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Proteomic analyses reveal the key roles of BrlA and AbaA in biogenesis of gliotoxin in *Aspergillus fumigatus*

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ABSTRACT

The opportunistic human pathogenic fungus *Aspergillus fumigatus* primarily reproduces by forming a large number of asexual spores (conidia). Sequential activation of the central regulators BrlA, AbaA and WetA is necessary for the fungus to undergo asexual development. In this study, to address the presumed roles of these key developmental regulators during proliferation of the fungus, we analyzed and compared the proteomes of vegetative cells of wild type (WT) and individual mutant strains. Approximately 1300 protein spots were detectable from 2-D electrophoresis gels. Among these, 13 proteins exhibiting significantly altered accumulation levels were further identified by ESI-MS/MS. Markedly, we found that the GliM and GliT proteins associated with gliotoxin (GT) biosynthesis and self-protection of the fungus from GT were significantly down-regulated in the Δ*abaA* and Δ*brlA* mutants. Moreover, mRNA levels of other GT biosynthetic genes including *gliM*, *gliP*, *gliT*, and *gliZ* were significantly reduced in both mutant strains, and no and low levels of GT were detectable in the Δ*brlA* and Δ*abaA* mutant strains, respectively. As GliT is required for the protection of the fungus from GT, growth of the Δ*brlA* mutant with reduced levels of GliT was severely impaired by exogenous GT. Our studies demonstrate that AbaA and BrlA positively regulate expression of the GT biosynthetic gene cluster in actively growing vegetative cells, and likely bridge morphological and chemical development during the life-cycle of *A. fumigatus*.

1. Introduction

Aspergillus fumigatus is a saprophytic soil fungus that causes invasive pulmonary aspergillosis (IPA) in immune-compromised patients [1,2]. *A. fumigatus* produces asexual spores (conidia) as the main means of dispersal, and asexual sporulation (conidiation) and production of certain mycotoxins is closely related [3,4]. Conidiation in *Aspergillus* is a precisely timed, genetically programmed event, and it requires activation of *brlA* [5–7]. BrlA is a C_2H_2 zinc-finger transcription factor (TF) that governs conidiation processes in conjunction with other regulators [8,9]. The *abaA* gene is activated by BrlA during the middle stages of conidiation, and it encodes a TF with an ATTS DNA-binding motif governing the formation of conidia from the phialide [10]. The *wetA* gene, activated by AbaA, functions in the late phase of conidiation and governs the synthesis of critical cell wall components and trehaloese biogenesis [11–13]. These genes have been proposed to define the central regulatory pathway that acts to control conidiation specific gene expression and determine the order of gene activation during conidiation in *Aspergillus nidulans* and *A. fumigatus* [5,6,13,14].

In *A. fumigatus*, a series of data suggest that BrlA, AbaA, and WetA might play certain roles during vegetative growth, in addition to their roles in spore development. First of all, *brlA* mRNA is clearly detectable during a certain period of vegetative growth (~18 ~ 24 h) [6,13,15]. Moreover, the absence of *abaA* causes delayed autolysis and cell death, whereas the overexpression of *abaA* accelerates these processes [13]. Finally, the deletion of *wetA* causes delayed germ-tube formation from conidia, and reduced hyphal branching and thallic density [13].

In the present study, to begin to understand their presumed roles during vegetative growth of *A. fumigatus*, we examined the

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proteomes of wild type (WT), and individual deletion mutants of *abaA*, *brlA*, and *wetA* upon 48 h of liquid submerged culture. Comparative proteomic analyses and identification of key altered proteins have revealed that BrlA and AbaA are necessary for the proper production of the GliM and GliT proteins that are associated with gliotoxin (GT) biosynthesis and self-protection of the fungus from GT, respectively [16–18]. Further studies reveal the roles BrlA and AbaA in proper formation of GT, and protection of the fungus from GT. Collectively, we propose a model depicting the key roles of the BrlA and AbaA developmental regulars in *A. fumigatus*.

2. Materials and methods

2.1. Fungal strain and culture conditions

In this study, *A. fumigatus* AF293 (wild type; WT) [19], Δ*abaA* (*pyrG1*, Δ*abaA*:*pyrG*$^{+}$), Δ*brlA* (*pyrG1*, Δ*brlA*:*pyrG*$^{+}$), and Δ*wetA* (*pyrG1*, Δ*wetA*:*pyrG*$^{+}$) [13] strains were used. Glucose minimal medium (MMG) or MMG with 0.1% (w/v) yeast extract (MMY) with supplements was used for general culture of fungal strains [20,21]. For liquid submerged culture, about 5×10^5 conidia/mL were inoculated into liquid MMY and incubated at 37 °C, 250 rpm.

2.2. Protein sample preparation

For sample preparation, conidia of WT and mutant strains were inoculated in 100 mL liquid MMY in 250 mL flask and grown at 37 °C for 2 days. Mycelial samples were collected and washed in a phosphate buffer saline (PBS, pH 7.4). Prepared mycelial samples were homogenized directly by motor-driven homogenizer (PowerGen125, Fisher Scientific) in sample lysis solution (7 M urea, 2 M thiourea containing 4% (w/v) 3-[(3-cholamidopropyl) dimethyammonio]-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol (DTT), 2% (v/v) pharmalyte and 1 mM benzamidine), incubated for 1 h at room temperature with vortexing, and centrifuged in a microcentrifuge for 1 h at 15,000 rpm at 4 °C. After centrifugation, insoluble material was discarded and soluble fraction was used for 2-DE gel electrophoresis.

2.3. 2-DE and image analysis

IPG dry strips (4-10 NL IPG, 24 cm, Genomine, Korea) were equilibrated for 12–16 h with 7 M urea, 2 M thiourea containing 2% CHAPS, 1% DTT, 1% pharmalyte and respectively loaded with 200 μg of sample. Isoelectric focusing (IEF) was performed at 20 °C using a Multiphor II electrophoresis unit and EPS 3500 XL power supply (Amersham Biosciences) following manufacturer's instruction. For IEF, the voltage was linearly increased from 150 to 3500 V during 3 h for sample entry followed by constant 3500 V, with focusing complete after 96 kVh. Prior to the second dimension, strips were incubated for 10 min in equilibration buffer (50 mM Tris-HCl, pH 6.8 containing 6 M urea, 2% SDS and 30% glycerol), first with 1% DTT and second with 2.5% iodoacetamide. Equilibrated strips were inserted onto SDS-PAGE gels (20 × 24 cm, 10–16%). SDS-PAGE was performed using Hoefer DALT 2D system (Amersham Biosciences) following manufacturer's instruction. 2-D gels were run at 20 °C for 1700 Vh. And then 2-D gels were Coomassie Brilliant Blue as described elsewhere [22]. Quantitative analysis of digitized images was carried out using the PDQuest (version 7.0, BioRad) software according to the protocols provided by the manufacturer. Quantity of each spot was normalized by total valid spot intensity. Protein spots were selected for the significant expression variation deviated over two fold in its expression level compared with control or normal sample.

2.4. Protein identification by nano-LC-ESI-MS/MS

All MS/MS experiments for peptide identification were performed using a nano-LC-MS system consisting of an Ultimate HPLC system (Dionex) and Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a nano-ESI source, as described previously [23,24]. Briefly, for each sample, 10 μL was loaded by the autosampler onto a C18 trap column (id 300 mm, length 5 mm, particle size 5 μm; LC Packings) for desalting and concentrating at a flow rate of 20 μL/min. Then the trapped peptides were separated on a 100-mm homemade microcapillary column composed of C18 (Aqua; particle size, 5 μm) packed into 75-μm silica tubing. The mobile phases, A and B, were composed of 0 and 80% acetonitrile, respectively, each containing 0.1% formic acid. The gradient began with 5% B for 15 min; ramped to 20% B over 3 min, to 60% over 45 min, and to 95% over 2 min; and remained at 95% B for another 7 min. The voltage applied to produce an electrospray was 2.5 kV. Argon was introduced as a collision gas at a pressure of 10 psi. The three most abundant MS ions were selected by data-dependent peak selection. The previously fragmented ions were excluded for 60 s. The proteins were identified by searching fungi subset (219,981 entries) of the National Center for Biotechnology Information (NCBI) protein databases (20100312) using the MASCOT 2.0 search algorithm (Matrix Science). The general parameters for search were considered to allow maximum one missed cleavage, the modifications of N-terminal Gln to pyroGlu, oxidation of methionine, acetylation of protein N terminus, carbamidomethylation of cysteine, and acrylamide modified cysteine. A peptide charge state of +2 or 3, and peptide/fragment mass tolerance of ±0.5 Da were used for the MS/MS ion search. Probability based MASCOT scores were estimated by comparison of search results against estimated random match population and reported as −10*Log(P) where P is the absolute probability. The significance threshold was set at $p < 0.05$.

2.5. Nucleic acid isolation and manipulation

Sample preparation and total RNA isolation were carried out as described [6,24]. Briefly, conidia (5×10^5 conidia/mL) of WT and mutant strains were inoculated in 100 mL liquid MMY in 250 mL flask and incubated at 37 °C, 250 rpm. Individual mycelial samples were collected as designated time points from liquid submerged cultures. Individual mycelial samples were collected; squeeze-dried and stored −80 °C until needed for total RNA isolation. The prepared samples were homogenized using a Mini Bead beater in the presence of 1 mL of TRIzol® reagent (Invitrogen) and 0.3 mL of silica/zirconium beads (Biospec). RNA extraction was performed according to the manufacturer's instruction (Invitrogen).

Quantitative PCR assays were performed according to the manufacturer's instruction (Qiagen, USA) using 96-well optical plates and a Rotor-Gene Q (Qiagen, USA). Each run was assayed in triplicates in a total volume of 20 μL containing the RNA template, 2 × 1-Step RT-PCR SYBR Mix (Doctor Protein, Korea), reverse transcriptase, and 10 pmole of each primer. The primers used for *gliM*, *gliP*, *gliT*, and *gliZ* were described in Gardiner et al. [16], and for EF1α were CCATGTGTGTCGAGTCCTTC (forward) and GAACGTACAGCAACAGTCTGG (reverse). Reverse transcription condition was 42°C/30 min and PCR conditions were: 95°C/5 min for one cycle, followed by 95°C/30 s and 55°C/30 s for 40 cycles. Amplification of one single specific target DNA was checked through melting curve analysis (+0.5 °C ramping for 10 s, from 55 °C to 95 °C). The expression ratios were normalized to EF1α expression, and were calculated according to the ΔΔCt method [25].

2.6. Phenotypic analysis

To assess the production of gliotoxin (GT), conidia of each strain were inoculated into 50 mL liquid MMY and incubated for 7 days at 37 °C and 280 rpm. GT was extracted with chloroform as described previously [26]. The chloroform extracts were air-dried and resuspended in 100 μL of methanol. Ten μL of each sample was applied to a thin-layer chromatography (TLC) silica plate containing a fluorescence indicator (Kiesel gel 60, E. Merck). TLC plate was developed with toluene:ethyl acetae:formic acid (5:4:1, v/v) until the solvent front reached about 15 cm. GT standard was purchased from Sigma (USA). Susceptibility of WT and mutant strains against GT was tested by the plate assays. Mycelial plugs cut from the growing edge of 5 day colonies of each strain were transferred to plates containing GT (25 μg/mL) and incubated at 37 °C for 3 days.

3. Results

3.1. Comparative proteome analysis

To better understand additional roles of AbaA, BrlA, and WetA, total intracellular proteins were extracted from the mycelia of 2-day old cultures of WT and mutant strains, and 2-DE gels were produced in biological triplicates, and the best gel from each strain was selected to serve as the reference map. As shown in Table 1, around 1428 ± 82 (WT), 1546 ± 185 (Δ*abaA*), 1317 ± 35 (Δ*brlA*), and 1287 ± 107 (Δ*wetA*) protein spots were identified from the 2-DE gels. The coefficient of variation (CV) for WT, Δ*abaA*, Δ*brlA*, and Δ*wetA* strains were about 5.7%, 11.9%, 2.6%, and 8.3%, respectively, and only minor differences were found in CV values from the replicate gels (Table 1). The 2-DE images of WT and mutant strains are shown in Fig. 1A and the molecular mass of the identified proteins ranged from 17.03 kDa (spot 2011) to 86.89 kDa (spot 7819) (Fig. 1A). Quantitative changes in proteins were assessed by comparison of the 2-DE gels using the PDQuest software (Fig. 1B and Table 2). Thirteen proteins were identified by nano-LC-ESI-MS/MS that showed a significantly altered abundance of ≥1.5 in the relative spot intensity (Table 2). The reliability of protein identification was assessed on the basis of MASCOT score and sequence coverage. MASCOT score and sequence coverages ranged 642 ~ 17,482 and 45.9% ~ 91.1%, respectively (Table 2). Heat shock protein 70 (Afu1g07440) and flavohemoprotein (Afu4g03410) were down-regulated in all mutant strains, whereas ATP sulphurylase (Afu3g06530) was up-regulated. ATP synthase F1 β subunit (Afu5g10550), inorganic diphosphatase (Afu3g08380), and cofilin (Afu5g10570) were down-regulated in Δ*brlA* and Δ*wetA* strains ranged from 1.16- to 4.16-fold. Proteins contributing to the amino acid and carbohydrate metabolism, spermidine synthase (Afu1g13490), cobalamin-independent methionine synthase (Afu4g07360), and transaldolase (Afu5g09230) were more abundant in Δ*abaA* and Δ*wetA* strains than WT. In particular, intensities of two GT-related proteins, GliM (Afu6g09680) and GliT (Afu6g09740), were significantly low in Δ*abaA* and Δ*brlA* strains (Table 2).

Table 1
Total protein numbers detectable in WT and mutant vegetative cells on 2-DE gel-based proteomics. Spots were identified and compared by the PDQuest software (BIO-RAD).

	WT	Δ*abaA*	Δ*brlA*	Δ*wetA*
Number of spots	1429 ± 82	1546 ± 185	1317 ± 35	1287 ± 107
Coefficient variance (%)	5.7	11.9	2.6	8.3

3.2. Verification of the GLI gene transcript levels

Because the protein levels of GliM and GliT were clearly different in WT and the mutants on 2-DE gels, we asked whether mRNA levels of these and other GT biosynthetic genes were affected in the mutants. To address this, we performed quantitative real time PCR using total RNA of WT and individual mutants, and analyzed the contribution of each regulator to the expression of major GT synthetic genes. In addition, when mRNA levels of the *gliM*, *gliP*, *gliT*, and *gliZ* genes were compared, expression of these genes was significantly lower in Δ*abaA* and Δ*brlA* strains than in WT and Δ*wetA* strains (Fig. 2A).

3.3. The key role of AbaA and BrlA in the synthesis of GT and protection against GT

In order to corroborate the roles of these asexual developmental regulators in the production and detoxification of GT, we further investigated the effects of the deletion of *abaA*, *brlA*, and *wetA*. To test whether low expression of GliM and GliT resulted in the reduced production of GT in the mutant strains, we determined the amounts of GT produced by WT, Δ*abaA*, Δ*brlA*, and Δ*wetA* strains. As shown in Fig. 2B, Δ*brlA* strain showed clear differences in the amounts of GT and produced undetectable amount of GT. Production of two other metabolites (Rf value is 0.43 and 0.35) exhibited a strain-dependent pattern. Production of an unknown more nonpolar metabolite (Rf = 0.43) reduced in Δ*abaA* and Δ*brlA* strains than in WT and Δ*wetA* strains, but the amount of another unknown metabolite (Rf = 0.35) was reversed, suggesting that AbaA and BrlA may play a similar role in secondary metabolism.

Beside the crucial function of GliT in GT biosynthesis itself, it confers the protection of *A. fumigatus* from the toxicity of GT [17,18]. To test whether reduced levels of GliT in Δ*abaA* and Δ*brlA* strains is associated with enhanced susceptibility of the mutants against GT, a series of 10-fold diluted conidia of WT, Δ*abaA*, Δ*brlA*, and Δ*wetA* strains were inoculated in the presence of exogenous GT and the growth were compared. Growth of three mutants was severely inhibited by exogenous GT and growth of Δ*brlA* mutants was completely blocked by the presence of GT (Fig. 2C). These data imply that asexual developmental regulators *abaA* and *brlA* are required for proper regulation of GliT expression, thereby the protection of the fungus of GT.

4. Discussion

To date a number of key asexual developmental regulators such as AbaA, BrlA, WetA, and Velvets have been identified and characterized in *A. fumigatus* [6,13,15,27]. AbaA plays a crucial role in the differentiation of phialides, the conidiogenous cells, which are required for formation of conidiospores. AbaA may also influence secondary metabolism by regulating expression of *veA*, *velB*, and *velC* [28]. BrlA functions in triggering the central regulatory pathway of conidiation. WetA plays a role in the completion of conidial wall and is essential for trehalose biogenesis, conidial viability, integrity, and stress tolerance [13]. In this study, we investigated the presumed roles of these regulators during vegetative growth of the fungus at protein level by 2-DE proteome analysis.

In the comparative proteomic analysis, stress-related proteins including heat shock protein 70 and flavohemoprotein were down-regulated in all mutant hyphae than WT. It has been reported that flavohemoprotein (Afu4g03410) is involved in reactive nitrogen species detoxification, and is 17-fold up-regulated in hypoxic growth condition [29]. Several microorganisms, including different bacterial and fungal species, make use of alternative electron

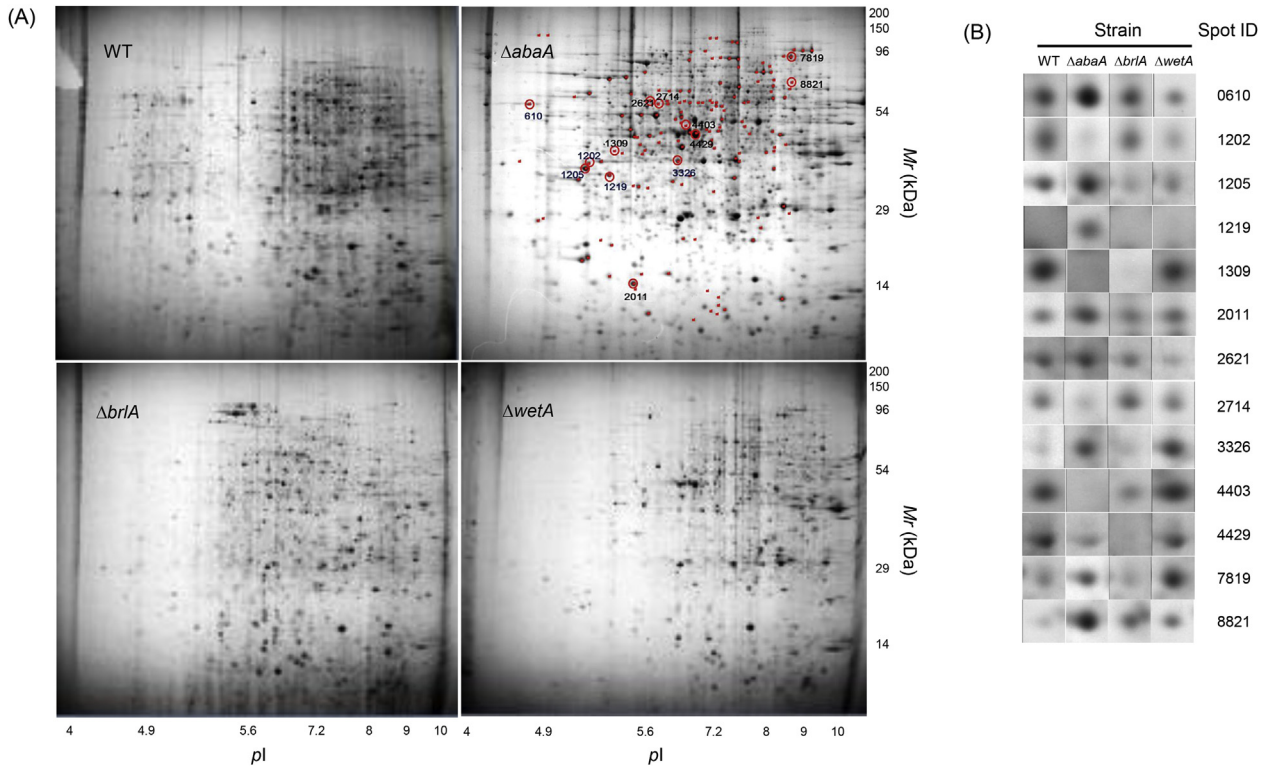

Fig. 1. Representative 2-DE gel of *A. fumigatus* WT (AF293) and mutant strains using pH 4-10 NL in the first dimension (A) and expression patterns of identified proteins (B). Proteins were separated in two dimensions and stained with Coomassie Brilliant Blue.

acceptors, such as nitrate, when oxygen is limited [30,31]. The reason of down-regulation of flavohemoprotein in mutant strains is currently unknown. However, ATP sulphurylase, which catalyzes the first intracellular reaction in the incorporation of inorganic sulfate into organic molecules, was up-regulated (Table 2). Undoubtedly, the most important finding in our present study is that the absence of *abaA* and *brlA* resulted in reduced expression of two GT-related proteins, GliM and GliT (Table 2). Moreover, mRNA levels of 4 GT biosynthetic genes were significantly reduced in Δ*abaA* and Δ*brlA* strains (Fig. 2A). These demonstrate that AbaA and BrlA may positively regulate expression of the GT gene cluster. GT biosynthetic gene cluster comprises 13 genes [16,17], and the borders of the cluster were determined by functionally assigning individual genes to GT biosynthesis and/or by co-regulation of their transcription. The *gliM* gene in this cluster is predicted to encode an *o*-methyltransferase [32], and *gliP* encodes a non-ribosomal peptide synthetase (NRPS). GliP catalyzes the first steps of GT biosynthesis, formation of a dipeptide and cyclization, which yields the diketopiperazine (DKP) scaffold [33]. Mutational inactivation of *gliP* results in the blocked production of GT and lowered expression of other GT genes [32]. The *gliT* gene encodes an oxidoreductase of the GT biosynthetic cluster and *gliT* expression is decreased when *gliZ* is deleted [17,34]. GliZ, a Zn_2Cys_6 transcription factor, is responsible for general GT induction and regulation, and deletion of *gliZ* results in a loss in GT production [35,36].

In this study, the absence of *brlA* resulted in a complete loss of GT production with altered production of other metabolites (Fig. 2A). Our results demonstrate that activation of BrlA and AbaA is necessary for the commencement of *gliM* and *gliT* expression in vegetative cells. GliT also plays a key role in detoxifying GT [17], and

Table 2
Identification of the proteins exhibiting significantly different levels in WT and mutant strains.

Spot id	Locus tag	GI number	Identification	MASCOT score	Sequence coverage (%)	Expression ratio (Mutant/WT)		
						Δ*abaA*	Δ*brlA*	Δ*wetA*
0610	5g10550	66851225	ATP synthase F1 β subunit	3582	91.1	1.13 ± 0.06	0.24 ± 0.01	0.44 ± 0.05
1202	3g04170	66846363	pyruvate dehydrogenase E1 beta subunit	1939	68.7	0.70 ± 0.03	1.85 ± 0.19	1.01 ± 0.03
1205	3g08380	66852413	inorganic diphosphatase	1404	67.2	1.58 ± 0.46	0.65 ± 0.16	0.86 ± 0.03
1219	1g13490	42820759	spermidine synthase	1104	85.7	2.58 ± 0.57	0.98 ± 0.16	1.27 ± 0.34
1309	6g09740	56609349	GliT	717	82.3	0.36 ± 0.01	0.26 ± 0.02	1.19 ± 0.09
2011	5g10570	66851223	cofilin	4182	78.6	1.54 ± 0.07	0.70 ± 0.03	0.70 ± 0.08
2621	2g00720	66846840	aldehyde dehydrogenase	1783	62.2	2.98 ± 0.08	2.37 ± 0.10	0.92 ± 0.06
2714	1g07440	45356863	heat shock protein 70	887	45.9	0.42 ± 0.03	0.73 ± 0.06	0.38 ± 0.02
3326	5g09230	66851352	transaldolase	1788	77.8	1.67 ± 0.25	0.80 ± 0.03	1.91 ± 0.04
4403	6g09680	129557256	*O*-methyltransferase GliM (MmcR)	642	58.9	0.17 ± 0.01	0.20 ± 0.02	3.23 ± 0.11
4429	4g03410	66844151	flavohemoprotein	17,482	83.5	0.77 ± 0.03	0.56 ± 0.02	0.93 ± 0.08
7819	4g07360	66849724	methionine synthase MetH/D	2258	72.6	1.23 ± 0.07	0.43 ± 0.06	2.80 ± 0.27
8821	3g06530	66852590	ATP sulphurylase	1072	65.0	6.55 ± 0.10	2.14 ± 0.30	2.52 ± 0.35

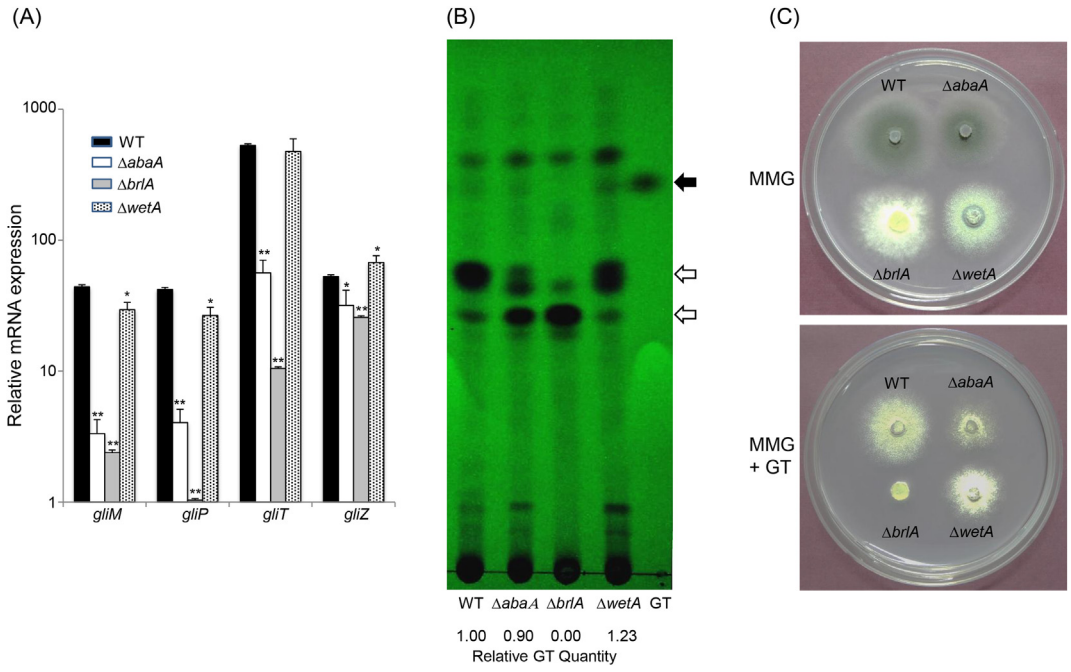

Fig. 2. Effects of the absence of three key asexual developmental regulators in production and detoxification of GT. (A) Q RT-PCR analysis of four GT-related genes in WT and relevant mutant strains. Statistical differences between WT and mutant strains were evaluated with Student's unpaired t-test. * = $P < 0.05$, ** = $P < 0.01$. (B) Determination of GT production in WT and mutant strains. The culture supernatant of each strain was extracted with chloroform and subjected to TLC. Chromatogram was analyzed with IMAGEJ (National Institutes of Health) and relative quantification of GT (black arrow) is indicated at the bottom. (C) Effects of exogenous GT on growth of WT and mutant strains.

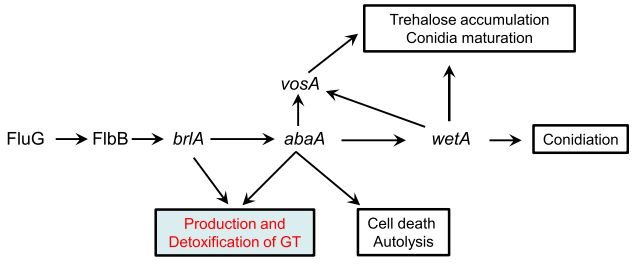

Fig. 3. A model for the roles of AbaA, BrlA, WetA and VosA in *A. fumigatus*.

may be involved in protecting the fungus from the deleterious effects of the epipolythiodioxopiperazine (ETP) toxins produced by other fungi [17,18]. This protection relies on the ability of GliT to keep GT in the sulfur-bridged form, which avoids generation of reactive oxygen species (ROS) and of protein conjugates [18]. Tolerance of the mutants against exogenous GT was significantly reduced compared to WT (Fig. 2B). In particular, colony growth of Δ*brlA* strain was completely impaired by exogenous GT. Collectively, we speculate that asexual developmental regulators play a key role in expression of GT-related genes during vegetative growth and likely in the lifecycle of the fungus. We propose a new model depicting the roles of asexual developmental regulators of *A. fumigatus* in GT production/detoxification (Fig. 3).

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References

[1] B.A. Cockrill, C.A. Hales, Allergic bronchopulmonary aspergillosis, Annu. Rev. Med. 50 (1999) 303–316.
[2] T.R.T. Dagenais, N.P. Keller, Pathogenesis of *Aspergillus fumigatus* in invasive Aspergillosis, Clin. Microbiol. Rev. 22 (2009) 447–465.
[3] A.M. Calvo, R.A. Wilson, J.W. Bok, N.P. Keller, Relationship between secondary metabolism and fungal development, Microbiol. Mol. Biol. Rev. 66 (2002) 447–459.
[4] J.H. Yu, N.P. Keller, Regulation of secondary metabolism in filamentous fungi, Annu. Rev. Phytopathol. 43 (2005) 437–458.
[5] T.H. Adams, M.T. Boylan, W.E. Timberlake, *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*, Cell 54 (1988) 353–362.
[6] J.H. Mah, J.H. Yu, Upstream and downstream regulation of asexual development in *Aspergillus fumigatus*, Eukaryot. Cell 5 (2006) 1585–1595.
[7] O. Yamada, B.R. Lee, K. Gomi, Y. Iimura, Cloning and functional analysis of the *Aspergillus oryzae* conidiation regulator gene *brlA* by its disruption and misscheduled expression, J. Biosci. Bioeng. 87 (1999) 424–429.
[8] B.N. Lee, T.H. Adams, The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I, Genes. Dev. 8 (1994) 641–651.
[9] J. Wieser, T.H. Adams, *flbD* encodes a Myb-like DNA-binding protein that coordinates initiation of *Aspergillus nidulans* conidiophore development, Genes. Dev. 9 (1995) 491–502.
[10] A. Andrianopoulos, W.E. Timberlake, The *Aspergillus nidulans abaA* gene encodes a transcriptional activator that acts as a genetic switch to control development, Mol. Cell Biol. (1994) 2503–2515.
[11] T.C. Sewall, C.W. Mims, W.E. Timberlake, Conidium differentiation in *Aspergillus nidulans* wild-type and wet-white (*wetA*) mutant strains, Dev. Biol. 138 (1990) 499–508.
[12] M.A. Marshall, W.E. Timberlake, *Aspergillus nidulans wetA* activates spore-specific gene expression, Mol. Cell Biol. 11 (1991) 55–62.
[13] L. Tao, J.H. Yu, AbaA and WetA govern distinct stages of *Aspergillus fumigatus* development, Microbiology (2011) 313–326.

[14] P.M. Mirabito, T.H. Adams, W.E. Timberlake, Interactions of three sequentially expressed genes control temporal and spatial specificity in *Aspergillus* development, Cell 57 (1989) 859–868.
[15] P. Xiao, K.S. Shin, T. Wang, J.H. Yu, *Aspergillus fumigatus flbB* encodes two basic leucine zipper domain (bZIP) proteins required for proper asexual development and gliotoxin production, Eukaryot. Cell 9 (2010) 1711–1723.
[16] D.M. Gardiner, B.J. Howlett, Bioinformatic and expression analysis of the putative gliotoxin biosynthetic gene cluster of *Aspergillus fumigatus*, FEMS Microbiol. Lett. 248 (2005) 241–248.
[17] M. Schrettl, S. Carberry, K. Kavanagh, H. Haas, G.W. Jones, J. O'Brien, et al., Self-protection against gliotoxin–a component of the gliotoxin biosynthetic cluster, GliT, completely protects *Aspergillus fumigatus* against exogenous gliotoxin, PLoS Pathog. 6 (2010) e1000952.
[18] D.H. Scharf, N. Remme, T. Heinekamp, P. Hortschansky, A.A. Brakhage, C. Hertweck, Transannular disulfide formation in gliotoxin biosynthesis and its role in self-resistance of the human pathogen *Aspergillus fumigatus*, J. Am. Chem. Soc. 132 (2010) 10136–10141.
[19] J.L. Brookman, D.W. Denning, Molecular genetics in *Aspergillus fumigatus*, Curr. Opin. Microbiol. 3 (2000) 468–474.
[20] E. Käfer, Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations, Adv. Genet. 19 (1977) 33–131.
[21] G. Pontecorvo, J.A. Roper, L.M. Hemmons, K.D. Macdonald, A.W. Bufton, The genetics of *Aspergillus nidulans*, Adv. Genet. 5 (1953) 141–238.
[22] O. Kniemeyer, F. Lessing, O. Scheibner, C. Hertweck, A.A. Brakhage, Optimisation of a 2-D gel electrophoresis protocol for the human-pathogenic fungus *Aspergillus fumigatus*, Curr. Genet. 49 (2006) 178–189.
[23] J.Y. Kim, H.J. Song, H.J. Lim, M.G. Shin, J.S. Kim, H.J. Kim, et al., Platelet factor-4 is an indicator of blood count recovery in acute myeloid leukemia patients in complete remission, Mol. Cell Proteomics 7 (2008) 431–441.
[24] H.S. Park, M. Ni, K.C. Jeong, Y.H. Kim, J.H. Yu, The role, interaction and regulation of the velvet regulator VelB in *Aspergillus nidulans*, PLoS One 7 (2012) e45935.
[25] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method, Methods 25 (2001) 402–408.
[26] J.W. Bok, N.P. Keller, LaeA, a regulator of secondary metabolism in *Aspergillus* spp, Eukaryot. Cell 3 (2004) 527–535.
[27] H.S. Park, Ö. Bayram, G.H. Braus, S.C. Kim, J.H. Yu, Characterization of the *velvet* regulators in *Aspergillus fumigatus*, Mol. Microbiol. 86 (2012) 937–953.
[28] Ö. Bayram, S. Krappmann, M. Ni, J.W. Bok, K. Helmstaedt, O. Valerius, S. Braus-Stromeyer, N.J. Kwon, N.P. Keller, J.H. Yu, et al., VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism, Science 320 (2008) 1504–1506.
[29] K. Kroll, V. Pähtz, F. Hillmann, Y. Vaknin, W. Schmidt-Heck, M. Roth, I.D. Jacobsen, et al., Identification of hypoxia-inducible target genes of *Aspergillus fumigatus* by transcriptome analysis reveals cellular respiration as an important contributor to hypoxic survival, Eukaryot. Cell 13 (2014) 1241–1253.
[30] M. Kobayashi, Y. Matsuo, A. Takimoto, S. Suzuki, F. Maruo, H. Shoun, Denitrification, a novel type of respiratory metabolism in fungal mitochondrion, J. Biol. Chem. 271 (1996) 16263–16267.
[31] T. Fujii, N. Takaya, Denitrification by the fungus *Fusarium oxysporum* involves NADH-nitrate reductase, Biosci. Biotechnol. Biochem. 72 (2008) 412–420.
[32] R.A. Cramer Jr., M.P. Gamcsik, R.M. Brooking, L.K. Najvar, W.R. Kirkpatrick, T.F. Patterson, et al., Disruption of a nonribosomal peptide synthetase in *Aspergillus fumigatus* eliminates gliotoxin production, Eukaryot. Cell 5 (2006) 972–980.
[33] C.J. Balibar, C.T. Walsh, GliP, a multimodular nonribosomal peptide synthetase in *Aspergillus fumigatus*, makes the diketopiperazine scaffold of gliotoxin, Biochemistry 45 (2006) 15029–15038.
[34] A.A. Brakhage, Regulation of fungal secondary metabolism, Nat. Rev. Microbiol. 11 (2013) 21–32.
[35] J.W. Bok, D. Chung, S.A. Balajee, K.A. Marr, D. Andes, et al., GliZ, a transcriptional regulator of gliotoxin biosynthesis, contributes to *Aspergillus fumigatus* virulence, Infect. Immun. 74 (2006) 6761–6768.
[36] D.H. Scharf, T. Heinekamp, N. Remme, P. Hortschansky, A.A. Brakhage, et al., Biosynthesis and function of gliotoxin in *Aspergillus fumigatus*, Appl. Microbiol. Biotechnol. 93 (2012) 467–472.