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Analysis of the global regulator Lae1 uncovers a connection between Lae1 and the histone acetyltransferase HAT1 in *Fusarium fujikuroi*

Eva-Maria Niehaus[1,2] · **Lena Rindermann**[1] · **Slavica Janevska**[1] · **Martin Münsterkötter**[3] · **Ulrich Güldener**[4] · **Bettina Tudzynski**[1]

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Abstract The fungus *Fusarium fujikuroi* causes "*bakanae*" disease of rice due to its ability to produce gibberellins (GAs), a family of plant hormones. Recent genome sequencing revealed the genetic capacity for the biosynthesis of 46 additional secondary metabolites besides the industrially produced GAs. Among them are the pigments bikaverin and fusarubins, as well as mycotoxins, such as fumonisins, fusarin C, beauvericin, and fusaric acid. However, half of the potential secondary metabolite gene clusters are silent. In recent years, it has been shown that the fungal specific *velvet* complex is involved in global regulation of secondary metabolism in several filamentous fungi. We have previously shown that deletion of the three components of the *F. fujikuroi velvet* complex, *vel1*, *vel2*, and *lae1*, almost totally abolished biosynthesis of GAs, fumonisins and fusarin C. Here, we present a deeper insight into the genome-wide regulatory impact of Lae1 on secondary metabolism. Over-expression of *lae1* resulted in de-repression of GA biosynthetic genes under otherwise repressing high nitrogen conditions demonstrating that the nitrogen repression is overcome. In addition, over-expression of one of five tested histone acetyltransferase genes, *HAT1*, was capable of returning GA gene expression and GA production to the GA-deficient Δ*lae1* mutant. Deletion and over-expression of *HAT1* in the wild type resulted in downregulation and upregulation of GA gene expression, respectively, indicating that HAT1 together with Lae1 plays an essential role in the regulation of GA biosynthesis.

Keywords Lae1 · *Fusarium fujikuroi* · Secondary metabolism · Gene regulation · Gibberellins · Histone acetyltransferases

Introduction

Filamentous fungi produce a wide range of small molecules called secondary metabolites (SMs), which include pigments, mycotoxins, antibiotics, and plant hormones. Most of the SMs are derived from only a few building blocks by key enzymes such as polyketide synthases (PKSs), non-ribosomal peptide synthetases (NRPSs), hybrid PKS-NRPS enzymes, terpene cyclases (TCs), or dimethylallyltryptophan synthases (DMATSs) (Keller et al. 2005; Niehaus et al. 2016a). In general, the key enzyme-encoding genes and additional biosynthetic genes such as monooxygenases, methyltransferases, and oxygenases are co-regulated and located adjacent to each other in gene clusters. SM gene clusters are controlled by complex regulatory networks including pathway-specific transcription factors (TFs) and/or global regulators, e.g., the nitrogen-responsive GATA factors AreA and AreB, the CCAAT-binding HAP complex, or the pH regulator PacC (Brakhage 2013). There are a growing number of examples

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✉ Bettina Tudzynski
tudzynsb@uni-muenster.de

[1] Institute for Plant Biology and Biotechnology, Westfälische Wilhelms University Münster, Schlossplatz 8, 48143 Münster, Germany

[2] Institute of Food Chemistry, Westfälische Wilhelms University Münster, Corrensstr. 45, 48149 Münster, Germany

[3] Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, Germany Research Center for Environmental Health (GmbH), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[4] Chair of Genome-oriented Bioinformatics, TUM School of Life Sciences Weihenstephan, Technical University of Munich, 85354 Freising, Germany

that show that SM gene clusters are also regulated by chromatin-level control, often causing silencing of cluster gene expression (Strauss and Reyes-Dominguez 2011).

One global regulator of secondary metabolism in filamentous fungi is the putative histone methyltransferase LaeA (loss of aflatoxin expression), a member of the *velvet* complex besides two additional proteins of the *velvet* family, VeA and VelB (Bayram et al. 2008). LaeA was first identified through complementation of a sterigmatocystin mutant in *Aspergillus nidulans* (Bok and Keller 2004). Later, orthologs were identified also in other *Aspergillus* species (Bok et al. 2005; Amaike and Keller 2009) and different ascomycetous species such as *Fusarium fujikuroi* (Wiemann et al. 2010), *Fusarium graminearum* (Kim et al. 2013), *Penicillium chrysogenum* (Hoff et al. 2010), *Fusarium verticillioides* (Butchko et al. 2012), *Trichoderma reesei* (Seiboth et al. 2012), *Cochliobolus heterostrophus* (Wu et al. 2012), and *Botrytis cinerea* (Schumacher et al. 2015). Deletion of *laeA*/*lae1* in these fungi resulted in downregulation of cluster genes, decreased SM production levels, altered asexual and sexual development, and reduced virulence for pathogenic fungi (Jain and Keller 2013). Microarray studies of *laeA*/*lae1* deletion and over-expression strains in several fungi clearly showed that LaeA transcriptionally regulates a large number of SM clusters. Over-expression of this global regulator gene resulted in activation of otherwise silent gene clusters and enabled subsequent identification of novel products, e.g., terrequinone A in *A. nidulans*, cyclopiazonic acid in *Aspergillus fumisynnematus*, and chaetoglobosin Z in *Chaetomium globosum* (Bok et al. 2006a; Hong et al. 2015; Jiang et al. 2016).

The precise mechanism by which LaeA controls so many processes in fungi is still unknown. As in all methyltransferases, LaeA contains an *S*-adenosyl methionine (SAM)-binding domain that is required for its function (Bok et al. 2006b). Although the substrate for methylation by LaeA has not been identified, there are a growing number of indications that LaeA is directly or indirectly involved in chromatin modifications. For instance, it has been shown that the loss of *laeA* in *A. nidulans* resulted in strongly elevated H3K9 trimethylation (H3K9me3) levels at the sterigmatocystin cluster, promoting formation of heterochromatin and reduced toxin production (Soukup et al. 2012). The deletion of genes encoding the heterochromatin protein 1 (Hep1) or the H3K9 methyltransferase ClrD in the Δ*laeA* background partially restores the wild-type (WT) production level (Reyes-Dominguez et al. 2010; Strauss and Reyes-Dominguez 2011). Furthermore, a suppressor screen for genes capable of restoring production of WT levels of SMs affected by deletion of *laeA* resulted in identification of the H4K12 histone acetyltransferase (HAT) EsaA, which is able to de-repress the sterigmatocystin, penicillin, terrequinone, and orsellinic acid gene clusters that are all targets of LaeA (Soukup et al. 2012).

The gibberellin (GA)-producing rice pathogen *F. fujikuroi* was the first fungus outside the class of *Eurotiomycetidae* for which an ortholog of LaeA, named FfLae1, has been identified and functionally characterized (Wiemann et al. 2010). As in *A. nidulans*, FfLae1 is part of the *velvet* complex together with the two *velvet* family proteins FfVel1 and FfVel2. Both FfLae1 and FfVel1 were shown to be essential for GA gene expression, whereas only FfVel1, but not FfLae1, affects the expression of bikaverin biosynthetic genes (Wiemann et al. 2010). In 2013, the genome of *F. fujikuroi* was released and provided insight into this fungal pathogen's potential to produce 47 SMs, most of them unknown due to transcriptional silencing (Wiemann et al. 2013). Since then, we have been able to identify some of the cryptic SMs by over-expressing pathway-specific TF genes (Von Bargen et al. 2013, 2015; Niehaus et al. 2014; Janevska et al. 2017). In addition, the products of two yet unknown and almost fully silenced gene clusters, beauvericin and (1*R*,4*R*,5*S*)-guaia-6,10(14)-diene, have been recently identified by manipulating the histone-modifying genes *hda1* and *kmt6*, respectively (Niehaus et al. 2016b; Studt et al. 2016a).

Here, we studied the impact of FfLae1 (from now on named Lae1) in *F. fujikuroi* on secondary metabolism at a genome-wide level. We performed microarray analysis comparing the expression profiles of the WT with those of the Δ*lae1* and a *lae1* over-expressing mutant (OE:*LAE1*). Beside GAs, manipulation of *lae1* resulted in upregulation of some of the known and five yet unknown gene clusters either in the deletion or the over-expression mutant. The most prominent result is the elevated expression of the GA biosynthetic genes and increased product levels under repressing nitrogen sufficent conditions. Furthermore, over-expression of *HAT1*, one of the five tested HAT-encoding genes, in the Δ*lae1* background resulted in full restoration of GA biosynthesis.

Material and methods

Fungal strains and culture conditions

The WT strain *Fusarium fujikuroi* IMI58289 (International Mycological Institute, Kew, UK) was used for experiments and for construction of deletion and over-expression mutants. The deletion strain Δ*lae1* (Wiemann et al. 2010) was also used in this study.

The strains were pre-incubated in 100-mL Darken medium in a 300-mL Erlenmeyer flask (Darken et al. 1959) for 3 days at 28 °C in the dark at 180 rpm. Of this culture, 0.5 mL was used as inoculum for cultivation in 100-mL synthetic ICI media (Imperial Chemical Industries Ltd., UK) in 300-mL flasks (Geissman et al. 1966). These cultures were incubated for 2–7 days in ICI containing either 6 or 60 mM glutamine, or 6 or 120 mM $NaNO_3$. For RNA isolation, the harvested and

lyophilized mycelia were used. HPLC analyses were done with cultures after 7 days of growing. For transformation, protoplasts were produced by using 0.5 mL of the starter culture for inoculation of 100-mL ICI medium having 10 g/L fructose instead of glucose and supplemented with 0.5 g/L ammonium sulfate (Tudzynski et al. 1999). For DNA isolation, the strains were grown at 28 °C in the dark on solidified complete medium (CM) (Pontecorvo et al. 1953) for 3 days on cellophane sheets (Alba Gewürze, Bielefeld, Germany).

Plasmid constructions

For the construction of complementation, over-expression, and deletion mutants, the yeast recombination system was used (Schumacher 2012). For construction of the *HAT1* deletion mutant, the 3′ and the 5′ flanking regions of the gene were amplified via PCR with HAT1-5F//HAT1-5R and HAT1-3F//HAT1-3R primers, respectively. The hygromycin resistance cassette, consisting of the hygromycin B phosphotransferase gene (*hph*) and the *trpC* promoter, was amplified from pSCN44 with the primer pair hph-F//hph-R (Staben et al. 1989). The hygromycin resistance cassette, both flanks and the *Eco*RI/*Xho*I digested shuttle vector pRS426 (Christianson et al. 1992), were brought together into the *Saccharomyces cerevisiae* strain FY834 (Winston et al. 1995). For the complementation of Δ*lae1*, the gene and about 1.5 kb of its native promoter were amplified by PCR using the following primer pairs: lae1-5F//lae1-Tgluc-5R and lae1-3F//lae1-3R. The obtained fragment was fused to the glucanase terminator BcT*gluc* from *Botrytis cinerea* B05.10 using the primer pair BcGlu-term-F2//Tgluc-nat1-R followed by the nourseothricin cassette, consisting of the nourseothricin acetyltransferase gene (*nat1*) under control of the *trpC* promoter. The *Eco*RI/*Xho*I restricted vector pRS426 was transformed together with the other obtained fragments in the FY834 strain, resulting in the plasmid p*lae1*^{C}. The construct p*lae1*^{SAM} with the mutated SAM domain of *lae1* (changing the amino acid sequence IMDI**G**T**G**TG to IMDI**A**T**A**TG) was generated the same way by PCR. However, the coding region of *lae1* was amplified in two parts from genomic DNA of *F. fujikuroi* by using the primer pairs lae1-5F//lae1-SAM-mod-R and lae1-SAM-mod-F//lae1-Tgluc-5R. Subsequently, it was fused to its native promoter and to the *B. cinerea* T*gluc* terminator similar to the construction of the complementation vector. The linearized vectors p*lae1*^{C} and p*lae1*^{SAM} were transformed into the Δ*lae1* mutant and integrated at the *lae1* locus by homologous recombination (Fig. S1). For constitutive expression of *lae1*, the gene was amplified using the primer pair lae1-OE-F//lae1-OE-GFP-R and the obtained fragment was cloned into a *Hin*dIII digested pRS426 plasmid with a hygromycin resistance cassette. For over-expression of *lae1*, the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) promoter was used (Wiemann et al. 2010). For the over-expression of the remaining genes (*HAT1*, *GCN5*, *GCN5-like*, *HAT5*, *HAT9*), the *Nco*I digested vector pNDN-OGG (Schumacher 2012) and the obtained fragments of the genes (primer pairs desired-gene-OE-F//desired-gene-OE-R) were cloned downstream of the *oliC* promoter of *A. nidulans*. A nourseothricin resistance cassette, consisting of the nourseothricin acetyltransferase gene (*nat1*) under control of the strong *trpC* promoter from *A. nidulans*, served as resistance marker. The primers we used are listed in Table S1.

Sequencing was performed to control the correct point mutation, the complementation vector, and the over-expression vectors. Therefore, the BigDye® Terminator v3.1 Cycle Sequencing Kit and the ABI Prism® 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) were used.

Standard molecular methods

DNA and RNA extraction was done as described in Niehaus et al. (2016b). For Southern blot analysis of Δ*HAT1*, the DNA was digested with *Eco*RV (Fermentas GmbH, St. Leon-Rot, Germany) and then separated in a 1% (*w*/*v*) agarose gel and transferred on to a Nytran® nylon transfer membrane (Whatman Inc., Sanford, ME, USA). Northern blot and quantitative real-time PCR qRT-PCR analyses of the SM genes were carried out as described by Niehaus et al. (2017).

Plasmid DNA of *S. cerevisiae* was extracted with the Easy Yeast Plasmid Isolation Kit (TaKaRa Bio USA, Inc., CA, USA). For the amplification of the *HAT1* knockout construct, a TaKaRa Polymerase Kit (TaKaRa Biotechnology (Dalian) Co., Ltd., Japan) and the primer pair HAT1-5F//HAT1-3R (Table S1) were used. PCR reactions for control of the transformed *HAT1* deletion mutants were done with 200 nM dNTPs, 5 pmol of each primer, 1 unit of BioTherm™ DNA polymerase (GENECRAFT GmbH, Lüdinghausen, Germany), and about 25 ng DNA. The reaction was initiated at 94 °C for 4 min, followed by 36 cycles of 1 min at 94 °C, 1 min at 57 °C, 1.30 min at 70 °C, and a final step at 70 °C for 10 min. The following primer pairs were used for control: HAT1-5F-diag//pCSN44-hph-trpC-T (5′ flank), HAT1-3R-diag//pCSN44-hph-trpC-P2 (3′ flank), and HAT1-WT-F//HAT1-WT-R (verifying the absence of the WT gene; Table S1). For checking the over-expression mutants, the following primer pairs were used: gene-of-interest-WT-R//pOliC-seq-F2 (Table S1).

Microarray analyses

The *F. fujikuroi* microarray analyses (Roche NimbleGen Systems, Madison, WI, USA) were performed as described previously (Wiemann et al. 2013). Microarray hybridizations were done at Arrows Biomedical (Münster, Germany), and RNA quality was checked using Agilent Bioanalyzer 2100 and RNA Nano 6000 Lab-Chip Kit (Agilent Technologies).

Expression data were analyzed as described before (Wiemann et al. 2013). Genes with an absolute log2-fold change above 1 or below −1 and an adjusted *P* value (FDR) below 0.05, based on biological duplicates, were regarded as significantly differentially expressed. The expression datasets are available in the Gene Express Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE90946).

To explore functional distributions of specific regulated gene sets, the Functional Catalog (FunCat) (Ruepp et al. 2004) was used to identify biological processes. We applied Fisher's exact test to determine statistically over-represented functional categories in differentially expressed gene sets. The retained *P* values were adjusted using Bonferroni procedure. Tested categories with an adjusted *P* value below 0.05 were regarded as significantly over-represented in the gene set.

Expression analysis via qRT-PCR

Expression of differentially expressed SM genes and of *lae1* itself was confirmed by qRT-PCR using iTaq Universal SYBR Green Supermix (Bio-Rad) and complementary DNA (cDNA) as template, in an iQ5 Biorad thermocycler. For synthesizing the cDNA with the SuperScript II reverse transcriptase (Invitrogen, Groningen, the Netherlands), 1 μg total RNA was used. The obtained cDNA was checked for absence of genomic DNA by PCR. The qRT-PCR analyses were performed by an annealing temperature of 60 °C with two independent biological and two technical replicates. The data were analyzed according to the ΔΔCt method (Pfaffl 2001), with the actin gene, ubiquitin gene, and the GDP mannose transporter serving as reference genes. The following primer pairs were used: rac-qPCR-F//rac-qPCR-R, fub-qPCR-F//fub-qPCR-R, and gmt-qPCR-F//gmt-qPCR-R (Table S1).

Fungal transformations

Transformation of *F. fujikuroi* was performed as described by Tudzynski et al. (1996). About 10^7 protoplasts were transformed with 10 μg of the following vectors: pOE:*LAE1*, p*lae1*^{C}, p*lae1*^{SAM}, pΔ*HAT1*, pOE:*HAT1*, pOE:*GCN5*, pOE:*GCN5-like*, pOE:*HAT5*, and pOE:*HAT9*, respectively. The transformants were regenerated on regeneration agar for 4–6 days in the dark at 28 °C containing 100 μg/mL hygromycin B (Calbiochem, Darmstadt, Germany) and/or 100 μg/mL nourseothricin (Werner-Bioagents, Germany).

Analyses of SMs

Beauvericin (BEA) was extracted from the harvested mycelium after incubation for 7 days in 120 mM $NaNO_3$ and analyzed via high-performance liquid chromatography with a diode array detector (HPLC-DAD) at λ = 210 nm as described previously (Niehaus et al. 2016b). Fusarubins (FSR), fusaric acid (FSA), and fusarins (FUSs) were analyzed in the supernatant of the 7-day-old ICI cultures supplemented with 6 mM $NaNO_3$ (FSR) and 60 mM glutamine (FSA, FUS), respectively. Bikaverin (BIK) was analyzed in the supernatant of both 6 and 60 mM glutamine. Before measuring the supernatant, mycelium was removed from the culture filtrates using 0.45-μm syringe filters (BGB®, Schloßböckelheim, Germany). Analyses were done with HPLC-DAD according to Wiemann et al. (2012). Total amounts of bioactive GAs (GA_3, GA_4, GA_7) were analyzed in the concentrated supernatant of 6 and 60 mM glutamine as described by Niehaus et al. (2016b).

Microscopical analysis

An AxioImager M1 (Carl Zeiss MicroImaging GmbH) was used for fluorescence microscopy of OE:*HAT1*:*GFP*. Images were made initially by differential interference contrast (DIC) microscopy. GFP fluorescence was done with the filter set 38 (excitation BP 470/40, beam splitter FT 495, emission BP 525/50). The Zeiss AxioCam MRm camera was taken for images and the AxioVison Rel 4.8 software package for analyzing the data.

Plate assay

For the plate assay, WT, Δ*HAT1*, and OE:*HAT1* were grown for 6 days at 28 °C in the dark on CM medium, CM medium supplemented with 40 mM H_2O_2 or 1 M NaCl, V8 vegetable juice (20% *v*/*v*, containing 30 mM $CaCO_3$; Campbell Foods, Puurs, Belgium), or 5% (*w*/*v*) Czapek Dox (CD) medium (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Results

Expression profiles of *lae1* deletion and over-expressing mutants

To gain a deeper insight into the functions of Lae1 in *F. fujikuroi*, genome-wide microarray analysis was performed to compare the expression profiles of the WT with those of the *lae1* deletion and over-expression mutants. For this purpose, we generated *lae1* over-expressing mutants (OE:*LAE1*) in addition to the already available deletion mutant (Wiemann et al. 2010). The WT, Δ*lae1*, and OE:*LAE1* strains were grown under nitrogen-limiting (6 mM glutamine) and nitrogen-sufficient (60 mM glutamine) conditions because the optimal conditions for the production of the different metabolites vary considerably regarding nitrogen availability in *F. fujikuroi* (Wiemann et al. 2013; Pfannmüller et al. 2017). The strains were grown in liquid synthetic medium in a duplicate for

3 days. Based on the selection criterion 4-fold (log2) change in expression at the 95% confidence interval, the expression of altogether 2452 genes was affected by deletion and/or over-expression of *lae1*. In the deletion mutant 242 and 473 genes were downregulated under low and high nitrogen conditions, respectively (Table 1, Fig. S2, and Table S2). Downregulation of 29 of these genes occurred under both nitrogen conditions. Of the altogether 686 downregulated genes, 171 genes were accordingly upregulated in the OE:*LAE1* mutant, either under one or both conditions. A smaller set of 493 genes was negatively affected by Lae1 and therefore upregulated in the deletion mutant. In the OE:*LAE1* mutant, 704 genes were upregulated and 851 genes were downregulated under high nitrogen, while 351 genes were upregulated and 142 genes downregulated at low nitrogen (Table 1, Fig. S2, and Table S2).

Lae1—a global regulator of genes with different functions

A functional distribution analysis of the upregulated or downregulated gene sets in the Δ*lae1* mutant indicated a strong enrichment of genes annotated in the categories "secondary metabolism"; "disease, virulence, and defense"; and "transport facilities" under low nitrogen. Enriched genes in categories secondary metabolism and "C-compound and carbohydrate metabolism" were downregulated under high nitrogen. The over-expression of *lae1* strongly affected similar gene categories, especially transport facilities and secondary metabolism, confirming a general de-regulation of these processes in the *lae1* mutants (Table S3).

In addition, genes belonging to several other gene families were also affected in the Δ*lae1* and/or OE:*LAE1* mutant (Table 1 and Table S2). For instance, there were 128 TF-encoding genes whose expression was significantly altered by deletion or over-expression of *lae1*. The most affected regulatory gene was *fub10* from the fusaric acid gene cluster (Studt et al. 2016b), which was dramatically downregulated in the Δ*lae1* mutant under inducing but also repressing (low nitrogen) conditions.

Furthermore, 208 transporter-, 93 dehydrogenase-, 75 monooxygenase/P450 monooxygenase-, 18 histone modifying enzyme-, and 350 secreted protein-encoding genes were upregulated or downregulated in Δ*lae1* (Table 1 and Table S2). Among the 18 differentially expressed histone modifying genes were those encoding putative histone acetyltransferases of the GNAT superfamily and SET domain-containing histone methyltransferases with yet unknown functions.

Among the monooxygenase genes regulated by Lae1 were several P450 monooxygenase-encoding genes which are involved in SM production, e.g., of GAs, fumonisins, and of an unknown putative NRPS23-derived product (Table S2). Also, some transporters belonging to SM gene clusters, e.g., those for the biosynthesis of fusaric acid, fumonisins, beauvericin, the siderophore fusarinine, and the yet unknown NRPS4 product, were regulated by Lae1. In addition, there were several sugar, amino acid, and purine transporters, but also several multidrug resistance and ABC transporters as well as ion channels that were affected by Lae1. Among the Lae1-

Table 1 Differentially regulated genes in the *lae1* mutants and their functional categories

Strain	Number of differentially regulated genes					
	Low nitrogen (6 mM glutamine)			High nitrogen (60 mM glutamine)		
	Total	Up	Down	Total	Up	Down
Δ*lae1*	483	241	242	746	273	473
SM gene clusters	5	1	4	8	3	5
Transcription factors	15	7	8	37	15	22
Dehydrogenases	25	4	21	42	5	37
Monooxygenases	16	4	12	23	4	19
Transporters	33	12	21	56	16	40
Histone modifiers	3	0	3	6	2	4
Secreted proteins	95	52	43	105	43	62
OE:*LAE1*	493	351	142	1555	704	851
SM gene clusters	7	6	1	9	6	3
Transcription factors	14	10	4	93	26	67
Dehydrogenases	4	2	2	60	21	39
Monooxygenases	15	11	4	49	24	25
Transporters	53	31	22	135	46	89
Histone modifiers	1	1	0	13	8	5
Secreted proteins	81	64	17	211	125	86

regulated amino acid permeases was the general amino acid permease FfGAP1, which was recently identified out of about 90 potential amino acid permeases in the genome by complementing the *S. cerevisiae* GAP1 deletion mutant (Pfannmüller et al. 2015).

Lae1-regulated SM gene clusters

Of the 47 putative SM gene clusters present in the genome of *F. fujikuroi*, gene members of 22 clusters were affected by *lae1* deletion or over-expression. For example, expression of all fusarin C cluster genes *fus1-fus9* (Niehaus et al. 2013) was elevated in the OE:*LAE1* mutant. Surprisingly, the most upregulation was observed under repressing low nitrogen conditions, indicating that the nitrogen regulation is overcome by over-expression of *lae1* (Table 2 and Table S2). The genes for fusaric acid biosynthesis (Studt et al. 2016b) were downregulated in the Δ*lae1* mutant and slightly upregulated in the OE:*LAE1* mutant under optimal high nitrogen conditions. Furthermore, over-expression of *lae1* resulted in activation of the recently identified beauvericin cluster which is almost silent in the WT (Niehaus et al. 2016b): the responsible genes (*bea1-bea3*) were strongly upregulated under both nitrogen conditions in the OE:*LAE1* mutant (Table 2 and Table S2).

Another observation was the upregulation of the genes *fsr1-fsr6*, responsible for the biosynthesis of the perithecial pigment fusarubins, in the OE:*LAE1* mutant under low glutamine (acidic) conditions. Previously, it has been shown that the fusarubin genes are only expressed in media with $NaNO_3$ due to the alkaline conditions caused by this nitrogen source (Studt et al. 2012). The elevated expression of the fusarubin genes with glutamine suggests that the pH regulation is circumvented by over-expressing *lae1*. In contrast, the expression of the key genes for the biosynthesis of the red pigment bikaverin (Wiemann et al. 2009) and the carotenoid neurosporaxanthin (Ansari et al. 2013) were increased in the Δ*lae1* mutant under normally repressing conditions (60 mM glutamine), and *PKS13*, the recently identified key gene for gibepyrone biosynthesis (Janevska et al. 2016), was downregulated in the over-expression mutant (Table 2 and Table S2). These data indicate that Lae1 acts as repressor of bikaverin, neurosporaxanthin, and gibepyrone biosynthesis.

We have previously shown that the expression of the GA and fumonisin biosynthetic genes depends on the presence of Lae1 (Wiemann et al. 2010). Here, we could confirm these data. Both gene clusters, which are known to be only expressed under nitrogen-limiting conditions in an AreA- and AreB-dependent manner (Mihlan et al. 2003; Michielse et al. 2014; Rösler et al. 2016b; Pfannmüller et al. 2017), were strongly downregulated in the Δ*lae1* mutant. However, the most surprising result was the upregulation of the genes, especially those of the GA gene cluster, in the OE:*LAE1* mutant under repressing high nitrogen conditions (Table 2 and Table S2).

Furthermore, two of the nine *F. fujikuroi* sesquiterpene cyclases (STCs) also responded to the deletion or over-expression of *lae1*. *STC1* which was recently shown to be responsible for (−)-germacrene D biosynthesis (Niehaus et al. 2017) was upregulated in the Δ*lae1* mutant under high nitrogen, whereas *STC3*, the key gene for eremophilene biosynthesis (Burkhardt et al. 2016), was downregulated in the Δ*lae1* mutant at low nitrogen (Table 2 and Table S2).

Besides the gene clusters with known products, there were also some cryptic putative gene clusters which were influenced by *lae1* deletion or over-expression. The yet uncharacterized *STC7* gene was upregulated in the OE:*LAE1* mutant under low, and the *NRPS4* gene was downregulated in the Δ*lae1* mutant under high nitrogen conditions (Table 2 and Table S2). Based on the co-expression of adjacent genes, the borders of the putative *STC7* and *NRPS4* gene clusters could be postulated (Fig. 1). The potential STC7 gene cluster probably contains genes encoding a MFS transporter and a hydrolase (*FFUJ_12026*, *FFUJ_12027*), while the putative *NRPS4* cluster consists of two additional genes encoding an ABC transporter (*FFUJ_08114*) and an amine oxidase (*FFUJ_08115*) (Fig. 1).

In summary, the microarray data clearly demonstrated the important role Lae1 plays in the regulation of SM biosynthesis in *F. fujikuroi*, the protein serving as activator of some and repressor of other gene clusters.

Confirmation of microarray data by qRT-PCR and HPLC-DAD analyses

To confirm the de-regulation of several gene clusters by deleting or over-expressing *lae1*, product measurements by HPLC-DAD and expression analyses by qRT-PCR under different nitrogen conditions (6 and 60 mM glutamine, 6 and 120 mM $NaNO_3$) were performed for some SMs (Fig. 2).

HPLC and qRT-PCR analyses confirmed elevated gene expression and increased GA production levels in the OE:*LAE1* mutant under inducing low nitrogen conditions. Surprisingly, we found also a partial de-repression under high nitrogen conditions (60 mM glutamine) (Fig. 2a). Bikaverin genes were more strongly expressed in the Δ*lae1* mutant under low and repressing high nitrogen conditions, confirming that Lae1 acts as a repressor of bikaverin biosynthesis. While no bikaverin was produced by the WT at high nitrogen (60 mM glutamine), the Δ*lae1* mutant produced low amounts under this condition, also seen as reddish coloration of the culture medium (Fig. 2b and Fig. S3a).

The yields and gene expression levels of the other tested SMs, fusarins, fusaric acid, and fusarubins were reduced or even totally abolished (fusarubins) in the Δ*lae1* mutant under

Table 2 Secondary metabolite (SM) key genes affected by *lae1* gene deletion or over-expression based on microarray analysis

Gene code	SM key enzyme	Product	Δ*lae1*-WT, N-	OE:*LAE1*-WT, N-	Δ*lae1*-WT, N+	OE:*LAE1*-WT, N+
FFUJ_09179	**DMATS1**	***r-N*-DMAT**	-1.2	-0.1	-2.3	-2.4
FFUJ_03654	**IaaH**	**Auxin**	-0.1	5.3	1	-1.3
FFUJ_08113	**NRPS4**		0.2	0.3	-2.3	2.7
FFUJ_10736	**NRPS6**	**Fusarinine**	-0.4	0.6	-2.3	-1.7
FFUJ_10934	**NRPS11**		2.1	-0.4	-0.5	-1
FFUJ_09296	**NRPS22**	**Beauvericin**	-0.1	3.3	0.8	7.2
FFUJ_02115	**NRPS34**	**Fusaric acid**	0	0	-8.7	2
FFUJ_00118	**PKS2**		0.4	0	-2.7	-1.1
FFUJ_03984	**PKS3**	**Fusarubins**	-0.1	4.2	0.1	-0.5
FFUJ_06742	**PKS4**	**Bikaverin**	0.3	-0.6	4.6	1.6
FFUJ_02105	**PKS6**	**Fusaric acid**	0	-0.5	-8.9	1.1
FFUJ_12090	**PKS8**		-3.7	1.8	0	0.1
FFUJ_14695	**PKS9**		-0.1	2.4	-1.4	-2.2
FFUJ_10058	**PKS10**	**Fusarins**	-0.6	7.1	-0.6	2.4
FFUJ_09241	**PKS11**	**Fumonisins**	-4.5	1.1	2.9	3
FFUJ_12020	**PKS13**	**Gibepyrones**	1.1	-4.3	0.8	-1.5
FFUJ_11199	**PKS16**		0.6	-0.4	-1.3	-2.6
FFUJ_00036	**STC1**	**(-) Germacrene**	1	-0.5	6.3	-0.3
FFUJ_04067	**STC3**	**Eremophilene**	-2	-1.8	-0.1	-0.3
FFUJ_12026	**STC7**		0.2	5.6	-0.2	0.6
FFUJ_14336	**DTC1**	**Gibberellins**	-4.4	0.1	-1.8	8.2
FFUJ_11802	**TeTC1**	**Carotenoids**	1.9	0.6	2.5	-0.2

Expression levels are represented by a blue or yellow background if they are ≤−2 or ≥+2, respectively. Shown are the key enzyme-encoding genes that are differentially expressed in the Δ*lae1* and/or OE:*LAE1* mutants compared to the wild type (WT) under low nitrogen (6 mM glutamine; N−) and high nitrogen (60 mM glutamine; N+) conditions. See "Material and methods" section for details on RNA isolation, sequencing, mapping, and quantification

the respective production condition, while elevated product levels were only found for fusarins and fusaric acid in the OE:*LAE1* mutant (Fig. 2c–e). Low expression levels for fusarin genes were also observed under repressing low nitrogen conditions in the OE:*LAE1* mutant. However, no detectable amounts of fusarins were found (Fig. 2c).

In addition, Lae1 was able to activate the silent beauvericin gene cluster. Whereas the WT produced only traces of beauvericin due to H3K27me3-mediated silencing (Studt et al. 2016a; Niehaus et al. 2016b), *bea* gene expression and beauvericin production were highly elevated in the OE:*LAE1* mutant under inducing (120 mM $NaNO_3$) conditions (Fig. 2f).

The SAM domain of Lae1 is essential for its function

To demonstrate whether Lae1 acts as a methyltransferase, similarly to LaeA in *A. nidulans* (Bok et al. 2006b; Patananan et al. 2013), we generated mutants carrying a SAM domain-mutated gene copy in which two conserved glycine residues in motif 1 were exchanged for alanine (Fig. 3a). Linearized plasmids with either the WT *lae1* gene

(p*lae1*^{C}) or the mutated gene (p*lae1*^{SAM}) were transformed into the Δ*lae1* mutant and the transformants were checked by diagnostic PCR (Fig. S1). The WT *lae1* gene copy fully restored the GA gene expression and GA production under inducing conditions. In contrast, the mutated gene copy *lae1*^{SAM} seemed to be non-functional because no GA gene expression and only very low product levels were detectable in the *lae1*^{SAM}-complemented strain (Fig. 3b, c). The fusarubin genes were much lower expressed in the *lae1*^{SAM} mutant, and the production levels were strongly reduced compared to the *lae1*^{C} strain (Fig. S4a, b). To confirm that the impaired function of *lae1*^{SAM} is not due to low gene expression, we analyzed the expressions of the *lae1*^{C} and *lae1*^{SAM}gene copies. Both alleles were similarly and even higher expressed than in the WT (Fig. S4c). These data indicate that the SAM domain is essential for full Lae1 activity in *F. fujikuroi*.

Over-expression of the putative histone acetyltransferase gene *HAT1* restores GA biosynthesis in the Δ*lae1* mutant

Studies on LaeA in *A. nidulans* showed that the histone acetyltransferase EsaA (HAT5) is able to partially complement the defects of the Δ*laeA* mutant (Soukup et al. 2012). To study whether the over-expression of the *EsaA* homolog or another HAT-encoding gene in the Δ*lae1* background is capable of returning the production of GAs, the most prominent SM of *F. fujikuroi*, a set of five different HAT-encoding genes (*HAT1*, *GCN5*, *GCN5-like*, *HAT5*, and *HAT9*) was selected for further studies. *HAT1* and *HAT9* were randomly chosen due to their expression profiles (Table S2). *HAT5* is the homolog of *EsaA* from *A. nidulans* and Gcn5 was recently identified as positive regulator of several SMs in *F. fujikuroi* (Rösler et al. 2016a). The gene *GCN5-like* encodes the closest homolog of Gcn5. The five genes were fused with the strong *oliC* promoter of *A. nidulans* and transformed into the Δ*lae1* background generating the mutants Δ*lae1*/OE:*HAT1*, Δ*lae1*/OE:*GCN5*, Δ*lae1*/OE:*GCN5-like*, Δ*lae1*/OE:*HAT5*, and Δ*lae1*/OE:*HAT9*. The WT, the Δ*lae1* mutant, and the verified transformants (Fig. S5) were analyzed for production of GAs and GA gene expression under optimal low nitrogen conditions (Fig. 4a). The over-expression of *HAT1* (Δ*lae1*/OE:*HAT1*) resulted in 7-fold higher GA formation compared to the Δ*lae1* mutant and about 75% of the WT GA levels (Fig. 4a). Accordingly, the expression of the key enzyme encoding gene *cps*/*ks* (*ent*-copalyl diphosphate synthase/*ent*-kaurene synthase) was elevated compared to the Δ*lae1* mutant (Fig. 4b). The production of fusarubins was partially restored in the Δ*lae1*/OE:*HAT1* mutant while the fusaric acid genes were not affected at all (Fig. S3b–d).

Fig. 2 Secondary metabolite (SM) production and gene expression is affected by deletion and over-expression of *lae1*. HPLC analyses of SM production levels and qRT-PCR expression analyses for gibberellins (GAs) (**a**), bikaverin (BIK) (**b**), fusarins (FUS) (**c**), fusaric acid (FSA) (**d**), fusarubins (FSRs) (**e**), and beauvericin (BEA) (**f**). SM quantitation was done as described in the "Material and methods" section. The production level of the WT was set to 100% for each SM, except for beauvericin. For expression and product analyses, the strains were grown for 3 and 7 days, respectively, in ICI medium with 6 or 60 mM glutamine. For fusarubin and beauvericin analyses, the strains were grown in ICI medium with 6 and 120 mM $NaNO_3$, respectively (alkaline pH conditions). Gene expression levels were measured for the key enzyme-encoding genes: *cps/ks* (GA), *bik1* (bikaverin (BIK)), *fus1* (fusarin (FUS)), *fub1* (fusaric acid (FSA)) *fsr1* (fusarubins (FSRs)), and *bea1* (beauvericin (BEA)). For the quantitation of expression levels, three different housekeeping genes were used. The amount of the WT was arbitrarily set as 1. Mean value and standard deviation are from a technical repeat; the experiment was done in three biological replicates. *n.d.* not detectable

Characterization of the histone acetyltransferase HAT1

Due to the unexpected restoration of GA biosynthesis in the Δ*lae1* background by over-expression of *HAT1*, we characterized this gene in more detail.

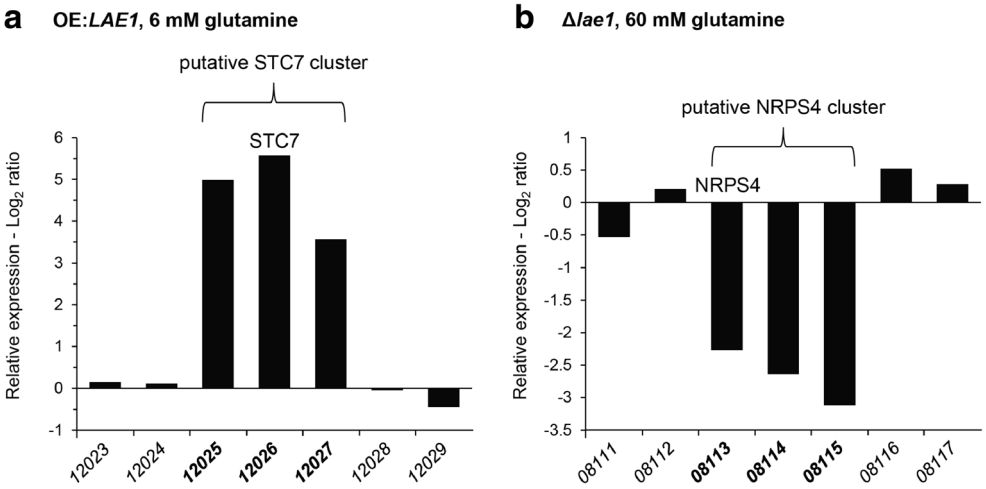
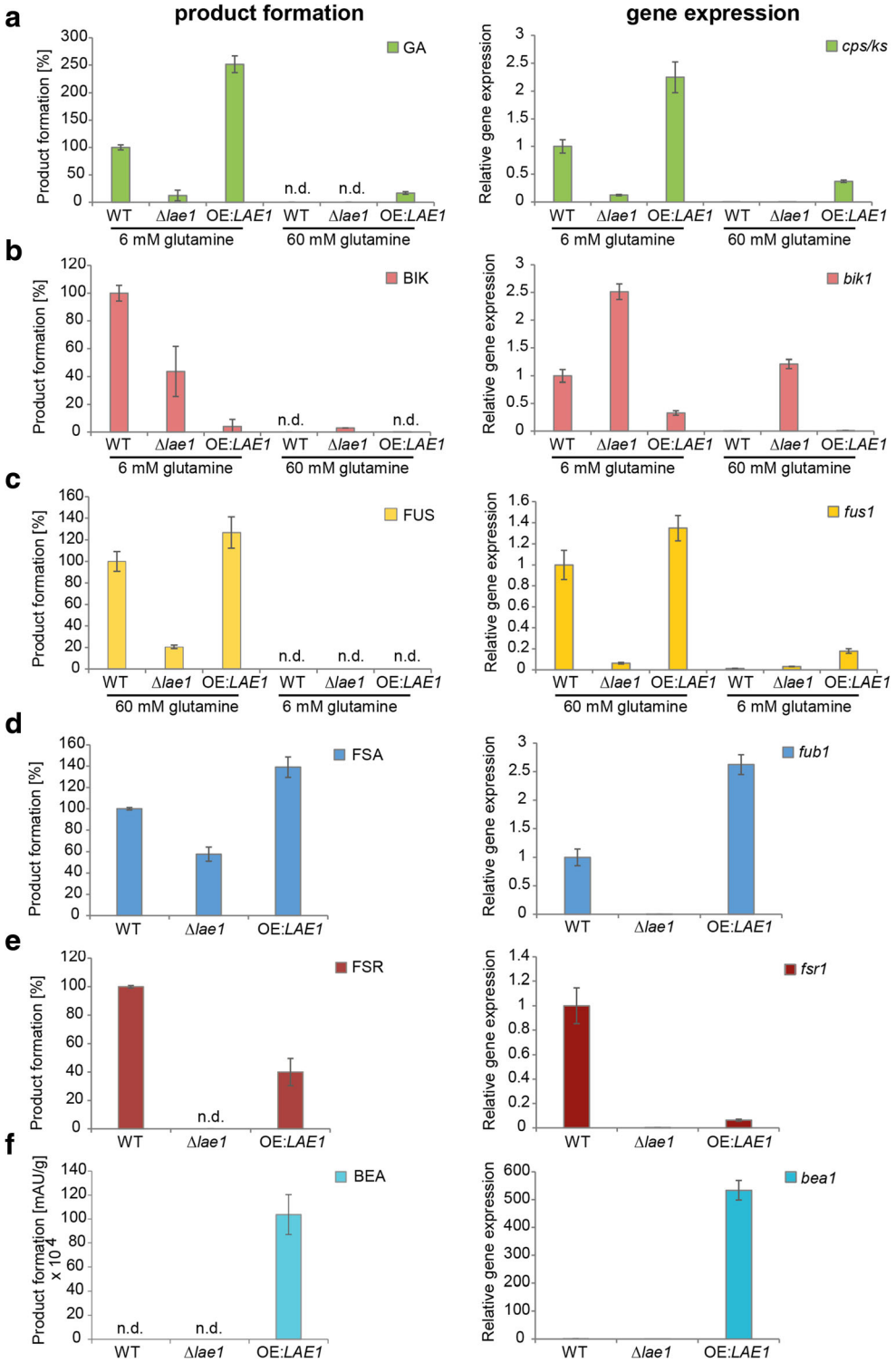


Fig. 1 Genome mining is able to identify potential secondary metabolite gene cluster of the Lae1 regulon, the putative STC7 (**a**) and NRPS4 (**b**) gene clusters. Shown are the expression ratios OE:*LAE1* to the WT (STC7) under low nitrogen and Δ*lae1* to the WT (NRPS4) under high nitrogen conditions. The expression is shown for the key enzyme-encoding *STC7* (*FFUJ_12026*) and *NRPS4* (*FFUJ_08113*) and adjacent genes


a
product formation
gene expression
GA
cps/ks
Product formation [%]
Relative gene expression
WT
Δlae1
OE:LAE1
6 mM glutamine
60 mM glutamine
n.d.
b
BIK
bik1
c
FUS
fus1
d
FSA
fub1
e
FSR
fsr1
f
BEA
bea1
Product formation [mAU/g] x 10 4

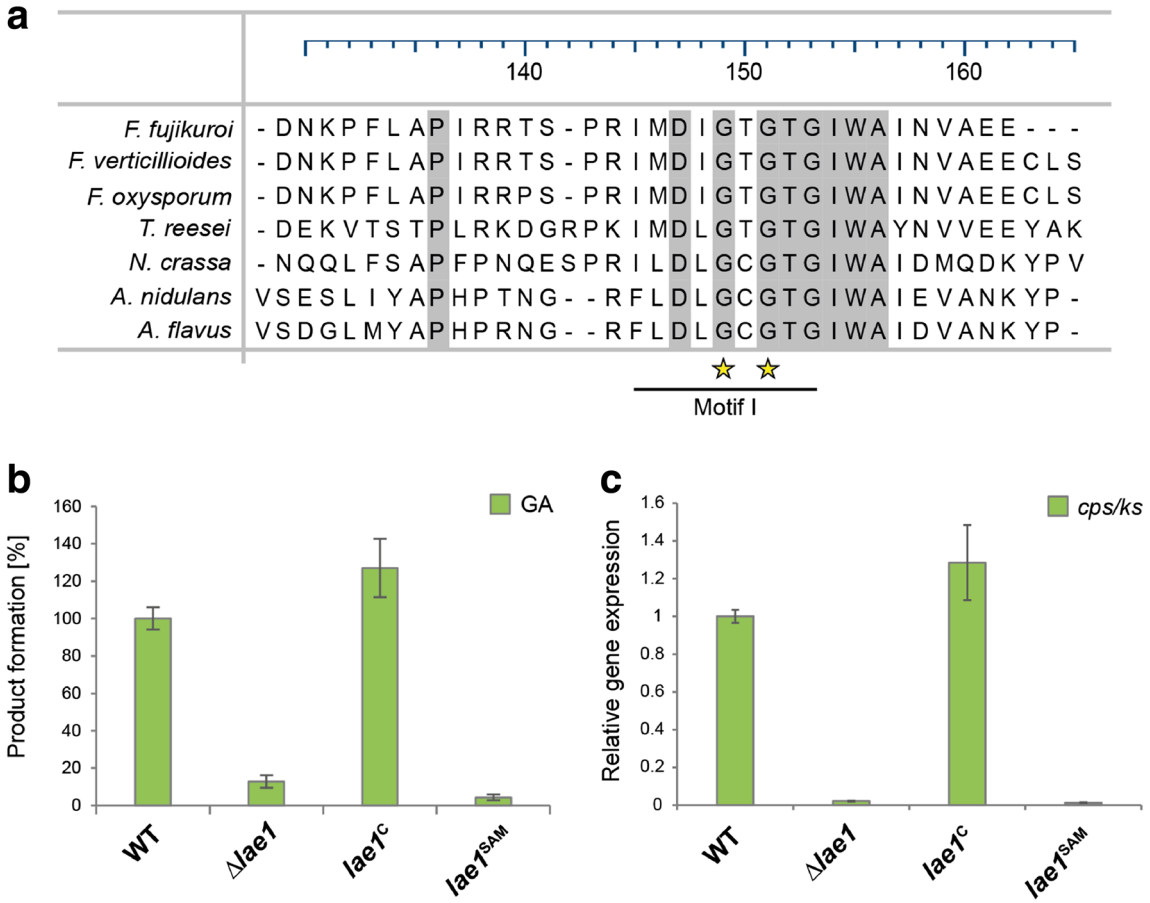


Fig. 3 The *S*-adenosyl L-methionine (SAM) domain of Lae1 is essential for its full activity. **a** Multiple sequence alignment of a part of the Lae1 SAM-binding domain from *F. fujikuroi* (*FFUJ_00592*) with characterized LaeA proteins from *F. verticillioides* (AHC70606.1), *Fusarium oxysporum* (A0A0J9UBD6), *T. reesei* (AFX86442.1), *Neurospora crassa* (ESA44297), *A. nidulans* (Q6TLK5), and *A. flavus* (AAX68412). The SAM-binding domain can be separated into four motifs: motif I, post-motif I, motif II, and motif III; only motif I is shown. The yellow stars marked the glycines that were replaced for alanine. **b** HPLC analysis of 7-day-old supernatants of ICI cultures supplemented with 6 mM glutamine. The production of GAs in the WT was arbitrarily set to 100%. The cultivation was done in triplicate. **c** qRT-PCR expression analysis of *cps/ks*, the key enzyme-encoding gene of GA biosynthesis, after growth for 3 days in ICI medium with 6 mM glutamine

HATs can be divided into two groups: type A HATs contain a bromodomain such as Gcn5 (Rösler et al. 2016a), while HATs of the type B group to which HAT1 belongs lack this domain.

The *HAT1* gene (*FFUJ_03208*) is 1440 bp long and encodes 480 amino acids. A multiple protein sequence alignment of different HAT1 proteins showed no significant overall similarity between them. However, there are highly conserved amino acid residues from human to *S. cerevisiae* in the characteristic functional domain of the HAT1 superfamily at the N-terminus (Neuwald and Landsman 1997) (Fig. 5a). The *F. fujikuroi* HAT1 has its highest similarity to the recently described HAT1 proteins of the filamentous fungi *Pestalotiopsis microspora* and *Metarhizium robertsii* (Zhang et al. 2016; Fan et al. 2017).

To demonstrate whether HAT1 is predominantly localized in the cytoplasm or the nucleus, an OE:*HAT1*:*GFP* fusion construct was generated and transformed into the WT strain. Microscopic evaluation clearly showed the nuclear localization of HAT1 (Fig. 5b). To further study the role of HAT1 in growth, stress response, development, and secondary metabolism in *F. fujikuroi*, *HAT1* deletion and over-expression mutants were generated (Fig. S6). None of the mutants had an effect on fungal growth, neither under normal nor under stress conditions (addition of H_2O_2 or NaCl) (Fig. 5c).

Next, the *HAT1* deletion and over-expression mutants were analyzed for their potential impact on GA formation. The Δ*HAT1* mutant produced fewer GAs and showed a reduced GA gene expression. In contrast, the OE:*HAT1* mutant produced more GAs compared to the WT similar to the OE:*LAE1* mutant (Fig. 6a). Consistently, the expression level of *cps/ks* was elevated (Fig. 6b). In contrast, the fusaric acid production and gene expression levels were elevated in the Δ*HAT1* mutant and slightly decreased in the over-expression mutant (Fig. 6c, d).

In summary, this study provides evidence that Lae1 controls large sets of genes mainly involved in secondary

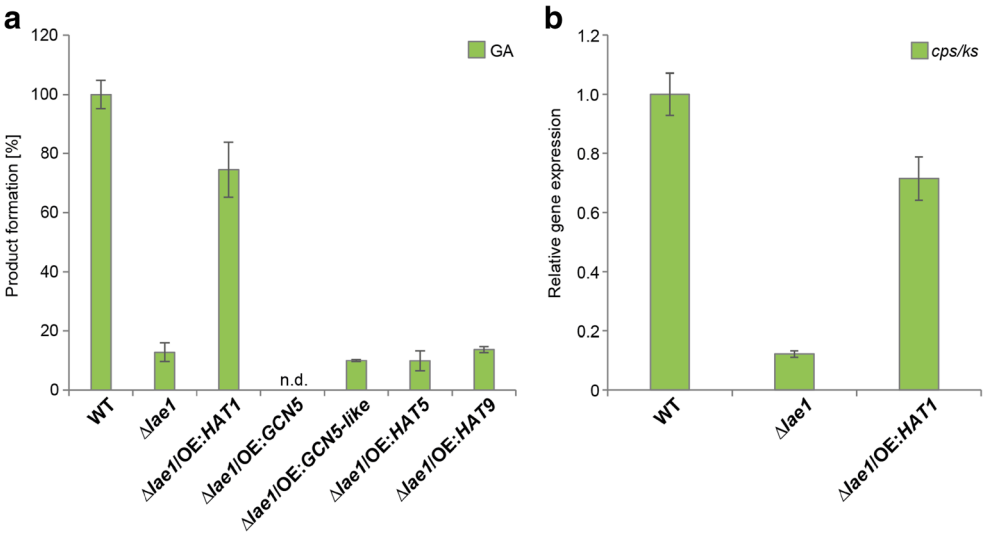


Fig. 4 Over-expression of *HAT1* in the Δ*lae1* background restores gibberellin (GA) production and GA gene expression. **a** HPLC analysis of 7-day-old extracted supernatants of ICI cultures supplemented with 6 mM glutamine. The production of GAs in the WT was arbitrarily set to 100%. The cultivation was done in triplicate. **b** qRT-PCR expression analysis of *cps*/*ks*, the key enzyme-encoding gene of GA biosynthesis, after growth for 3 days in ICI medium with 6 mM glutamine. *n.d.* not detectable

metabolism, transport, and cell defense. We focused on its impact on the regulation of SM biosynthesis. Lae1 acts as an activator for the majority of SMs (GA, fusarins, fusaric acid, fusarubin, and the otherwise silent beauvericin genes), but as repressor for bikaverin, gibepyrone, and carotenoid biosynthesis. The over-expression of *lae1* is able to partially circumvent the nitrogen repression of GA and pH regulation of bikaverin biosynthesis. Over-expression of five HAT-encoding genes in the Δ*lae1* background identified one of them (HAT1) as a suppressor of the Δ*lae1* mutant, which was able to partially restore the GA and fusarubin production in the Δ*lae1* mutant. Deletion of *HAT1* led to reduced GA and elevated fusaric acid gene expression and product formation, while over-expression of *HAT1* had the contrary effect.

Discussion

In this work, we extended our previous studies on the role of Lae1 and analyzed Lae1-mediated alterations in genome-wide gene expression by use of microarrays. Our detailed functional analyses using *lae1* deletion and over-expression strains clearly demonstrated that Lae1 has a major impact on secondary metabolism in *F. fujikuroi*. These data are comparable to those from other fungi, where deletion of the *laeA*/*lae1* gene caused a drastic decrease and the over-expression an increase in SM gene expression and subsequently, in production levels (Kim et al. 2013; Lee et al. 2013; Hong et al. 2015). Due to the global impact of LaeA and its homologs on secondary metabolism and other biotechnologically important processes, e.g., the production of extracellular enzymes, LaeA became a major focus for strain improvement of biotechnologically important fungi (Karimi-Aghcheh et al. 2013; Bok and Keller 2016). For example, it was shown that LaeA regulates the production of penicillin in *P. chrysogenum* (Kosalková et al. 2009; Hoff et al. 2010). Furthermore, LaeA controls the expression of *mlcR*, encoding the pathway-specific TF of the compactin (ML-236B) gene cluster in *Penicillium citrinum* (Baba et al. 2012; Zheng et al. 2014). Over-expression of *laeA* orthologs also resulted in higher production of aflatoxin in *Aspergillus flavus* (Kale et al. 2008), trichothecenes in *F. graminearum* (Kim et al. 2013), T-toxin in *C. heterostrophus* (Wu et al. 2012), and monacolin K and pigments in *Monascus pilosus* (Lee et al. 2013). Over-expression of *laeA* was also successfully used to activate otherwise silent gene clusters, which led to the identification of their yet unknown products, e.g., the antitumor compound terrequinone A from *A. nidulans* and chaetoglobosin Z in *Chaetomium globosum*, respectively (Bok et al. 2006a; Jiang et al. 2016). LaeA-mediated regulation of SM gene expression can also be used to predict beginnings and ends of SM gene clusters due to the sharp demarcation of transcriptional control by LaeA (Bouhired et al. 2007). In the present work, we predicted the potential borders of the yet unknown STC7 and NRPS4 gene clusters based on the Lae1-dependent co-regulation of adjacent genes (Fig. 1).

Comparison of the expression profiles of both the Δ*lae1* and OE:*LAE1* mutants with those of the WT revealed an enrichment especially of genes of the functional categories secondary metabolism, "transport," and "gene regulation" (Table S3). Similarly, a RNA-seq analysis comparing the expression profile of the Δ*fglaeA* mutant with that of the WT strain revealed an enrichment of differentially regulated genes belonging to the categories "metabolism" and gene regulation. Among them were genes lying in 56 putative gene clusters, e.g., those for trichothecene, zearalenone, and culmorin biosynthesis (Kim et al. 2013).

In *F. fujikuroi*, 22 of the 47 predicted SM gene clusters were affected in one or both mutants. Most of these clusters with

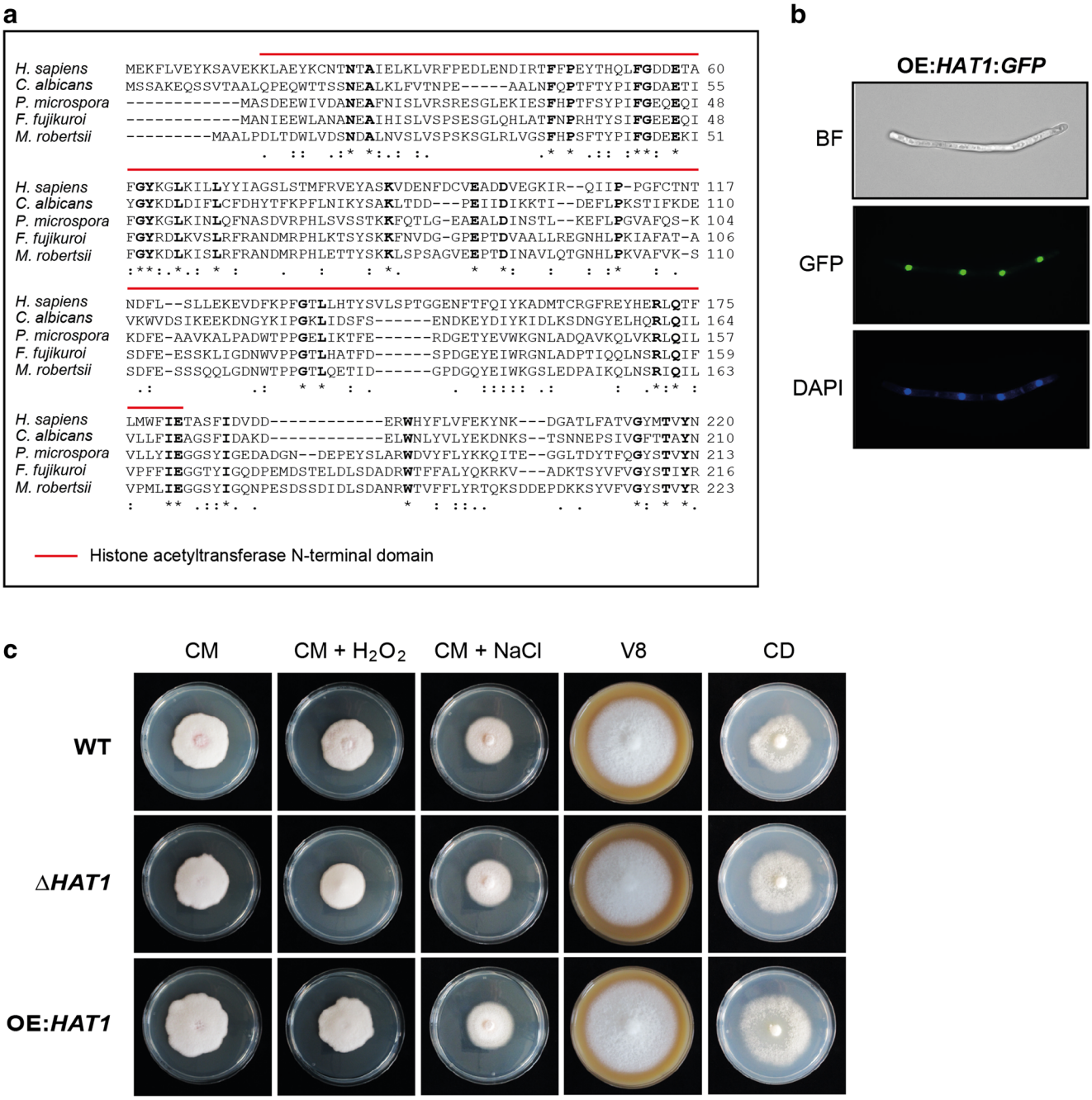


Fig. 5 Functional characterization of HAT1 *in F. fujikuroi.* **a** Multiple sequence alignment of HAT1 proteins from *M. robertsii* (XP_007822160.1), *H. sapiens* (NP_003633.1), *F. fujikuroi* (FFUJ_03208), *C. albicans* (XP_713589.1), and *P. microspora* (ANS59908.1) was performed using Clustal Omega (EMBL-EBI). Asterisks and dots indicate the identical and similar amino acids, respectively. **b** The subcellular localization of the HAT1:GFP fusion protein was determined after 24 h of growth in liquid ICI medium supplemented with 6 mM glutamine. Nuclei were visualized using HOECHST 33342 staining (DAPI). *BF* bright field. **c** Plate assay with the WT, Δ*HAT1* and OE:*HAT1* mutants on CM, CM supplemented with 40 mM H_2O_2 or 1 M NaCl, V8 and on CD media. The strains were grown for 6 days

known products were downregulated in the Δ*lae1* and upregulated in the OE:*LAE1* mutant under one of the two nitrogen conditions, in particular the genes for fusarubin (PKS3), fusarin (PKS10), fumonisin (PKS11), and GA (DTC1) biosynthesis (Table 2). Also, some orphan clusters with unknown products were upregulated in the OE:*LAE1* mutant, e.g., the putative STC7 and PKS9 clusters. However, the (−)-germacrene D (STC1), bikaverin (PKS4), and the carotenoid (TeTC1) clusters were upregulated in the Δ*lae1* mutant under high nitrogen conditions, and the gibepyrone (PKS13) cluster

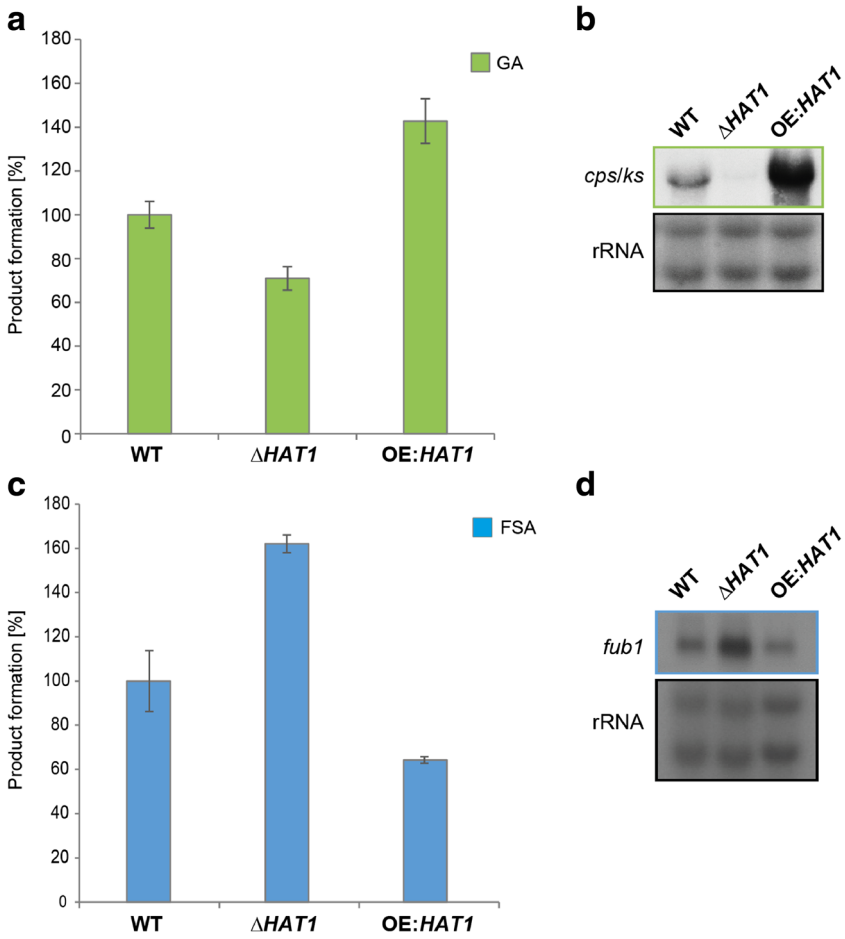


Fig. 6 *HAT1* deletion and overexpression in the WT affects gibberellin (GA) biosynthesis. **a** HPLC analysis of GA production levels was performed after 7 days of growth in ICI medium supplemented with 6 mM glutamine. The production in the WT was arbitrarily set to 100%, and the cultivation was done in triplicate. **b** Northern blot analyses of cultures grown for 3 days in ICI medium with 6 mM glutamine. The *cps/ks* gene was used as a probe. **c** HPLC analysis of fusaric acid (FSA) production levels after 7 days of growth in ICI with 60 mM glutamine. **d** Northern blot analysis of the FSA key gene *fub1* in 3-day-old cultures

was downregulated in the OE:*LAE1* mutant indicating that Lae1 is not always a positive regulator of secondary metabolism.

Interestingly, some SM biosynthetic genes were upregulated in the OE:*LAE1* mutant under otherwise repressing conditions. Examples are the fumonisin and GA genes under high nitrogen, and fusarin C genes under low nitrogen conditions. Furthermore, the fusarubin genes were significantly upregulated in the OE:*LAE1* mutant under otherwise repressing acidic pH conditions (Studt et al. 2012). A de-regulation of the PacC-mediated repression of the bikaverin genes has been previously observed in the Δ*vel1* mutant. Vel1 was shown to act as strong repressor of bikaverin gene expression, and its deletion led to an activation of gene expression under repressing alkaline conditions (Wiemann et al. 2010). These data indicate that some strong regulatory mechanisms, such as nitrogen and pH regulation, could be at least partially overcome by over-expression of *lae1*. However, the strong upregulation of gene expression did not always correlate with similarly elevated production levels (Fig. 2). The discrepancy between expression and product formation for some SM cluster genes might be due to the changes of expression levels over time. For instance, both the bikaverin and fusarubin biosynthetic genes are only temporally expressed in a short time frame (Wiemann et al. 2009; Studt et al. 2012).

The most striking result was the activation of the almost silent beauvericin gene cluster by over-expressing *lae1*: three of the four cluster genes (*bea1–bea3*) were highly expressed in the OE:*LAE1* mutant in accordance with high beauvericin production levels (Fig. 2f). Recently, we have shown that these genes are also strongly upregulated by deletion of *hda1* encoding one of the four histone deacetylases, and by knockdown of *kmt6* encoding the histone methyltransferase Kmt6 which is responsible for trimethylation of H3K27 (Niehaus et al. 2016b; Studt et al. 2016a). These data support the hypothesis made for LaeA in *A. nidulans* (Reyes-Dominguez et al. 2010; Bayram and Braus 2012) that Lae1 is involved in histone modifications. First of all, LaeA and its homologs are localized to the nucleus and contain a SAM-binding site, and its mutation led to inactivation of LaeA in *A. nidulans* (Bok et al. 2006b) and Lae1 in *F. fujikuroi* (this work). Furthermore, loss of LaeA led to increased accumulation of the heterochromatin mark H3K9me3 at the sterigmatocystin gene cluster, indicating that LaeA counteracts H3K9 trimethylation and the formation of repressive chromatin (Reyes-Dominguez et al. 2010). In *T. reesei*,

Lae1-mediated gene regulation correlates with changes in the H3K4me3 mark shown by chromatin immunoprecipitation (ChIP)-seq analyses (Karimi-Aghcheh et al. 2013). Furthermore, deletion of several histone-modifying genes in fungi, e.g., the fungus-specific sirtuin-type histone deacetylase HstD, was shown to coordinate fungal development and secondary metabolism via the regulation of *laeA* expression in filamentous fungi (Kawauchi et al. 2013).

F. fujikuroi is best known as a source for the biotechnological production of GAs which are used as plant growth regulators in agriculture (Bömke and Tudzynski 2009).The loss of global regulators, such as Lae1, Vel1, and Vel2 (Wiemann et al. 2010), the GATA TFs AreA and AreB (Michielse et al. 2014), the global TFs Sge1 (Michielse et al. 2015), the component of the H3K4-methylating COMPASS complex Ccl1 (Studt et al. 2017), the histone deacetylases Hda1 and Hda2 (Studt et al. 2013), and the HAT Gcn5 (Rösler et al. 2016a), resulted in abolished or strong reduction of both GA gene expression and GA production levels. However, over-expression of none of these regulators except for *lae1* led to elevated GA biosynthesis. In this work, we showed that the expression of GA biosynthetic genes (shown for *cps/ks*) is significantly increased in the OE:*LAE1* mutant under both inducing (6 mM glutamine) and repressing high nitrogen conditions in accordance with elevated production levels (Table 2 and Fig. 2a). These data show that Lae1 is able to circumvent the strong AreA- and AreB-mediated nitrogen repression of GA biosynthesis.

Recently, a multicopy suppressor screen for genes capable of restoration of SM production in the Δ*laeA* mutant in *A. nidulans* identified the HAT Esa1 which is able to activate several SM gene clusters through H4K12 acetylation (Soukup et al. 2012). A strong impact of HATs on secondary metabolism was also shown for *A. parasiticus*: the initiation of histone H4 acetylation at the aflatoxin promoters correlates with the accumulation of aflatoxin (Roze et al. 2007). For *F. fujikuroi*, we have shown that Gcn5 is an essential HAT which is responsible for H3K4, H3K9, H3K18, and H3K27 acetylation (Rösler et al. 2016a). Previously, we revealed by ChIP-seq that H3K9 acetylation is enriched at the GA cluster under inducing low nitrogen conditions but reduced at high nitrogen (Wiemann et al. 2013).

To establish whether Gcn5 or another HAT is able to restore the lack of GA and fusarubin gene expression in the Δ*lae1* mutant, we over-expressed five HAT-encoding genes in the Δ*lae1* background. In contrast to *A. nidulans*, the EsaA homolog HAT5 did not restore the capability to produce GAs or fusarubins. However, HAT1 was able to restore the GA production and to counteract the Δ*lae1* phenotype. The OE:*HAT1* mutant was also able to partially restore the fusarubin formation, though not in the same extent as seen for GAs.

The yeast HAT1 homolog was originally purified from cytoplasmic extracts in *S. cerevisiae* and shown to be responsible for the acetylation on lysines 5 and 12 of newly synthesized histone H4. More recent studies indicated that HAT1 in *S. cerevisiae* is predominantly a nuclear enzyme which may be directly involved in the chromatin assembly process (Ai and Parthun 2004; Parthun 2012). A predominant nuclear localization has been also shown for HAT1 in *F. fujikuroi*, suggesting that this acetyltransferase might be involved in chromatin remodeling.

Only recently, the first HAT1 homologs have been characterized in filamentous fungi (Zhang et al. 2016; Fan et al. 2017). In *P. microspora*, deletion of *HAT1* resulted in a delay of conidia production and reduced formation of the SM pestalotiollide B (Zhang et al. 2016). In contrast, unexpected activations of orphan SM genes have been found upon the disruption of *HAT1* in *M. robertsii* resulting in the characterization of 11 new natural products, including eight isocoumarin derivatives and two nonribosomal peptides (Fan et al. 2017).

In *F. fujikuroi*, deletion or over-expression of the *F. fujikuroi HAT1* gene did not affect production of the conidia (data not shown). However, deletion and over-expression of this gene resulted in significantly reduced and elevated GA gene expression, respectively, as it was observed for *lae1* deletion and over-expression. These data suggest that HAT1 in *F. fujikuroi* probably acts in similar pathways as Lae1. Besides the GAs, HAT1 affected also the fusaric acid gene cluster. However, in contrast to the GA genes, *fub* gene expression and fusaric acid formation were elevated in the Δ*HAT1* mutant and downregulated in the OE:*HAT1* mutant. Therefore, both the Δ*HAT1* and OE:*HAT1* mutants are likely to be valuable tools in natural product studies, for example, in activating yet unknown SM gene clusters.

In conclusion, we showed that in *F. fujikuroi*, the global regulator Lae1 is mainly involved in the regulation of SM gene clusters, but also affects expression of genes encoding TFs, histone modifiers, transporters, and proteins of several other functional categories. While Lae1 acts as a positive regulator for the majority of the Lae1-regulated SM gene clusters, e.g., those for GAs, fusaric acid, fusarubins, fusarin, beauvericin, and fumonisin biosynthesis, some others are negatively regulated by Lae1, e.g., the genes for biosynthesis of bikaverin, carotenoids, gibepyrone, and (−)-germacrene D, the product of STC1. Over-expression of *HAT1* in the Δ*lae1* mutant resulted in partial restoration of GA and fusarubin biosynthesis. Deletion and over-expression of *HAT1* in the WT led to decreased and increased expression of GA genes, respectively, suggesting that this enzyme acts in the same regulatory network as Lae1 in *F. fujikuroi.*

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Compliance with ethical standard

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

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