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Sirtuin A regulates secondary metabolite production by *Aspergillus nidulans*

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Late-stage cultures of filamentous fungi under nutrient starvation produce valuable secondary metabolites such as pharmaceuticals and pigments, as well as deleterious mycotoxins, all of which have remarkable structural diversity and wide-spectrum bioactivity. The fungal mechanisms regulating the synthesis of many of these compounds are not fully understood, but sirtuin A (SirA) is a key factor that initiates production of the secondary metabolites, sterigmatocystin and penicillin G, by *Aspergillus nidulans*. Sirtuin is a ubiquitous NAD^+-dependent histone deacetylase that converts euchromatin to heterochromatin and silences gene expression. In this study, we have investigated the transcriptome of a *sirA* gene disruptant (SirAΔ), and found that SirA concomitantly repressed the expression of gene clusters for synthesizing secondary metabolites and activated that of others. Extracts of SirAΔ cultures grown on solid agar and analyzed by HPLC indicated that SirA represses the production of austinol, dehydroaustinol and sterigmatocystin. These results indicated that SirA is a transcriptional regulator of fungal secondary metabolism.

Key Words: *Aspergillus nidulans*; austinol; biosynthetic products; gene cluster; histone deacetylase; stationary growth phase; sterigmatocystin; transcriptional regulator

Introduction

Filamentous fungi produce various secondary metabolites (SM) including useful compounds such as pharmaceuticals and antibiotics, and toxic mycotoxins such as aflatoxin and trichothecenes (Yu and Keller, 2005). The biosynthetic genes in fungal genomes are often organized in clusters, and nucleotide sequencing of fungal genomes has predicted vast numbers of such clusters, the biosynthetic products of which remain mostly unidentified (Khaldi et al., 2010). Fungal mechanisms have been investigated with the intention to increase the production efficiency of SM for the pharmaceutical industry, prevent fungal toxin production, and understand human and plant pathogenesis. The ascomycetes genus *Aspergillus* encodes many biosynthetic gene clusters for SM synthesis (Inglis et al., 2013). The model ascomycete *Aspergillus nidulans* produces several SM (Nielsen et al., 2011) that include the antibiotic penicillin G (PN) (MacCabe et al., 1990) and the carcinogen sterigmatocystin (ST), which is related to the agricultural contaminant aflatoxin (Brown et al., 1996). Their production is regulated by histone acetylation and methylation (Gacek and Strauss, 2012).

Sirtuin is a ubiquitous NAD^+-dependent histone deacetylase that deacetylates acetyllysine residues of histones, and leads to heterochromatin formation that silences gene expression (Dang et al., 2009; Moazed, 2001). Sirtuin also deacetylates non-histone substrates and is involved in various cellular processes, such as aging, disease, cancer development, stress responses, and genome integrity (Michan and Sinclair, 2007). Sirtuin-dependent mechanisms are emerging that regulate various physiological events in filamentous fungi. *A. nidulans* HstA is a predicted NAD^+-dependent histone deacetylase which represses the expression of genes involved in the production of PN and norsolorinic acid (Shwab et al., 2007). We previously found that *A. nidulans* sirtuin A (SirA), a homolog of the *Saccharomyces cerevisiae* Sir2p, deacetylates the acetylated 16th lysine residue on histone H4 in the promoter regions of genes that synthesize PN and ST (Shimizu et al., 2012). *Aspergillus oryzae* AoHstD is a counterpart of *S. cerevisiae* NAD^+-dependent histone H3K56 deacetylase Hst4, and is involved in conidia formation, kojic acid production (Kawauchi et al., 2013) and stress tolerance (Kawauchi and Iwashita, 2014). *Magnaporthe oryzae* MoSir2 deacetylates non-histone

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substrates to regulate plant pathogenicity (Fernandez et al., 2014).

Since heterochromatin prevails over all genomes, and sirtuins seem to regulate global transcription, little is known about the role of filamentous fungal sirtuins in global transcriptional regulation. The present study analyzes the transcriptome of the *sirA* gene disruptant of *A. nidulans* and investigates the role of SirA in global metabolic regulation. The results indicated that SirA both up- and down-regulates the expression of various SM gene clusters during the late stage of liquid culture. Analysis of fungal culture extracts using HPLC indicated that SirA represses the production of ST, austinol (AUS), and dehydroaustinol (DAUS) in a solid medium. These findings indicate that SirA is a global repressor of SM metabolic genes that function during the stationary growth phase of *A. nidulans*.

Materials and Methods

Strains, cultures, and media. *Aspergillus nidulans* A26 (*biA1*) (Fungal Genetic Stock Center, University of Missouri, Columbia, MO, USA) conidia (2×10^8) were shaken at 120 rpm in 500-mL flasks containing 200 mL of liquid GMM medium (Takasaki et al., 2004) at 30°C. Solid GMM medium containing 1.5% agar and overlaid with a cellophane membrane was inoculated with 1×10^6 of *A. nidulans* conidia and incubated at 37°C. Mycelia that grew and extended over the membrane were harvested for quantitative PCR. Secondary metabolites were determined from *A. nidulans* cultured in plates containing agar without a cellophane membrane. Biotin (0.25 mg L^{-1}), was added to the culture medium for auxotrophic mutants.

DNA microarray analysis. *Aspergillus nidulans* strains A26 and SirAΔ (*biA1*, *argB2*, Δ*sirA*::*argB*) (Shimizu et al., 2012) were cultured in 200 mL of GMM at 30°C for 24, 48 and 72 h, and then the total RNA was purified using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Transcriptomes were analyzed using a custom-synthesized *A. nidulans* GeneChip (Masuo et al., 2010). The signal $\log_2$ ratio between signals obtained for the A26 and SirAΔ cultures at each time point was normalized, processed as described (Masuo et al., 2010), and published in the data repository Gene Expression Omnibus (GEO) under accession number GSE85319. Genes that were ≥2-fold up- or down-regulated at one or more time points were grouped into three sets using *k*-means clustering analyses (Soukas et al., 2000). The number of sets was determined using principal component analysis (van den Berg et al., 2006). Gene ontology (GO) was analyzed using a tool published in AspGD (http://www.aspgd.org/).

Determination of secondary metabolites. *Aspergillus nidulans* strains A26 and SirAΔ were cultured in 200 mL of liquid GMM medium at 30°C and 120 rpm, or in plates containing GMM agar at 37°C for four and five days. Fractions were collected from portions of liquid culture (20 mL) and agar (30 cm^2) that were agitated for 12 h with 20 mL of ethyl acetate. After drying *in vacuo* and dissolution in 500 μL of methanol, the ethyl acetate fractions were analysed by high-performance liquid chromatography (HPLC) using a 1200 Infinity series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a 250 × 4.6-mm Purospher® Star RP-18 end-capped column (particle size = 5 μm, Millipore, Billerica, MA, USA). The solvent gradient comprised acetonitrile (solvent B) in 0.05% ammonium formate/water (pH 4.0) (solvent A): 40% B from 0 to 5 min, 40% to 80% B from 5 to 10 min, hold at 80% B from 10 to 15 min, 80% to 100% B from 15 to 25 min, hold at 100% B from 25 to 27 min, 100 to 60% B from 27 to 29 min, and hold at 60% B from 29 to 35 min. The flow rate was 0.8 mL min^{-1} and absorption at 210 nm was monitored. The temperature of the column oven was 40°C. Mass spectra were acquired using an LCMS-8030 instrument (Shimadzu, Kyoto, Japan) under the following conditions: capillary voltage, 4.5 kV; detection range, *m* /*z* 50–600; desolvation line, 250°C; heat block, 400°C; nebulizer nitrogen gas 3 L/min; drying gas, 15 L/min. Ion source polarity was set in the positive or negative mode. The conditions for chromatography were as described above for HPLC.

Table 1. Primers included in quantitative PCR analyses.

Primer	Nucleotide sequence	Target gene
ausA RT F	5′-ACAGACCGAGAGCCAAAGAG-3′	*ausA*
ausA RT R	5′-GTGCGTGTGGTAGCAAAGAG-3′	
aflR RT F	5′-CTGCCTTGCGAGTATATGGTTTC-3′	*aflR*
aflR RT R	5′-TTGGTGATGGTGCTGTCTTG-3′	
stcJ RT F	5′-AGCCAATACCGGACACG-3′	*stcJ*
stcJ RT R	5′-TGTAGTGTGGACTTGCCGC-3′	
stcU RT F	5′-CATTTCCATTCAAGCCGATGT-3′	*stcU*
stcU RT R	5′-CCAGGTATCCGAAGTGCTCAA-3′	
actA RT F	5′-GAAGTCCTACGAACTGCCTGATG-3′	*actA*
actA RT R	5′-AAGAACGCTGGGCTGGAA-3′	

Table 2. Numbers of genes up- or down-regulated for ≥2-fold in SirAΔ.

	24 h	48 h	72 h
Up	533	1,429	266
Down	544	1,262	521

Quantitative PCR. The A26 and SirAΔ strains were cultured on liquid or solid GMM as described above for 48 or 24 h, and then the total RNA was extracted as described above. Single-strand cDNA was obtained from reverse transcription reactions using PrimeScript™ Reverse Transcriptase (Takara, Kyoto, Japan) and then quantitative real-time PCR proceeded as described (Shimizu et al., 2009). Table 1 shows the gene-specific primers. The amounts of transcripts were normalized against those of the actin gene (*actA*) and are shown as relative values.

Results and Discussion

Global gene expression control by SirA

To understand the role of SirA in the fungal regulation of global transcription, we cultured the *sirA* gene disruptant SirAΔ and A26 strain in a GMM liquid medium for 24, 48, and 72 h, prepared the total RNA, and then investigated time-dependent changes in their transcriptomes us-

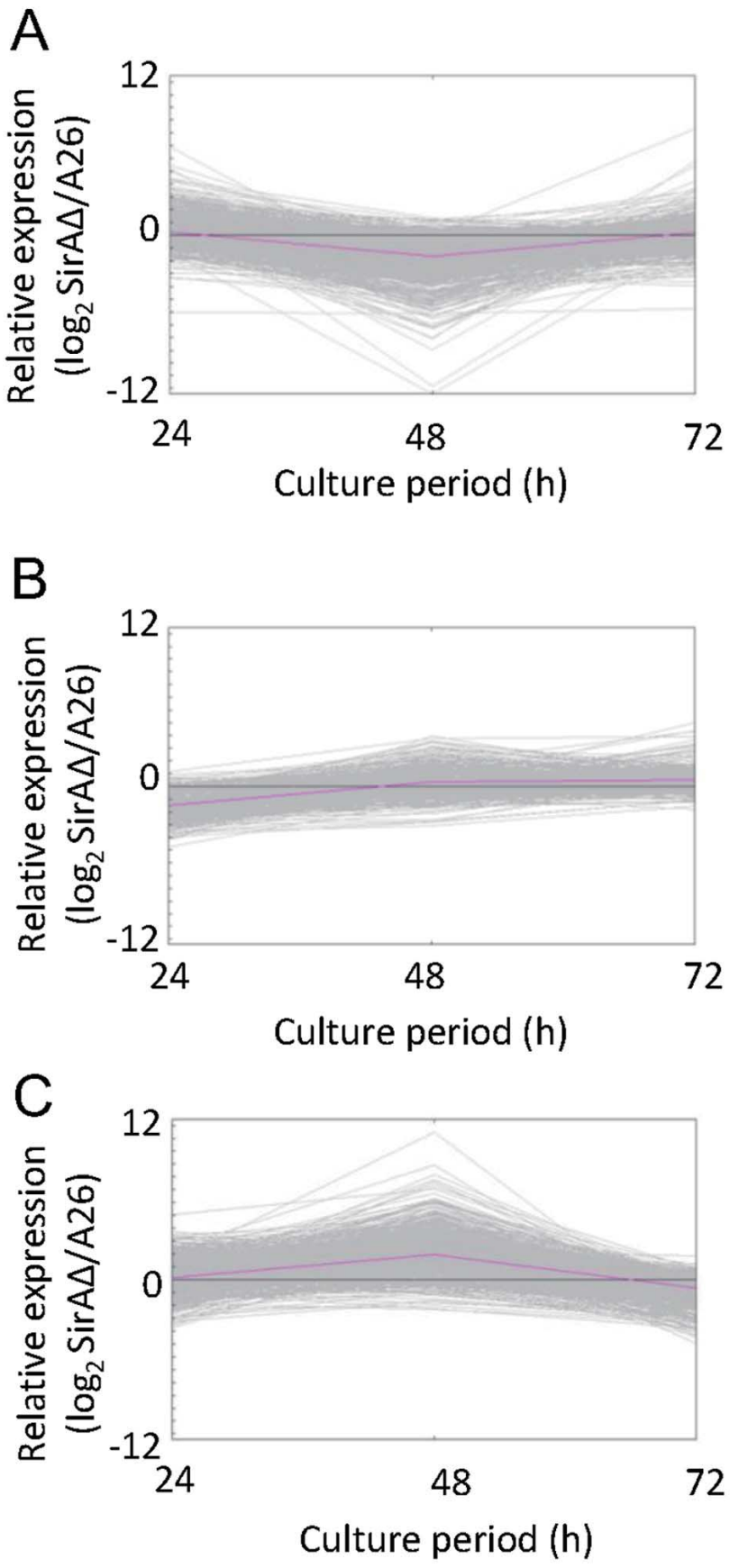


Fig. 1. Sets of differentially expressed genes.

Three sets of genes were grouped using *k*-means clustering. A–C, expression profiles of genes in Sets 1 to 3. Signal log ratios ($\log_2$ SirAΔ/A26) are shown by the *y* axis, and cultivation periods are shown by the *x* axis.

Table 3. Gene ontology terms enriched among regulated genes in SirAΔ.

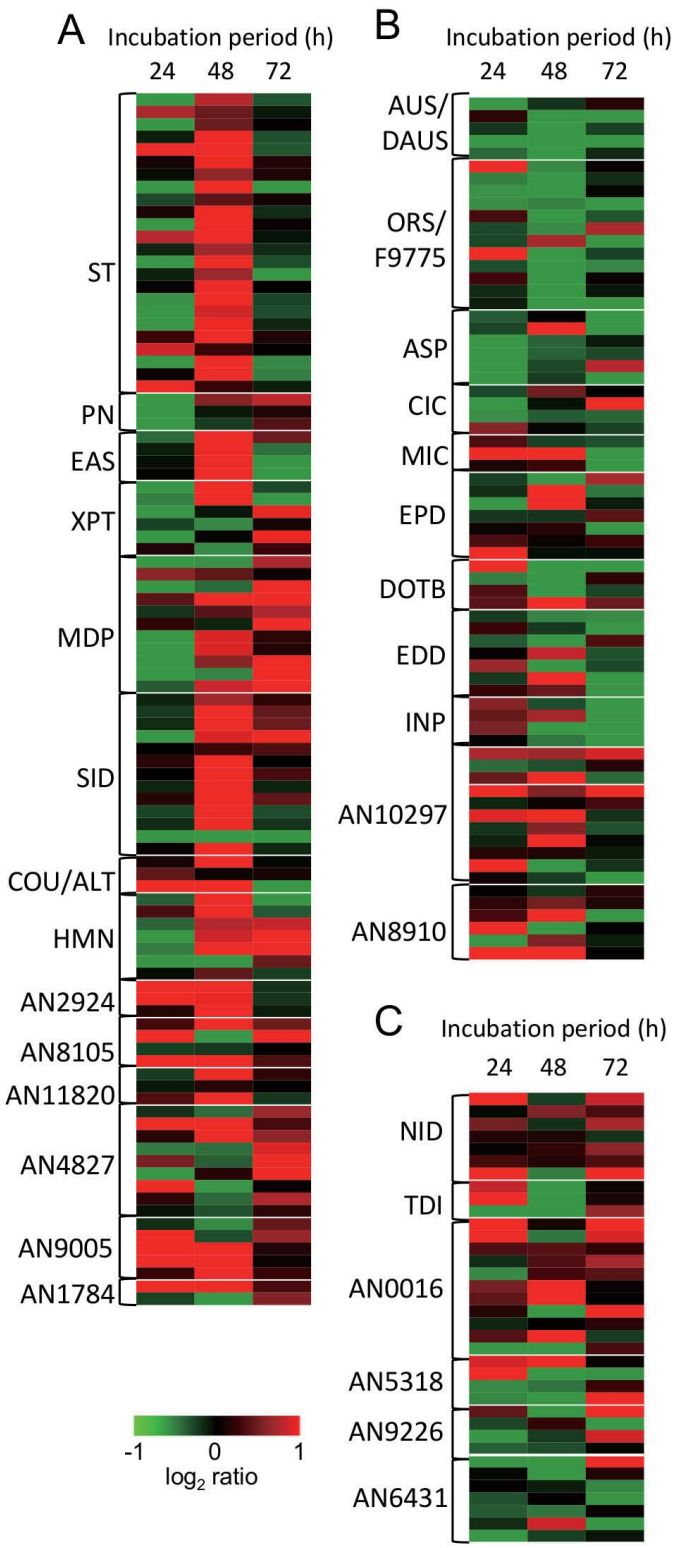
GO ID	GO term	Cluster frequency (%)*	Gene number /Total gene number**	*P* value
Set 1 (1434 genes)				
Biological process				
44710	Single-organism metabolic process	24.4	334/1983	2.17E-07
44699	Single-organism process	35.7	487/3133	6.65E-07
10033	Response to organic substance	3.3	45/153	2.42E-05
6082	Organic acid metabolic process	8.2	112/552	8.98E-05
71310	Cellular response to organic substance	2.8	38/124	9.54E-05
44711	Single-organism biosynthetic process	10.4	142/750	0.00013
19752	Carboxylic acid metabolic process	6.4	87/412	0.00048
44283	Small molecule biosynthetic process	5.8	80/372	0.00067
6526	Arginine biosynthetic process	0.6	8/9	0.0007
44281	Small molecule metabolic process	12.1	166/940	0.00124
43436	Oxoacid metabolic process	6.4	87/425	0.00194
42221	Response to chemical	7.2	99/510	0.00447
1901135	Carbohydrate derivative metabolic process	5.3	73/346	0.00453
6090	Pyruvate metabolic process	1.2	16/37	0.00467
6022	Aminoglycan metabolic process	1.3	60/269	0.00645
6040	Amino sugar metabolic process	1.3	18/46	0.00645
6520	Cellular amino acid metabolic process	3.5	18/46	0.00747
44763	Single-organism cellular process	25.1	48/201	0.00773
Set 2 (455 genes)				
Biological process				
44550	Secondary metabolite biosynthetic process	6.9	29/208	7.37E-07
1900813	Monodictyphenone metabolic process	1.9	8/15	1.07E-05
1900815	Monodictyphenone biosynthetic process	1.9	8/15	1.07E-05
19748	Secondary metabolic process	7.6	32/286	2.48E-05
46189	Phenol-containing compound biosynthetic process	2.6	11/37	3.93E-05
42537	Benzene-containing compound metabolic process	2.1	9/24	6.28E-05
18958	Phenol-containing compound metabolic process	2.6	11/46	0.00044
42180	Cellular ketone metabolic process	2.8	12/72	0.00805
Set 3 (1504 genes)				
Biological process				
55085	Transmembrane transport	9.0	130/687	0.00592
Molecular function				
16491	Oxidoreductase activity	14.5	210/1124	5.70E-06
3824	Catalytic activity	6.0	87/407	0.00062

*Ratio of numbers of genes with each GO term versus total numbers of genes found in the gene sets.
**Numbers of genes with each GO term and those encoded by whole fungal genome.

ing custom DNA microarrays created on GeneChip (Masuo et al., 2010). No difference was observed between the growth rates of SirAΔ and A26 strains. A significant level of signals were obtained for 9,336 genes (Table S1), which comprised 87% of the genes covered by the GeneChip. The ratios of signals between SirAΔ and A26 were calculated at each time point. SirAΔ either up- or down-regulated 3,393 (36%) genes ≥2 fold compared with A26 at one or more specific time points (Table 2). Principle component analysis and *k*-means clustering categorized these genes into three sets according to time-dependent changes in the signal $\log_2$ ratio (Table S1).

Set 1 comprised 1,434 genes (42%) that were down-regulated in SirAΔ cultured for 48 h (Fig. 1A). Sets 2 and 3 comprised 455 (13%) and 1,504 (44%) genes that were up-regulated in SirAΔ at the late stage of the cultures (48 and 72 h; Figs. 1B and C). Signal $\log_2$ ratios between SirAΔ and A26 in Set 2 continuously increased during the culture periods while those in Set 3 increased at 48 h and decreased at 72 h of culture. These results indicated that SirA selectively increased and decreased gene expression. Sirtuin-dependent global transcriptional repression of the genes in Sets 2 and 3 is consistent with the notion that the fungus produces SirA and histone deacetylase activity during the stationary growth phase (Shimizu et al., 2012).

Gene ontology analysis using annotations in the AspGD database (http://www.aspgd.org/) indicated that significant numbers of the genes in Set 1 were categorized under the GO term, "biological process", where they were related to "single-organism" ($P < 8 \times 10^{-3}$) such as "arginine biosynthetic process" ($P = 7 \times 10^{-4}$), "oxoacid metabolic process" ($P = 2 \times 10^{-3}$), "pyruvate metabolic process" ($P = 5 \times 10^{-3}$, and "cellular amino acid metabolic process" ($P = 7 \times 10^{-3}$) (Table 3). These categories include genes for carbon source (glucose) and amino acid metabolism, indicating that SirA up-regulates the genes for the primary metabolism during the late stage of culture. This finding was in contrast to those derived from *S. cerevisiae* sirtuin Sir2p and Hst1p that repress the expression of genes for glycolysis, and, hence, shift metabolism upon exiting the exponential growth phase (Li et al., 2013), and agreed with findings of previous transcriptome analyses indicating that



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Cluster	Up	Down	Detected	All
ST	14	0	24	27
PN	2	0	3	3
EAS	4	2	4	5
XPT	4	1	6	7
MDP	6	0	11	12
SID	10	1	13	13
COU/ALT	2	1	3	8
HMN	4	2	7	8
AN2924	3	0	3	4
AN8105	3	1	4	10
AN11820	2	0	3	5
AN4827	5	1	9	9
AN9005	3	0	5	6
AN1784	1	1	2	4
AUS/DAUS	0	4	5	14
ORS/ F9775	0	10	12	19
ASP	1	3	6	6
CIC	1	0	4	11
MIC	1	2	3	3
EPD	2	2	7	8
DOTB	1	3	4	9
EDD	1	5	7	11
INP	0	4	4	7
AN10297	4	2	11	16
AN8910	2	2	6	9
NID	0	0	7	8
TDI	0	3	3	5
AN0016	5	2	11	15
AN5318	2	2	4	5
AN9226	1	2	4	14
AN6431	1	4	7	8

Fig. 2. Transcriptome analysis of *A. nidulans* SM genes.

Aspergillus nidulans A26 and SirAΔ were cultured in a liquid GMM medium at 30°C for 24, 48 and 72 h. Heat maps show signal $\log_2$ ratios between SirAΔ and A26 transcripts of genes detected by microarray analysis. Heat maps for SM gene clusters involve genes grouped in Sets 2 and 3 (A and B), and SM genes grouped into Set 1 (C). A and B, Heat maps for SM gene clusters in which >50% of the genes were up-regulated ≥2 fold (A) and were not up-regulated (B) in SirAΔ cultures incubated for 48 or 72 h. (D) Numbers of SM cluster genes that are up- or down-regulated for ≥2-fold in SirAΔ Gene clusters are represented by their products and by representative genes when their products were not identified. Abbreviations: ASP, aspernidine A; AUS, austinol; DAUS, dehydroaustinol; CIC, cichorine; COU/ALT, citreoisocoumarin, 6,8-dihydroxy-3-(4-hydroxy-3-oxopentyl)-isocoumarin and alternariol; DOTB, 2,4-dihydroxy-6-[(3*E*,5*E*,7*E*)-2-oxonona-3,5,7-trienyl] benzaldehyde; EAS, emericellamide; EDD, 2-ethyl-4,6-dihydroxy-3,5-dimethyl benzaldehyde; EPD, ent-pimara-8(14),15-diene; INP, fellutamide B; MDP, monodictyphenone; MIC, microperfuranone HMN, 6-hydroxy-7-methyl-3-nonylisoquinoline-5,8-dione; NID, nidulalin A; ORS/ F9775, orcinol and F9775; PN, penicillin G; SID, fungal siderophore; ST, sterigmatocystin; TDI, terrequinone; XPT, xanthone and shamixanthone. Table S3 lists all genes in these clusters.

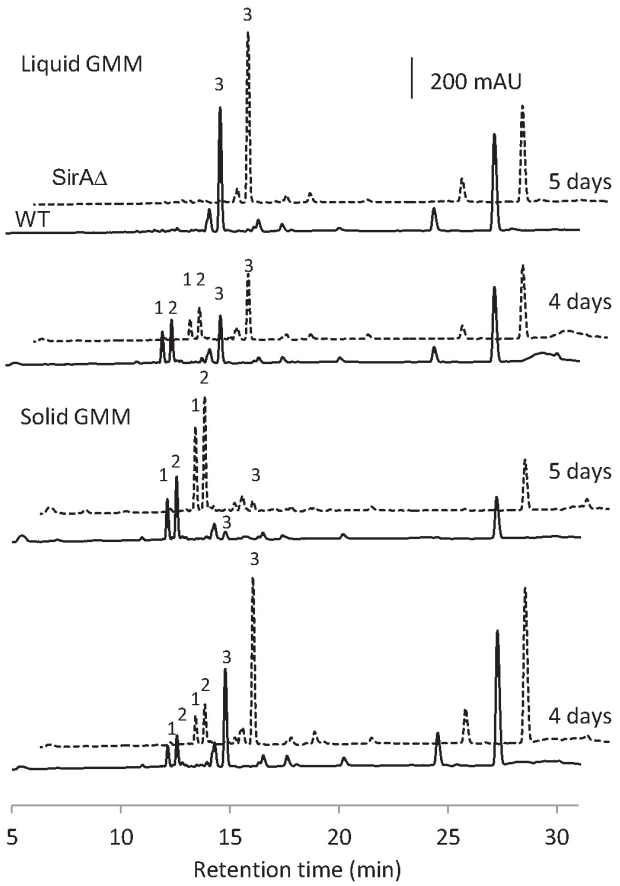

Fig. 3. Amounts of SM produced by *A. nidulans* determined by HPLC. Solid lines, extracts of *A. nidulans* A26; broken lines, SirAΔ cultured in both liquid and solid GMM for four and five days. The numbers 1, 2 and 3 represent austinol, dehydroaustinol and sterigmatocystin, respectively.

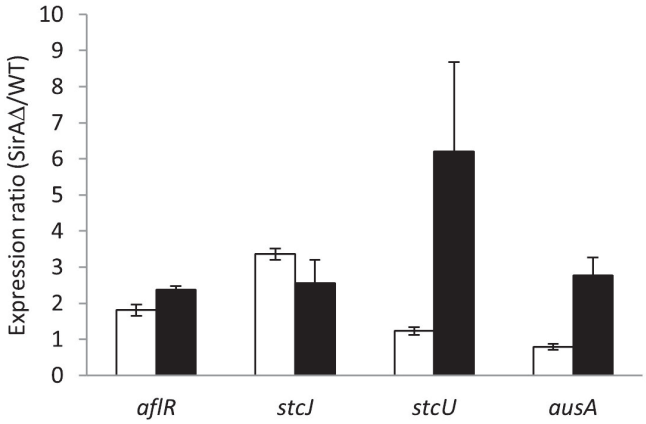

Fig. 4. Amounts of transcripts of genes for producing sterigmatocystin, austinol and dehydroaustinol of SirAΔ determine by real-time PCR.

SirAΔ and A26 were cultured in liquid GMM medium for 48 (unfilled bars) and solid GMM medium for 24 h (filled bars). Values are relative to those of A26. *ausA*, gene for synthesizing austinol and dehydroaustinol; *aflR*, regulatory gene in cluster for synthesizing sterigmatocystin; *stcJ* and *stcU*, genes involved in sterigmatocystin synthesis. Data are presented as means ± standard error of three experiments.

S. cerevisiae Sir2p and Hst1p, as well as the *A. oryzae* sirtuin isozyme AoHstD, up-regulate multiple genes (Bedalov et al., 2003; Kawauchi et al., 2013). We recently identified another sirtuin isozyme (SirE) of *A. nidulans* that represses the primary metabolism, including the tricarboxylic acid cycle as well as the glycolytic and pentose phosphate pathways. Thus, the sirtuin-dependent up-regulation of the primary metabolism is a standard mechanism in *A. nidulans*. Since general sirtuins are considered to silence gene expression, these sirtuins are likely to indirectly up-regulate the gene for the primary mechanism.

The enriched genes in Set 2 were classified under the GO terms, "SM biosynthetic process" ($P < 7 \times 10^{-7}$) and "secondary metabolic process" ($P < 2 \times 10^{-5}$). The latter contained 32 genes associated with the production of the secondary metabolites used for synthesizing SM, such as ST, PN, xanthone and shamixanthone (XPT), the fungal siderophore (SID), emericellamide (EAS), austinol and dehydroaustinol (AUS/DAUS), aspernidine A (ASP), cichorine (CIC), ent-pimara-8(14),15-diene (EPD) (Andersen et al., 2013), the aromatic amino acid, fumiquinazoline C, ergot alkaloid and an unknown polyketide (Table S2). Other genes in Set 2 were assigned to a "monodictyphenone (MDP) metabolic process" ($P < 1 \times 10^{-5}$), and a "MDP biosynthetic process" ($P < 1 \times 10^{-5}$). Production of the *A. nidulans* secondary metabolite MDP is facilitated by *mdp* gene clusters (Chiang et al., 2010). The most abundant term for the genes in Set 3 was "oxidoreductase activity" ($P = 6 \times 10^{-6}$) in the "molecular function" category. This group comprised 21 genes assigned to the GO term, "secondary metabolic process" and 20 genes encoding (predicted) cytochrome P450, among which predictions indicate that seven are located within or near gene clusters that produce fungal SM and are involved in their biosynthesis (asterisks in Table S2) (Kelly et al., 2009). These results indicated that the genes in Sets 2 and 3 enrich SM production and that SirA is a global transcription repressor for fungal SM synthesis at the late stage of culture.

SirA represses gene clusters associated with secondary metabolites

The genes in Sets 2 and 3 included 85 that were annotated with an SM-related function (Table S2). Among these, 73 reside in the known 25 SM gene clusters or those predicted by Inglis et al. (2013). Figures 2A and B shows heat maps of the transcription of genes in these clusters. Over 50% of the genes in 14 clusters were up-regulated ≥2 fold in SirAΔ cultures incubated for 48 or 72 h (Figs. 2A, D, and Table S3). These clusters included genes for synthesizing ST, PN, EAS, XPT, MDP, SID, citreoisocoumarin, 6,8-dihydroxy-3-(4-hydroxy-3-oxopentyl)-isocoumarin and alternariol (COU/ALT), and 6-hydroxy-7-methyl-3-nonylisoquinoline-5,8-dione (HMN) (Ahuja and Chiang, 2012), indicating that SirA represses the expression of these SM gene clusters. The repression of ST and PN agrees with our findings that SirA deacetylates histone on their gene promoters and consequently represses their gene expression and production (Shimizu et al., 2012). SirAΔ also expressed genes in the predicted SM gene clusters involving non-ribosomal pep-

tide synthetases (NRPS) (AN2924, AN8105, AN11820, AN4827 clusters) and polyketide synthases (PKS) (AN9005, AN1784 clusters) (Figs. 2A, D, and Table S3). These results indicated that SirA represses the expression of these gene clusters.

Other SM gene clusters regulated by SirA

Although SirAΔ up-regulated the SM genes found in Sets 2 and 3 during the late growth phase, >50% of the genes in 11 SM gene clusters were not up-regulated in SirAΔ, and these clusters included genes that synthesize AUS/DAUS, orcinols and F9775 (ORS/F9775), ASP, CIC, microperfuranone (MIC), EPD, 2,4-dihydroxy-6-[(3*E*,5*E*,7*E*)-2-oxonona-3,5,7-trienyl] benzaldehyde (DOTB) (Ahuja and Chiang, 2012), 2-ethyl-4,6-dihydroxy-3,5-dimethylbenzaldehyde (EDD) (Ahuja and Chiang, 2012) and fellutamide B (INP) (Yeh et al., 2016), predicted NRPS (AN10297), and predicted PKS (AN8910) (Figs. 2B, D, and Table S3). Thus, our transcriptome could not distinguish whether or not SirA regulates the production of these SM. Among the genes in Set 1 that were up-regulated by SirA, 52 were assigned to the GO term, "secondary metabolic process" (Table S4). Among these, nine were located in six SM gene clusters that are predicted to synthesize nidulalin A (NID), terrequinone (TDI), and NRPS gene clusters (AN0016, AN5318, AN9226 clusters) and the PKS gene cluster (AN6431 cluster) (Figs. 2C, D, and Table S3). In the TDI, AN5318, and AN9226 clusters, >50% of genes were down-regulated in SirAΔ at the late stage of the culture, indicating that SirA up-regulates the expression of these SM gene clusters. On the other hand, in the NID, AN0016, AN6431 gene clusters, the ratio of genes up-regulated ≥2 fold in SirAΔ was ≤50%. These results indicated that SirA differentially regulates SM gene clusters. Such differential regulation of genes that synthesize SM was also found among *A. oryzae* genes that are regulated by AoHstD (Kawauchi et al., 2013).

SirA regulates production of sterigmatocystin and austinol

The production of SM in SirAΔ and A26 cultured in a liquid GMM medium for four and five days was analyzed by HPLC. SirAΔ produced 1.5- and 1.4-fold more ST (eluted with the same retention time as standard ST) than A26 (Fig. 3). This result was consistent with those of the microarray analysis (Fig. 2A) and our previous study (Shimizu et al., 2012). The results of culture on the solid GMM medium for four and five days were similar, as SirAΔ produced 1.7- and 1.2-fold more ST than A26, respectively (Fig. 3). The up-regulated expression of *aflR*, *stcJ* and *stcU* that are involved in ST synthesis, in SirAΔ (Fig. 4), was consistent with the production levels of ST determined by HPLC (Fig. 3). The growth rate and level of conidiophore development on solid GMM were indistinguishable between SirAΔ and A26. These results indicated that SirA represses ST production in both liquid and solid cultures.

The signals of compounds migrating at 12.1 min were m/z = 459.1, 481.1, and 497.1, which matched those of $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ from AUS (molecular mass = 458.50, $C_{25}H_{30}O_8$) (Lo et al., 2012). The signals of compounds migrating at 12.6 min were m/z = 457.2 and 495.2, which matched those of $[M+H]^+$ and $[M+K]^+$ from DAUS (molecular mass = 456.49, $C_{25}H_{28}O_8$) (Lo et al., 2012). Their absorption maxima at 246 and 234 nm, determined by photodiode array measurements on HPLC, were identical to those of the respective compounds, which confirmed that they were indeed AUS and DAUS (Bok et al., 2009). After culture for four days in liquid GMM, SirAΔ accumulated 30% and 19% less AUS and DAUS, respectively, relative to A26. This result was consistent with those of the microarray analysis indicating that fewer transcripts of their synthesizing genes accumulated in SirAΔ (Fig. 2B). The signals for AUS and DAUS disappeared after culture for five days, indicating they were consumed by the fungus. By contrast, fungal cultures on solid GMM gradually accumulated AUS and DAUS, and SirAΔ produced 1.4- and 1.3-fold more AUS and DAUS than A26 after culture for five days, (Fig. 3). Quantitative PCR detected 2.7-fold more transcripts of the representative *ausA* gene that synthesizes AUS and DAUS in SirAΔ than in A26 under our culture conditions (Fig. 4), indicating that SirA represses AUS and DAUS production in a solid medium at the level of transcription.

In addition to ST and PN synthesis (Shimizu et al., 2012), gene regulation of *ausA* and other genes for AUS/DAUS synthesis is a novel function of SirA that was noted in the present study. Culture in liquid and solid media affected SM gene expression, which is often found during the production of other SM (Andersen et al., 2013), and the present findings indicated that *A. nidulans* SirA discriminates culture conditions upon expression of the AUS/DAUS synthetic genes. An increase in cellular NAD^+, which is a substrate for sirtuin-dependent histone deacetylation, stimulates the activity of mammalian and yeast sirtuins (Haigis and Guarente, 2006; Kato and Lin, 2014). However, we previously found that the amount of NAD^+ in cultured *A. nidulans* remains unchanged during the late growth phase for up to 72 h (Shimizu et al., 2012). This implies that SirA is not activated by the amount of NAD^+ at the growth stage. The time-dependent changes in the transcriptomes (Fig. 2) indicated that SirA activity changes according to the culture period, the regulation mechanism of which will pose the next opportunity to deepen the understanding of the function of SirA in global transcriptional regulation.

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Supplementary Materials

Supplementary tables are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/jgam).

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

NT supervised all laboratory experiments.

EI designed, acquired, analyzed, interpreted all results and wrote the manuscript.

NT and MS designed all experiments, acquired and interpreted the data and helped to prepare the manuscript.

RS helped with HPLC analysis.

KIO and SM helped to write the manuscript.

All authors have read and approved the final submission.

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