, VeC, Gcn5, Hda1/2
TeTC1	FFUJ_11802	Carotenoids	No	Lae1, Kmt6
STC1*	FFUJ_00036	Germacrene D	No	Csm1, Gcn5
STC2	FFUJ_00969		No	AreA
STC3	FFUJ_04067	Eremophilene	No	Lae1
STC4	FFUJ_10353	Koraiol	No	AreB, Kmt6
STC5	FFUJ_11739	Guaiadiene	1 (+)	Kmt6, Gcn5
STC6	FFUJ_12585	Acorenol	No	AreA, AreB,
STC7*	FFUJ_12026		No	Lae1
STC8	FFUJ_09423		No	Kmt6
STC9*	FFUJ_14833		No	AreA, Gcn5
Dimethylallyltryptophan synthase (DMATS)				
DMATS1	FFUJ_09176	r-*N*-DMAT	1 (?)	AreA

References: Pfannmüller et al. (2017); Janevska et al. (2016); Studt et al. (2013, 2016a, 2016b); Rösler et al. (2016a); Niehaus et al. (2013, 2014, 2016, 2017a, 2017b); Michielse et al. (2014)

ps-TF pathway-specific TF, *VeC Velvet* complex consisting of Ve1, Ve2, Lae1, *(+)* activator, *(−)* repressor, *(?)* function unknown, * subtelomeric cluster position

Microarray analyses under different conditions revealed that the *PKS-NRPS1* cluster is silent in *F. fujikuroi* (Janevska et al. 2017a). Over-expression of *TF22* (*FFUJ_02222*), one of the two cluster-specific TF genes, led to an activation of all five cluster genes, including the PKS-NRPS key gene and the second TF-encoding gene (Fig. 2i). The mutant strains showed severe growth defects demonstrating that the produced compound is toxic to the fungus. The metabolic profile

of the OE::*TF22* mutant revealed a new peak which was not present in the WT. Structure elucidation confirmed that this product is trichosetin (Fig. 1i). Furthermore, over-expression of *eqxD* from *F. heterosporum* into the OE::*TF22* mutant of *F. fujikuroi* led to the accumulation of the *N*-methylated equisetin, the final product of the *F. heterosporum* cluster (Janevska et al. 2017a). In contrast to *TF22*, over-expression of *TF23* (*FFUJ_02223*) did not result in an activation of the cluster genes. Instead, TF23 was essential for inducing the cluster-encoded MFS transporter gene (*FFUJ_02224*) by the final product trichosetin. Therefore, TF23 plays an important role in detoxification of trichosetin and self-protection of the producing fungus (Janevska et al. 2017a).

However, not all TF genes which are located adjacent to key enzyme-encoding genes act as positive regulators of those clusters. Attempts to identify the products of PKS13 (gibepyrones), PKS16 (unknown product), STC1 (germacrene D), or NRPS22 (BEA) by over-expressing the TF genes in near proximity to the key genes have failed (Table 2). The strong upregulation of the TF-encoding genes did not result in elevated expression of the remaining cluster genes. Similarly, the TF adjacent to the dimethylallyltryptophan synthase-encoding gene *DMATS1* (*FFUJ_09179*) is not functional or at least does not regulate *DMATS1* expression. However, over-expression of the key gene *DMATS1* resulted in production of a new metabolite, a reversely *N*-prenylated tryptophan with a rare form of prenylation (Arndt et al. 2017).

Global regulators affect growth, differentiation, and secondary metabolism

Besides cluster-specific TFs, the expression of SM biosynthetic genes is controlled by a hierarchical network of global regulators that respond to multiple environmental signals (Lind et al. 2015). The best studied regulatory complex is the fungal-specific *velvet* complex. The founding member of this group is the *Aspergillus nidulans velvet* protein VeA. *VeA1* mutant strains obtained by classic mutagenesis were described more than 40 years ago, but only in 2002, the *veA* gene was cloned by complementation of the *veA1* mutant strain with a multicopy plasmid library (Calvo 2008). Deletion of *veA* resulted in loss of the ability to form cleistothecia and the production of SMs, e.g., sterigmatocystin or penicillin, demonstrating a regulatory connection between secondary metabolism and development (Bayram and Braus 2012). In 2004, another global regulator of secondary metabolism was identified: LaeA (loss of *aflR* expression) (Bok and Keller 2004). Besides sterigmatocystin and penicillin, the *A. nidulans laeA* mutant was unable to produce lovastatin and some other SMs (Bok and Keller 2004). In 2008, it was found that LaeA and two proteins of the *velvet* family, VeA and VelB, form a trimeric complex that is essential for coordination of secondary metabolism and development (Bayram et al. 2008). Since then, the *velvet* complex and its impact on secondary metabolism has been studied in *F. fujikuroi* (Wiemann et al. 2010) and several other fungi, e.g., *Penicillium chrysogenum*, *Trichoderma reesei*, and *Aspergillus fumigatus* (Hoff et al. 2010; Karimi-Aghcheh et al. 2013; Perrin et al. 2007; Dhingra et al. 2012).

Deletion of *vel1*, *vel2*, and *lae1* (the orthologs of *veA*, *velB* and *laeA*) in *F. fujikuroi* revealed common and different functions of the complex partners: while deletion of the three components of the *F. fujikuroi velvet* complex almost totally abolished biosynthesis of GAs, FUM, FUS, and FSA, Vel1 could simultaneously act as repressor of BIK biosynthesis (Wiemann et al. 2010; Niehaus et al. 2014b) (Fig. 3a). After genome sequencing, the role of Lae1 on secondary metabolism was analyzed again on a genome-wide basis (Niehaus et al. 2017c). The expression profile of the WT was compared with those of the Δ*lae1* and a *LAE1* over-expressing mutant (OE:*LAE1*). Both deletion and over-expression of *LAE1* resulted in upregulation of some of the known, and five yet unknown, gene clusters. Thus, the expression of all GA, FUS, FUM, FSR, and the recently identified silent BEA genes was significantly increased in the OE:*LAE1* mutant under inducing and in some cases also under repressing conditions. For instance, the strongest upregulation of FUS genes was observed under repressing low nitrogen conditions indicating that the nitrogen regulation is overcome by over-expression of *LAE1*. Similarly, the FSR genes, which are only expressed at alkaline pH in the WT (Studt et al. 2012), were upregulated in

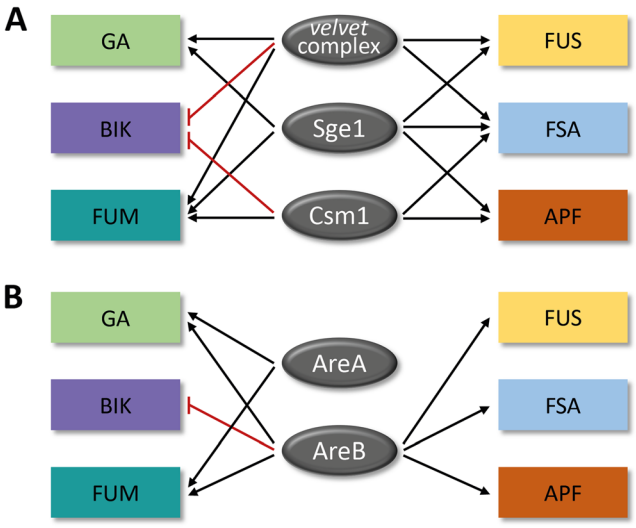

Fig. 3 Global regulators that affect the biosynthesis of the major *F. fujikuroi* secondary metabolites (SMs). Schematic representation of the clusters responsible for the biosynthesis of the nitrogen-repressed SMs gibberellins (GA), bikaverin (BIK), and fumonisins (FUM), as well as of the nitrogen-induced SMs fusarins (FUS), fusaric acid (FSA), and apicidin F (APF). The regulatory network is shown for the *velvet* complex, Sge1 and Csm1 (**a**) and for the nitrogen regulators AreA and AreB (**b**). Black arrows: direct or indirect gene cluster activation; red bars: direct or indirect repression (color figure online)

the OE:*LAE1* mutant under acidic conditions suggesting that the pH regulation is circumvented by over-expressing *LAE1*. The most prominent result was the elevated expression of the GA biosynthetic genes and increased product levels under otherwise repressing nitrogen sufficent conditions (Mihlan et al. 2003; Pfannmüller et al. 2017).

In contrast, the expression of the BIK genes (Wiemann et al. 2009) was increased in the Δ*lae1* mutant under normally repressing high nitrogen indicating that Lae1 acts as repressor of BIK biosynthesis, similar to what was shown before for Vel1 (Wiemann et al. 2010). Lae1 and Vel1 also act as repressors for PKS13 which was recently shown to be responsible for gibepyrone biosynthesis (Janevska et al. 2016). Besides, some cryptic putative gene clusters, e.g., the yet uncharacterized *STC7* and *NRPS4* genes, were upregulated in the Δ*lae1* and OE::*LAE1* mutants. Based on the co-expression of adjacent genes, the borders of the putative STC7 and NRPS4 gene clusters could be predicted (Niehaus et al. 2017c).

Another global regulator affecting secondary metabolism in some *Fusarium* species is Sge1, a homolog of the morphological switch regulators Wor1 and Ryp1 in *Candida albicans* and *Histoplasma capsulatum*, respectively (Michielse and Rep 2009; Michielse et al. 2015; Brown et al. 2014). In contrast to other fungi, this TF is not required for conidiogenesis and pathogenicity in *F. fujikuroi* (Michielse et al. 2015). Microarray analysis of the Δ*sge1* mutant in comparison to the WT revealed that Sge1 functions as a global activator of secondary metabolism in *F. fujikuroi*. Under inducing low nitrogen conditions, the transcript and product levels of GA and FUM biosynthesis were significantly reduced, while under high nitrogen, the optimal condition for FSA, FUS, and APF (Niehaus et al. 2013; Niehaus et al. 2014a; Niehaus et al. 2014b), the gene expression and production levels for these three SMs were significantly reduced in the Δ*sge1* mutant (Michielse et al. 2015) (Fig. 3a). Interestingly, the almost total loss of APF biosynthesis in the Δ*sge1* mutant could not be overruled by over-expression of the cluster-specific TF gene *APF2*, suggesting that Sge1 affects the accessibility of this SM gene cluster for its activating TF, possibly through modification of the histone landscape (Michielse et al. 2015).

Over-expression of *SGE1* in the WT background led to elevated FUM, FSA, and APF productions under favorable and FUS and FSA productions even under non-favorable conditions, indicating that nitrogen regulation can be overruled in this mutant. Most significantly, Sge1 was also required for expression of the yet uncharacterized non-canonical NRPS34 gene and six adjacent genes. All these genes were downregulated in the Δ*sge1* mutant and upregulated in the OE:*SGE1* strain (Michielse et al. 2015). The product of the new cluster was identified by comparing the metabolite profiles between the OE:*SGE1* mutant and the *NRPS34* deletion strain. Surprisingly, instead of a new compound, a peak with the corresponding mass for FSA was identified in the OE:*SGE1* mutant. As the new gene cluster is located in close proximity to the already known FSA biosynthetic gene cluster (Niehaus et al. 2014b), we suggested that both cluster segments are functionally linked and involved in FSA biosynthesis (Studt et al. 2016b) (Fig. 2d). During the course of this study, a second segment of the *FUB* gene cluster was also reported for two related fungi, *F. verticillioides* and *Fusarium oxysporum* (Brown et al. 2015). In all three species, the five *FUB* genes of the first cluster segment and the seven genes of the second segment are separated by three to four genes which are not involved in FSA biosynthesis (Fig. 2d). The extended FSA gene cluster encodes two TFs with specific functions, similar to the trichosetin (PKS-NRPS1) gene cluster (Janevska et al. 2017a). One of them, i.e., Fub10, regulates the expression of all cluster genes while the over-expression of the second TF, *FUB12*, did not result in a significant upregulation of *FUB* gene expression and FSA product formation. Instead, Fub12 is involved in the regulation of the derivatization of FSA into the less toxic derivatives dehydrofusaric acid and fusarinolic acid (Studt et al. 2016b).

Recently, another TF was found to be a major regulator of secondary metabolism and conidiation in *F. fujikuoi*, the GATA-type TF Csm1 (Niehaus et al. 2017b). This conserved GATA TF is an ortholog of *A. nidulans* NsdD which was shown to be an activator of sexual development and key repressor of conidiation in *A. nidulans* (Lee et al. 2014). In *Botrytis cinerea*, this TF was described as light-regulated repressor of macroconidia formation (Ltf1) and regulator of secondary metabolism (Schumacher et al. 2014). Deletion of *CSM1* in *F. fujikuroi* resulted in strong elevation of microconidia formation compared to the WT indicating that Csm1 also acts as repressor of conidiogenesis in this fungus. Furthermore, expression patterns were compared between the *F. fujikuroi* WT and the Δ*csm1* mutant under different nitrogen conditions. This microarray analysis revealed a strong impact of Csm1 on the expression of 19 out of the 47 gene clusters present in the genome of *F. fujikuroi*. Among them were the genes involved in biosynthesis of known products, e.g., APF, BEA, BIK, FSR, FSA, fujikurins, gibepyrones, and FUM (Fig. 3a). For some of them, we observed a de-regulated gene expression under non-favorable nitrogen or pH conditions (Niehaus et al. 2017b). For instance, the expression of APF, BEA, FSA, and FUS biosynthetic genes was elevated under usually repressing low nitrogen conditions, and the expression of the BIK and FSR biosynthetic genes was de-regulated under otherwise repressing pH conditions. Besides, some putative silent gene clusters, e.g., those with *STC1*, *NRPS4*, *NRPS11*, and *NRPS20* as key enzyme-encoding genes, were strongly upregulated in the Δ*csm1* mutant. The STC1 cluster with the sesquiterpene cyclase gene *STC1* and two additional co-regulated genes was analyzed in more detail. Heterologous expression of *STC1* in *Escherichia coli* led to the

identification of the product as the volatile bioactive compound (−)-germacrene D (Niehaus et al. 2017b). The function of the remaining genes is currently under investigation.

It is currently not known whether the global TFs can directly bind the promoters of cluster genes, or whether they affect the expression of gene clusters rather indirectly, e.g., by regulating the expression of other TFs and chromatin modifiers.

The role of the global nitrogen regulators AreA and AreB on secondary metabolism in *F. fujikuroi*

It has been known for a long time that the commercial production of GAs delivers highest yields under low nitrogen conditions (Borrow et al. 1964; Bu'Lock et al. 1974; Jefferys 1970). However, the molecular mechanism of nitrogen regulation of GA biosynthesis remained elucive. The GAs were the first fungal SMs for which an essential role of the major nitrogen regulator, the GATA-type TF AreA, was shown: deletion of *areA* resulted in almost total loss of GA biosynthesis (Tudzynski et al. 1999) and GA gene expression (Mihlan et al. 2003). These findings were unexpected because AreA was only known as global regulator required for the activation of alternative nitrogen assimilation pathways which are not expressed when preferred nitrogen sources, such as glutamine and ammonium, are available (Caddick et al. 1994; Arst and Cove 1973; Marzluf 1997).

Later, genome-wide microarray analyses under low and high nitrogen conditions revealed that not only the GA biosynthetic genes, but half of the SM gene clusters in *F. fujikuroi* depend on nitrogen availability (Wiemann et al. 2013). However, not all nitrogen-repressed SM gene clusters are regulated in the same way. For instance, the expression of the BIK cluster genes, though co-regulated with the GA genes, do not depend on the presence of AreA (Wiemann et al. 2009).

Recently, a second GATA-type TF, termed AreB, was shown to be involved in nitrogen-dependent regulation of SM genes in *F. fujikuroi* (Michielse et al. 2014; Pfannmüller et al. 2017). Whereas the AreB orthologs in *A. nidulans* and *P. chrysogenum* were generally regarded as the negative counterparts to AreA, acting as major repressors of AreA-activated nitrogen catabolism genes (Haas et al. 1997; Wong et al. 2008), its function appears to be more complex in *F. fujikuroi*. In this fungus, AreB can positively or negatively regulate SM gene clusters under both nitogen-limiting and nitrogen-sufficient conditions (Michielse et al. 2014). For instance, AreB is essential for the expression of APF, FUS, and FSA genes under high nitrogen and for GA and FUM genes under low nitrogen (Pfannmüller et al. 2017) (Fig. 3b). In addition to their activity as TFs, both AreA and AreB are likely required for chromatin remodeling processes at the GA gene cluster (Pfannmüller et al. 2017) (Fig. 4a). The most surprising function of AreB in regulating secondary metabolism is the one as repressor of BIK biosynthesis (Fig. 3b). In accordance with elevated *BIK* transcription, the cultures of the Δ*areB* mutant were deeply red colored under both low and repressing high nitrogen conditions, and significantly elevated amounts of BIK were produced (Pfannmüller et al. 2017).

In addition, AreB acts as repressor of some orphan SM clusters, e.g., the PKS9, PKS20, NRPS4, and NRPS21 clusters, which are never expressed in the WT. Therefore, the Δ*areB* mutant can be used to activate these unknown gene clusters and to identify their potential products in near future.

Activation of silent gene clusters by genetic manipulation of histone modifying genes

Chromatin-based regulation through histone modifications, such as acetylation and methylation, has now been shown to precisely affect the expression of physically linked SM genes belonging to one gene cluster in several fungi (Brakhage 2013).

Due to the high number of silent gene clusters in *F. fujikuroi*, several efforts were made to activate them either by inactivation of HDACs or by deletion or silencing of histone methyltransferase genes. Actively trancribed genes typically carry high levels of histone acetylation catalyzed by histone acetyltransferases, while histone deacetylation often results in gene silencing (Bannister and Kouzarides 2011). Deletion of three HDAC-encoding genes, *HDA1*, *HDA2*, and *HDA4*, indicated that Hda1 and Hda2 are involved in regulation of secondary metabolism, while Hda4 regulates growth and development but is dispensable for SM production in *F. fujikuroi* (Studt et al. 2013). Single deletions of both *HDA1* and *HDA2* led to up- and downregulation of several SM gene clusters, indicating that HDACs not only are involved in gene silencing but also participate in the activation of gene expression (Table 2). For instance, the expression of GA biosynthetic genes and GA production levels were reduced in both single deletion mutants, resulting in a loss of *bakanae* symptom development. Furthermore, Hda1 and Hda2 were essential for WT-like expression of BIK, FSR, and FSA biosynthetic genes (Studt et al. 2013). The most striking result was the activation of a silent and orphan gene cluster in the Δ*hda1* mutant (Niehaus et al. 2016a). Comparison between the SM profiles of the WT and the Δ*hda1* mutant revealed a novel peak in the mutant, which was identified as the cyclooligomer depsipeptide BEA (Figs. 1j and 4b). This mycotoxin is well known from the insect pathogen *Beauveria bassiana* (Xu et al. 2008) but was also detected in some *Fusarium* species (Zhang et al. 2013; Moretti et al. 1996). Phylogenetic analysis revealed that NRPS22 from *F. fujikuroi* grouped together with BeaS, the key enzyme for BEA biosynthesis in *B. bassiana*. Deletion of the respective *NRPS22* (*BEA1*) gene in the Δ*hda1* mutant

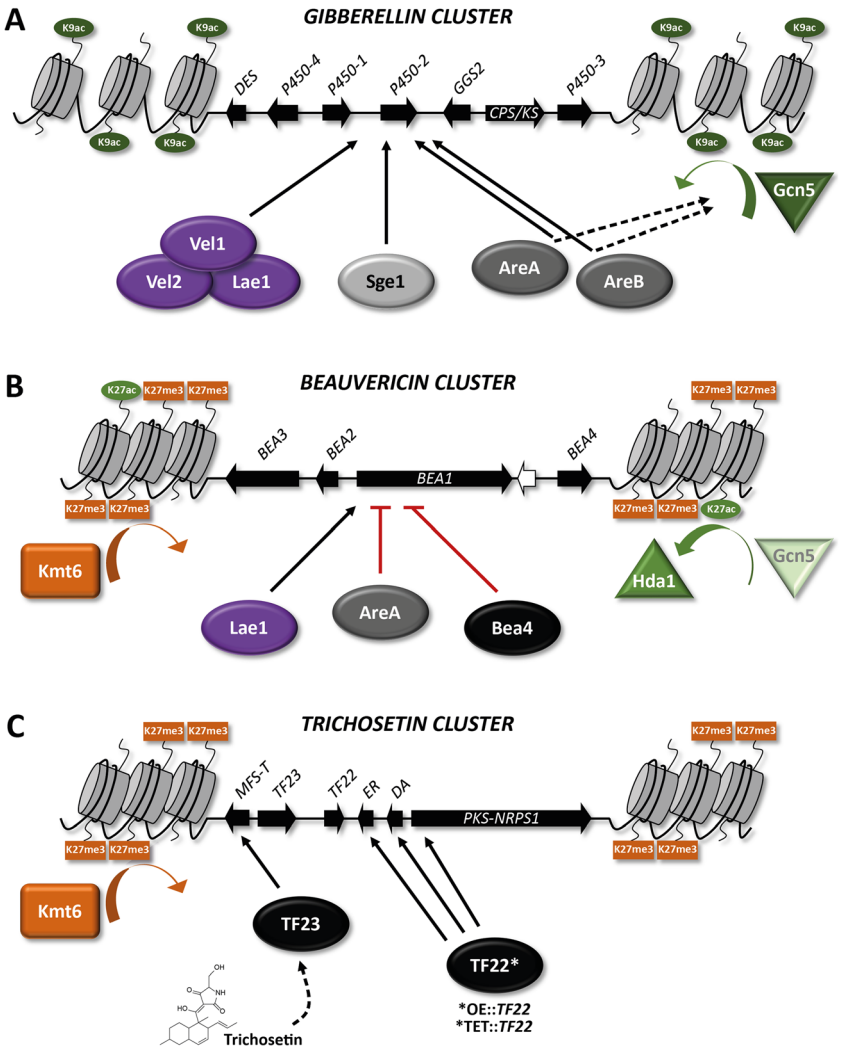

Fig. 4 Interplay of pathway-specific transcription factors, global regulators, and histone modifying enzymes that regulate gibberellin (**a**), beauvericin (**b**), and trichosetin (**c**) biosynthesis in *F. fujikuroi*. Black arrows indicate (direct or indirect) transcriptional activation, while red bars show (direct or indirect) gene cluster repression. Dotted arrows represent unclear interactions. The histone landscape around the secondary metabolite gene clusters is shown schematically: the trimethylation of lysine 27 at histone H3 (K27me3) is a repressive mark (facultative heterochromatin), while the acetylation of H3K9 (K9ac) and H3K27 (K27ac) is likely responsible for active transcription at these gene clusters

abolished BEA biosynthesis, confirming that it is the ortholog of *beaS*. Together with the key enzyme-encoding gene, three additional genes are involved in BEA biosynthesis and regulation, encoding a ketoisovalerate reductase (KivR; Bea2), an ATP-binding cassette (ABC) transporter (Bea3), and a $Zn(II)_2Cys_6$ TF (Bea4) (Fig. 2j) (Niehaus et al. 2016a). *BEA2* and *BEA3* were strongly upregulated in the Δ*hda1* mutant together with *BEA1*, while *BEA4* expression was unaffected by the deletion of *HDA1*.

Recent chromatin immunoprecipitation combined with high-throughput sequencing (ChIP-Seq) showed that many of the silent SM gene clusters in *F. fujikuroi*, mainly located at subtelomeric regions, are enriched for the silencing methylation mark at lysine 27 of histone 3 (H3K27me3) (Studt et al. 2016a). The gene for the putative H3K27 methyltransferase, *KMT6*, was recently identified in two fungi, *F. graminearum* and *Epichloë festucae* (Connolly et al. 2013; Chujo and Scott 2014). Deletion of the *KMT6* homologs in these two ascomycetes resulted in upregulation of several yet uncharacterized SM-related genes demonstrating that H3K27me3 is a promising target for the induction of otherwise silent SM gene clusters. It is worth to mention that several other model fungi, e.g., *A. nidulans* and the human pathogen *A. fumigatus*, lack *KMT6* and all other components of the known polycomb repressive complex 2 (PRC2) (Connolly et al. 2013).

Surprisingly, the *KMT6* gene is an essential gene in *F. fujikuroi* and could not be deleted. Therefore, a knockdown approach was performed to reduce the H3K27me3 level at a genome-wide level. Several yet unknown and silent SM key enzyme-encoding genes were upregulated in the $KMT6^{kd}$ mutant (Table 2). Among them was again the BEA (NRPS22) cluster (Studt et al. 2016a) which was shown to be repressed by a whole set of negatively acting regulators: Hda1, Kmt6, the cluster-specific TF Bea4 and the ABC transporter Bea3 (Niehaus et al. 2016a) (Fig. 4b). The latter seems to act as a regulator of gene expression rather than as transporter.

Besides the BEA cluster, several additional silent gene clusters were activated by downregulating *KMT6* (Table 2). Among them were the PKS-NRPS1 (Fig. 4c), NRPS4, STC4,

STC8, and STC5 clusters. All of them are located in regions of high H3K27me3 levels, either at subtelomeric regions (NRPS22, PKS-NRPS1, NRPS4) or in central regions of the chromosomes (STC4, STC5, STC8) (Studt et al. 2016a). The sesquiterpene cyclase STC5 was analyzed in more detail and shown to be responsible for the production of the sesquiterpene hydrocarbon (1*R*,4*R*,5*S*)-guaia-6,10(14)-diene (Studt et al. 2016a; Burkhardt et al. 2016). The product of a second silent gene cluster, PKS-NRPS1, was identified as trichosetin by an alternative approach as described above: the overexpression of the cluster-specific TF gene (Janevska et al. 2017a) (Fig. 4c).

An important role for the regulation of secondary metabolism was shown for the histone acetyltransferase (HAT) Gcn5, a member of the SAGA complex. This HAT is responsible for acetylation of several histone 3 lysines in *F. fujikuroi*, i.e., H3K4, H3K9, H3K18, and H3K27 (Rösler et al. 2016b) (Fig. 4a, b). In total, the transcription of 28 out of the 47 bioinformatically identified key genes of putative SM gene clusters was affected by *GCN5* deletion (Table 2). The expression of the majority (18 out of 28) of these SM clusters, e.g., those for GA and FSR biosynthesis, was downregulated or abolished in Δ*gcn5* compared to the WT, suggesting a mainly activating role of Gcn5 in SM gene regulation. However, some gene clusters, e.g., those for BIK and FSA biosynthesis, as well as some apparently silent gene clusters (NRPS4, PKS-NRPS1, STC5, STC9, DMATS1) were upregulated under at least one tested condition in the mutant (Rösler et al. 2016b).

The methylation marks of H3K4 and H3K36 have been described as hallmarks of euchromatin in budding and fission yeasts as well as higher eukaryotes (Rando and Chang 2009; Wagner and Carpenter 2012). The euchromatic localization of H3K4me2 could be verified by ChIP-Seq (Wiemann et al. 2013), while H3K36me3 was found to cover whole chromosomes in *F. fujikuroi* (Janevska et al. 2017b). Set2 is the major H3K36-specific methyltransferase, and it has already been studied in *Neurospora crassa* and *F. verticillioides* (Adhvaryu et al. 2005; Gu et al. 2017). In addition, only filamentous fungi, but not yeasts, contain a second H3K36-specific methyltransferase gene, *ASH1*, which was functionally analyzed in *F. fujikuroi* for the first time (Janevska et al. 2017b). Thus, *F. fujikuroi* Set2 and Ash1 were shown to deposit their methylation at euchromatic and subtelomeric regions, respectively. Intriguingly, deletion of *ASH1* resulted in the loss of subtelomeric regions and of the accessory chromosome XII, likely counteracting H3K27me3 at these regions and mediating DNA repair mechanisms. Although secondary metabolism was de-regulated in Δ*set2* and Δ*ash1* mutants, the effects are probably indirect because the expression of SM biosynthetic genes did not correlate with H3K36me3 and/or H3K27me3 levels at the analyzed gene clusters (Janevska et al. 2017b).

Furthermore, the loss of H3K4me3 upon *CCL1* deletion had an impact on secondary metabolism in *F. fujikuroi* (Studt et al. 2017). Ccl1 is a critical component for the trimethylation of H3K4 by the complex of proteins associated with Set1 (COMPASS). Also in this case, many of the effects are likely to be indirect, although enhanced levels of H3K4me2 at the BIK cluster correlated with an elevated BIK biosynthesis in Δ*ccl1* (Studt et al. 2017). However, further analyses are required to fully understand the impact of histone modifications on fungal secondary metabolism.

Conclusion

Sequencing of large numbers of fungal genomes revealed the enormous genetic capacity of fungi to produce a battery of low molecular weight natural products. However, most of the SM gene clusters remain silent under standard laboratory conditions and, therefore, their products are still unknown. The increasing interest in identification of novel fungal SMs with potential applications in medicine, agriculture, and the food industry resulted in efforts to activate them by the use of different molecular techniques. This review summarizes the recent work on secondary metabolism of the rice pathogenic fungus *F. fujikuroi*. Whereas only three gene clusters for GA, BIK, and carotenoid biosynthesis were identified and experimentally characterized before the genome was sequenced in 2013, the products of 20 out of the 47 potential SM gene clusters are now known due to genetic manipulations of key enzyme-encoding genes, pathway-specific and global regulators, and histone-modifying enzymes. By these approaches, we were able to shed more light on the multi-level regulation of secondary metabolism. For example, we gained much more knowledge on the complex regulation of GA biosynthesis, the most prominent SM of *F. fujikuroi* studied over a long period. At the beginning, only the repression of GA formation by high levels of nitrogen was described. Now, a number of transcription factors and histone modifiers were shown to affect the expression of GA genes (Fig. 4a). Besides the major nitrogen regulators, AreA and AreB, all three components of the *F. fujikuroi velvet* complex are essential for high gene expression. In addition, global regulators such as Sge1 and the acetylation status of histones were shown significantly to affect GA gene expression (Fig. 4a). One of the most surprising results of these studies was that each single gene cluster is regulated by a specific and unique

network of regulators, probably due to their specific biological function.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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