

Among Developmental Regulators, StuA but Not BrlA Is Essential for Penicillin V Production in *Penicillium chrysogenum*^{▽†}

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In filamentous fungi, secondary metabolism is often linked with developmental processes such as conidiation. In this study we analyzed the link between secondary metabolism and conidiation in the main industrial producer of the β -lactam antibiotic penicillin, the ascomycete *Penicillium chrysogenum*. Therefore, we generated mutants defective in two central regulators of conidiation, the transcription factors BrlA and StuA. Inactivation of either *brlA* or *stuA* blocked conidiation and altered hyphal morphology during growth on solid media, as shown by light and scanning electron microscopy, but did not affect biomass production during liquid-submerged growth. Genome-wide transcriptional profiling identified a complex StuA- and BrlA-dependent regulatory network, including genes previously shown to be involved in development and secondary metabolism. Remarkably, inactivation of *stuA*, but not *brlA*, drastically downregulated expression of the penicillin biosynthetic gene cluster during solid and liquid-submerged growth. In agreement, penicillin V production was wild-type-like in *brlA*-deficient strains but 99% decreased in *stuA*-deficient strains during liquid-submerged growth, as shown by high-performance liquid chromatography (HPLC) analysis. Thus, among identified regulators of penicillin V production StuA has the most severe influence. Overexpression of *stuA* increased the transcript levels of *brlA* and *abaA* (another developmental regulator) and derepressed conidiation during liquid-submerged growth but did not affect penicillin V productivity. Taken together, these data demonstrate an intimate but not exclusive link between regulation of development and secondary metabolism in *P. chrysogenum*.

The filamentous fungus *Penicillium chrysogenum* plays an important role in biotechnology as the main industrial producer of the β -lactam antibiotic penicillin, a secondary metabolite. A better understanding of parameters which directly or indirectly influence biosynthesis of penicillin is fundamental for optimizing industrial production of penicillin and possibly secondary metabolites in general. Previous studies revealed an association of fungal secondary metabolism with developmental processes such as asexual reproduction, termed conidiation (12). Both processes are associated with the stationary growth phase (3). In agreement, it recently has been shown that inactivation of either VelaA or LaeA, both components of the velvet-like complex, decreases conidiation and penicillin production in *P. chrysogenum* (27). On the other hand, inactivation of the G α subunit of a heterotrimeric G protein, Pga1, increases conidiation but decreases production of penicillin and the mycotoxin roquefortine, another secondary metabolite, in *P. chrysogenum* (19). Regulation of conidiation has been extensively studied in the ascomycete *Aspergillus nidulans*, but little is known about regulation of conidiation in *P. chrysogenum*. In response to nutrient sensing, *A. nidulans* conidia

(spores) swell and form germ tubes and subsequently septated hyphae. After gaining so-called “developmental competence” and exposure to air, hyphae initiate conidiation, which starts with the growth of an aerial stalk followed by the formation of metulae and phialides. These phialides give rise to chains of uninucleate conidia (2). Notably, nutritionally sufficient submerged culture allows vegetative growth but represses conidiation in *A. nidulans* and *P. chrysogenum*.

Conidiogenesis is under complex genetic control. In *A. nidulans*, FluG (assumed to be associated with the production of a small diffusible molecule) and FlbA (regulator of G-protein signaling) are key upstream activators of conidiation, both acting via inhibition of the repressive Zn₂C₆-type zinc finger transcription factor SfgA and FadA (ortholog of *P. chrysogenum* Pga1), respectively (36–38, 57). Four additional downstream transcription factors, FlbB, FlbC, FlbD, and FlbE, individually are required for transcriptional activation of *brlA* and subsequent conidiation (20, 21, 75, 76). Furthermore, a Pcl-like cyclin (54) and the cyclin-dependent kinase encoded by *nimX*^{Cdc2} of *A. nidulans* seem to mediate cell cycle events during developmental cell type formation (54, 55). BrlA together with AbaA and WetA forms a regulatory cascade, required for the formation of conidiophores and conidia. The transcription factors StuA and MedA are developmental modifiers required for correct conidiophore morphogenesis through spatial and temporal regulation of *brlA* and *abaA* expression (5, 39, 44). BrlA is a C₂H₂-type zinc finger transcription factor, which begins to accumulate shortly after induction of development (52). Two overlapping transcripts orig-

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TABLE 1. *P. chrysogenum* orthologs of genes previously implicated in conidiogenesis of *A. nidulans* or *A. fumigatus*

Gene	ID	Species	<i>P. chrysogenum</i> gene	ID	e value ^a	Reference
<i>abaA</i>	AAA33286	<i>A. nidulans</i>	<i>abaA</i>	Pc16g09610	0.0	5
<i>abr1</i>	XP_756089.2	<i>A. fumigatus</i>	<i>abrA</i>	Pc21g16380	0.0	68
<i>abr2</i>	ACJ13064.1	<i>A. fumigatus</i>	<i>yA</i>	Pc22g08420	0.0	68
<i>alb1</i>	EAL94057	<i>A. fumigatus</i>	<i>wA</i>	Pc21g16000	0.0	68
<i>arp1</i>	XP_756093.1	<i>A. fumigatus</i>	<i>arpA</i>	Pc21g16420	4e-65	68
<i>arp2</i>	XP_756091.1	<i>A. fumigatus</i>	<i>arpB</i>	Pc21g16430	7e-100	68
<i>asp f4</i>	XP_749515	<i>A. fumigatus</i>	<i>asp f4</i>	Pc16g11310	6e-77	16
<i>ayg1</i>	EDP55259.1	<i>A. fumigatus</i>	<i>aygA</i>	Pc21g16440	6e-162	68
<i>brlA</i>	AAB48671	<i>A. nidulans</i>	<i>brlA</i>	Pc06g00470	2e-147	1
<i>catA</i>	AAC49254	<i>A. nidulans</i>	<i>catA</i>	Pc20g06360	0.0	45
<i>dewA</i>	CBF73692	<i>A. nidulans</i>	<i>dewA</i>	Pc16g06690	3e-17	64
<i>fadA</i>	AAC49476	<i>A. nidulans</i>	<i>pga1</i>	Pc13g12240	0.0	19
<i>flbA</i>	AAA73955	<i>A. nidulans</i>	<i>flbA</i>	Pc15g00600	0.0	36
<i>flbB</i>	CAM35586.1	<i>A. nidulans</i>	<i>flbB</i>	Pc12g01640	7e-138	18
<i>flbC</i>	ACP28867	<i>A. nidulans</i>	<i>flbC</i>	Pc12g12190	5e-124	75
<i>flbD</i>	AAA61913	<i>A. nidulans</i>	<i>flbD</i>	Pc13g03170	5e-93	21
<i>flbE</i>	ACP28868	<i>A. nidulans</i>	<i>flbE</i>	Pc16g12460	2e-47	20
<i>fluG</i>	AAC37414	<i>A. nidulans</i>	<i>fluG</i>	Pc20g02420	1e-120	37
<i>laeA</i>	AAQ95166	<i>A. nidulans</i>	<i>laeA</i>	Pc16g14010	9e-134	7
<i>medA</i>	AAC31205	<i>A. nidulans</i>	<i>medA</i>	Pc22g13450	0.0	10
<i>nimX</i>	AAA20597	<i>A. nidulans</i>	<i>nimX</i>	Pc20g10270	7e-145	54
<i>pclA</i>	CAC06384	<i>A. nidulans</i>	<i>pclA1</i>	Pc16g08700	1e-118	55
			<i>pclA2</i>	Pc03g00090	8e-63	
			<i>pclA3</i>	Pc22g26190	2e-69	
<i>phiA</i>	CAA09585	<i>A. nidulans</i>	<i>phiA</i>	Pc22g00190	3e-52	40
<i>ppoC</i>	AAT36614	<i>A. nidulans</i>	<i>ppoC</i>	Pc18g00240	0.0	69
<i>rodA</i>	AAA33321	<i>A. nidulans</i>	<i>rodA</i>	Pc22g14290	5e-52	63
<i>rodB</i>	XP_753093.1	<i>A. fumigatus</i>	<i>rodB</i>	Pc21g18350	2e-19	51
<i>sfgA</i>	AA99779	<i>A. nidulans</i>	<i>sfgA</i>	Pc21g18560	3e-177	57
<i>stuA</i>	CBF70741	<i>A. nidulans</i>	<i>stuA</i>	Pc13g04920	9e-180	41
<i>tmpA</i>	AAP13095	<i>A. nidulans</i>	<i>tmpA</i>	Pc21g07830	2e-170	38
<i>veA</i>	AAD42946	<i>A. nidulans</i>	<i>veA</i>	Pc13g13200	2e-112	61
<i>velB</i>	ABQ17967	<i>A. nidulans</i>	<i>velB</i>	Pc22g22320	2e-77	11
<i>vosA</i>	ABI51618	<i>A. nidulans</i>	<i>vosA</i>	Pc22g06890	3e-104	46
<i>wetA</i>	AAA33330	<i>A. nidulans</i>	<i>wetA</i>	Pc22g03220	3e-138	39

^a Homology (at the amino acid level) of the *P. chrysogenum* gene product to the corresponding *Aspergillus nidulans* or *Aspergillus fumigatus* gene product.

inate from the *brlA* locus, *brlA* α and *brlA* β , both having essential functions for normal development (25). *brlA*-deficient mutants are blocked in development at an early stage in the transition from polar growth of the conidiophore stalk to the swelling of the vesicle. The stalks somehow elongate indeterminately and fail to differentiate, which gives the mutants a “bristle” appearance (14). Furthermore, the mutants fail to accumulate other developmentally regulated transcripts like *abaA* and *wetA* (9). Forced expression of *brlA* in submerged cultures is sufficient to transcriptionally activate *abaA* and *wetA* and to induce conidiation (1). StuA is a basic helix-loop-helix-like transcription factor with an APSES DNA-binding motif (17). Expression of *stuA* is upregulated with the onset of developmental competence and remains at that level during conidiation (41). Similarly to *brlA* expression, *stuA* expression results in two overlapping and differentially regulated transcripts. Conidiophores of *stuA* null mutants have shortened stalks and reduced vesicles and lack metulae and phialides. In contrast to *brlA* mutants, *stuA* mutants still produce few conidia, which directly bud from the vesicle as shown in *A. nidulans* and *A. fumigatus* (42, 58). In *A. nidulans*, deficiency in StuA, but not BrlA, also impairs sexual development (35, 78).

BrlA orthologs are present only in *Aspergillus*, *Neosartorya*, *Penicillium*, and *Talaromyces* species. In contrast, StuA is evolutionary widely conserved and orthologs have been shown to reg-

ulate development in *A. fumigatus*, *Penicillium marneffei*, *Neurospora crassa*, *Fusarium oxysporum*, *Glomerella cingulata*, *Saccharomyces cerevisiae*, and *Candida albicans* (6, 8, 22, 50, 58, 62, 67, 72). In *Aspergillus fumigatus*, 6 of 22 putative secondary metabolite-encoding gene clusters were found to be StuA dependent (15, 70), including clusters for biosynthesis of gliotoxin, pseurotin A, and the alkaloids fumigaclavin and fumitremorgin, whereas BrlA affects expression of only 2 of these gene clusters: fumigaclavin positively and fumitremorgin negatively. In *A. nidulans*, the synthesis of the mycotoxin sterigmatocystin is influenced positively by FluG and FlbA but negatively by FadA. In contrast, penicillin production is positively affected by FadA (26, 57, 65).

The genetic program controlling development in *P. chrysogenum* appears to be highly similar to that of aspergilli, as the *P. chrysogenum* genome encodes orthologs of all regulators identified in *A. nidulans* (Table 1). In this study, we demonstrate that in *P. chrysogenum* inactivation of either *brlA* or *stuA* blocks conidiation but affects expression of genes involved in development and secondary metabolism partially differently. In particular, penicillin production was found to be absolutely dependent on *stuA* but not *brlA*.

MATERIALS AND METHODS

Strains and culture conditions. *Escherichia coli* strain K-12 Fusion-Blue (*recA endA*) was used for plasmid construction (In-Fusion Dry-Down PCR cloning kit;

Clontech). Reproduction and maintenance of plasmids were done according to standard protocols (53). *P. chrysogenum* carrying $\Delta Pcku70$ (herein referred to as the $\Delta ku70$ mutant) (28), derived from *P2niaD18* (herein referred to as the *niaD18* mutant) carrying a mutated *niaD* gene (29), served as the parental strain in all transformation experiments (see Table S1 in the supplemental material). Additionally, most assays included the *niaD18* mutant as an additional control. Fungal strains were cultivated in liquid complete CCM (43) or minimal MM1 medium for 48 to 120 h at 25°C and 230 rpm or on solid minimal medium MM2 as already described (23). For sporulation assays, strains were grown on solid complete medium CM2 [3.5 g/liter (NH₄)₂SO₄, 2 g/liter K₂SO₄, 0.2 g/liter KH₂PO₄, 1 g/liter of soy flour, 5 g/liter limestone powder, 50 g/liter lactose, 20 g/liter agar] for transcript analysis on CM2 on sterile membranes (Supor-200; Pall Life Sciences, USA) or in liquid production medium (28). Cultures were inoculated with 5×10^4 spores for point inoculations on plates, with 2×10^6 spores for inoculation of whole plates (sporulation assay and microarrays) and 1×10^7 spores for liquid cultures. In studies including nonsporulating mutants, cultures were inoculated with 5 μ l, 200 μ l, and 500 μ l of liquid 72-h CCM cultures for point inoculation, whole plate assays, and liquid cultures, respectively.

Transformation of *P. chrysogenum*. Transformation was performed as described by Windhofer et al. (77) with some modifications. Liquid CCM medium cultures were incubated for 48 h at 25°C and 230 rpm. Protoplasts were transformed by linear PCR, restriction fragments, or circular plasmids 4.103, 4.104, 4.122, 4.127, 4.128, 4.196, 4.181, 4.182, and 4.177 (see Table S2 in the supplemental material). Transformants were selected on either terbinafine (0.9 μ g/ml) or phleomycin (45 μ g/ml) containing MM2 supplemented with 0.5 g/liter arginine and either 2.5% xylose or 2.5% glucose depending on the promoter used for the selection marker gene. Screening of positive clones was performed by PCR (data not shown). To obtain homokaryotic transformants, colonies from single homokaryotic spores were picked and homologous integration of the constructs was verified by Southern blot analysis.

Identification of *P. chrysogenum* genes *stuA* and *brlA*. The genomic sequence of *P. chrysogenum* ATCC 28089 (71) served as a source for the sequences used here. The sequences for the *P. chrysogenum* genes *brlA* (CAP79040.1) and *stuA* (CAP91561.1) were obtained from the public database NCBI Entrez (<http://www.ncbi.nlm.nih.gov/sites/gquery>). Protein sequence alignments were performed with the ClustalW program (66).

Preparation of nucleic acids, hybridization protocol, and PCR. Fungal genomic DNA for Southern blot analysis was isolated from hyphal cells, grown for 3 days at 25°C and 230 rpm in liquid CCM medium, based on protoplasting as previously described (80). RNA extraction for microarray and quantitative real-time PCR (qRT-PCR) analysis from mycelia grown on solid complete CM2 or in liquid production medium was done using Trizol reagent (no. 15596-018; Invitrogen), the RNeasy midikit (no. 75144; Qiagen) and the DNase I kit (no. 79254; Qiagen) for DNase I treatment according to manufacturer's protocols. For PCR analysis, transformants were grown on solid complete CM2 medium for 6 days at 25°C and mycelia or spores were washed off with H₂O. A 5- μ l portion of this suspension was used for DNA extraction with the REDExtract-N-Amp seed PCR kit (no. 086K6825; Sigma).

For clonings, PCR amplifications were performed with the Accu Prime Pfx DNA polymerase (no. 12344-024; Invitrogen) with "proofreading" activity, for screening approaches with the REDTaq DNA polymerase (no. R4775; Sigma), according to the manufacturer's instructions.

Southern hybridization was carried out with Duralon-UV membranes (no. 420101; Stratagene) and digoxigenin-labeled probes (PCR DIG probe synthesis kit, no. 11636090910; Roche). The attached probes were detected with the DIG luminescence detection kit (no. 11363514910; Roche). All techniques were used according to the method of Sambrook and Russell (53).

Quantification of penicillin (penicillin V) and biomass production. For quantification of penicillin productivity, 5 g culture broth from shake flask cultures grown for 144 h at 25°C and 230 rpm in a production medium were analyzed by high-performance liquid chromatography (HPLC) (56). For quantification of biomass production, 5-ml portions of culture broth (grown as mentioned above) from each sample were filtered and dried for 18 h at 100°C.

Quantification of conidiation. Spores (2×10^6) were spread on four independent CM2 plates per strain and incubated for 168 h at 25°C. Areas of 1 cm in diameter were cut out from each plate, pooled, and vortexed together with five small glass balls in 5 ml M3 (8.5 g/liter NaCl, 1 ml/liter Tween 80) for 1.5 min. Spores of each sample were counted three times in the Thoma chamber.

Microarray analysis. The custom-designed Affymetrix GeneChip DSM_PENa520255F, representing the genome of *P. chrysogenum* Wisconsin54-1255, was used for gene expression quantification (71). RNA was isolated from the strains carrying the $\Delta ku70$, $\Delta brlA$, and $\Delta stuA$ mutations after 36, 48, 60, 72,

and 96 h of growth on solid complete CM2 plates separated from the medium by dialysis membranes. RNA of three biological replicates was pooled for sample preparation and microarray hybridization (28). Differentially expressed genes were determined using the R/Bioconductor packages limma and affyGUI (60, 74); raw data were preprocessed using the implemented RMA algorithm (31, 32). Correlation analysis was performed using GeneSpring 7.2 (Silicon Genetics) and a correlation coefficient of ≥ 0.9 . Heat maps were generated with the program TreeView by Michael Eisen (version 1.60; Howard Hughes Medical Institute, University of California at Berkeley [<http://rana.lbl.gov/EisenSoftware.htm>]).

Quantitative real-time PCR (qRT-PCR). RNA was extracted from three biological replicates of the *niaD18*, $\Delta ku70$, $\Delta brlA$ and $\Delta stuA$ strains in a time course experiment on solid CM2 plates (see microarray analysis). Furthermore, from three biological replicates of the $\Delta ku70$ and *stuA*-OE strains, RNA was isolated in a time course experiment in liquid production medium after 48, 72, 96, 120 and 144 h. From all RNA samples, cDNA was synthesized with the High-Capacity cDNA archive kit (no. 4322171; ABI). qRT-PCR was carried out with 4 to 8 ng/ μ l cDNA. Detection was based on the SYBR green I dye detection system (no. 4309155; ABI) and measured with a 7900HT Fast-Realtime PCR system (Applied Biosystems). qRT-PCR assays were performed in triplicate for each sample. Data analysis was performed according to the method of Nowrousian et al. (48). All values were normalized to the housekeeping gene *actA* (Pc20g11630) encoding γ -actin.

Scanning electron microscopy. Strains were grown on solid complete CM2 plates at 25°C for 48 h and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). A brief washing in the same buffer was followed by 1 h of postfixation with 1% aqueous OsO₄, gradual dehydration with ethanol, and critical point drying with liquid CO₂ (Bal-Tec CPD 030; Balzers, Liechtenstein). Specimens were mounted on aluminum stubs with Leit C (Göcke, Plano GmbH), sputtered with 10 nm Au/Pd (MED 020; Balzers, Liechtenstein), and examined with a Zeiss field emission scanning electron microscope (FESEM, Gemini 982).

RESULTS AND DISCUSSION

Identification of the *P. chrysogenum* *stuA* and *brlA* orthologs.

P. chrysogenum *brlA* and *stuA* were identified by homology searches as described in Materials and Methods. *P. chrysogenum* *brlA* encodes a protein of 433 amino acids showing 63% and 62% identity to the orthologs of *A. nidulans* and *A. fumigatus*, respectively (see Fig. S1 in the supplemental material). *P. chrysogenum* *stuA* encodes a protein of 818 amino acids. Alignment of the predicted *P. chrysogenum* StuA protein shows 62% to 67% identity with the corresponding published amino acid sequences of *A. flavus*, *Neosartorya fischeri*, *A. nidulans*, and *A. fumigatus*, with *A. flavus* StuA being the most similar ortholog. Interestingly, compared to the *A. nidulans* and *A. fumigatus* orthologs, which are annotated as proteins 622 and 635 amino acids in length, respectively, the annotated StuA orthologs of *P. chrysogenum*, *A. flavus*, and *N. fischeri* contain an "N-terminal extension" of about 200 amino acids. TBLASTN searches revealed that the amino acid sequence of the N-terminal extension also is highly conserved in the *A. nidulans* and *A. fumigatus* orthologs, indicating misannotation of these two StuA-encoding orthologs (see Fig. S2 in the supplemental material).

Generation of *brlA* and *stuA* deletion strains. Deletion of either the *brlA* or the *stuA* locus was performed by replacement of the respective coding region with the terbinafine resistance gene *ergA* under the control of the xylose-inducible *xyIP* promoter (59, 80) using the bipartite marker technique (47) in a $\Delta ku70$ mutant (28), as described in Fig. S3A and B and "Generation of transformation fragments" in the supplemental materials. A total of 42 *brlA* knockout strains out of 221 transformants and 27 *stuA* knockout strains out of 50 transformants were verified by Southern blot analysis after reisolation. South-

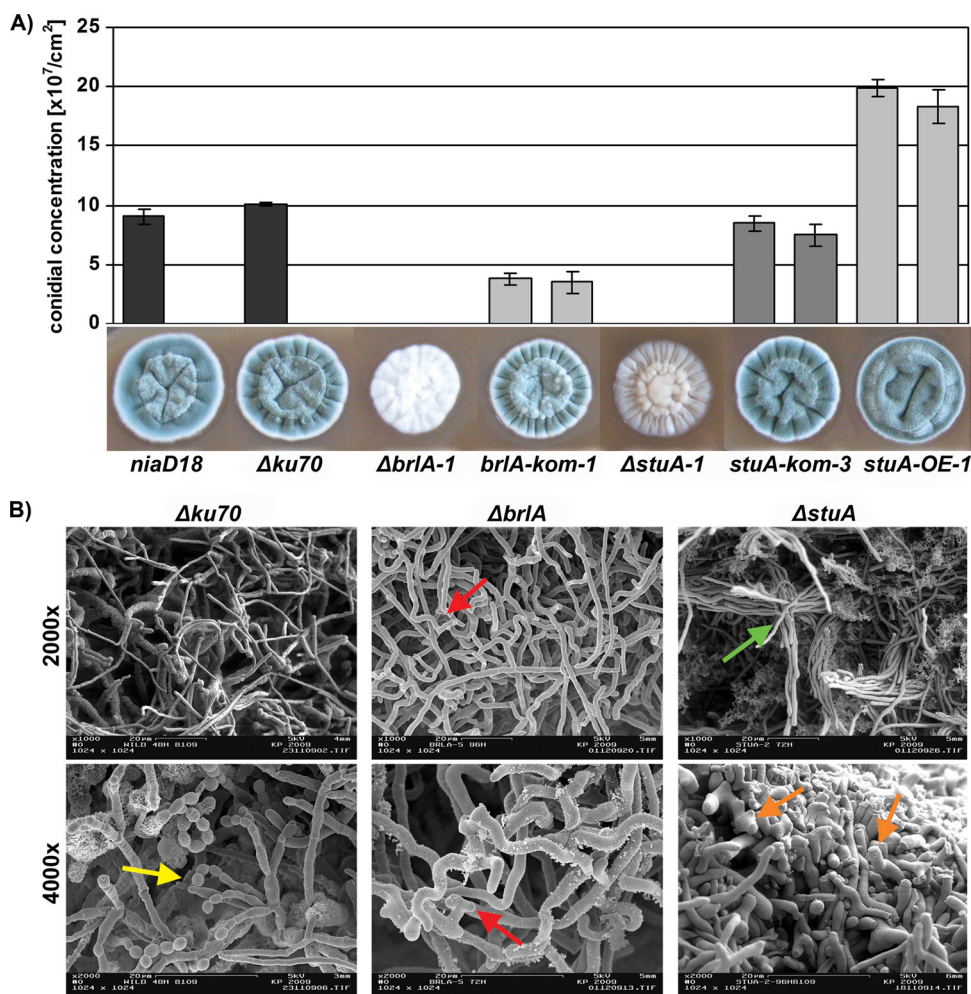


FIG. 1. Characterization of $\Delta brlA$ and $\Delta stuA$ strains compared to the recipient $\Delta ku70$ and *niaD18* mutant strains. (A) Colony phenotypes on solid complete CM2 after growth for 168 h at 25°C. Quantification of conidial formation in mutant and complemented strains was performed in triplicate and repeated twice with two independent primary transformants of each strain; bars show standard deviations. (B) Scanning electron microscopy of $\Delta brlA$ and $\Delta stuA$ mutants. Both mutants lack conidia and conidiophores (yellow arrow indicates conidia on conidiophores in the $\Delta ku70$ mutant); the $\Delta brlA$ strain displayed zigzag growth and increased and abnormal branching (red arrows); $\Delta stuA$ strain hyphae were bundled at the surface of the cultures (green arrow) and showed increased fragmentation (orange arrow).

ern blot analysis confirmed the homologous integration of the selection marker as well as the loss of the gene to be deleted (see Fig. S3A and B in the supplemental material). In conclusion, the homologous *brlA* and *stuA* recombination frequencies correspond to about 19 and 54%, respectively, clearly reinforcing the qualification of the *P. chrysogenum* $\Delta ku70$ mutant as a valuable recipient for gene deletion experiments. For simplicity, only three deletants each of *brlA* and *stuA* were used for further analysis.

For reconstitution of the $\Delta brlA$ mutant, a functional copy of the *brlA* gene was inserted ectopically. This was achieved by transforming the $\Delta brlA$ mutant with plasmid p4.122 containing the *brlA* gene and the *ergA* selection marker gene driven by the *P. chrysogenum* gene γ -actin promoter (Pc20g11630). This promoter exchange enables a second selection on terbinafine with glucose instead of xylose as a carbon source in the selection medium, which represses the *xyIP*-driven expression of *ergA* (80). Four transformants, designated *brlA-kom*, were proved to carry the *brlA* gene after single-spore reisolation and Southern

blot analysis (see Fig. S3C in the supplemental material). For reconstitution, the $\Delta stuA$ strain was transformed with plasmid p4.196 carrying a functional copy of the *stuA* gene and the Tn5 phleomycin selection marker gene of *E. coli* driven by the *ipnA* promoter of *P. chrysogenum*. Three transformants were identified as positive complementation strains (designated *stuA-kom*), after single-spore reisolation and Southern blot analysis (see Fig. S3D). Interestingly, further PCR and Southern blot analyses (data not shown) demonstrated that in all three *stuA-kom* strains the entire plasmid p4.196 integrated homologously at the *stuA* locus by single crossover.

Inactivation of either *brlA* or *stuA* blocks conidiation and causes aberrant hyphal morphology. Primary transformants lacking *brlA* or *stuA* did not produce conidia and therefore had to be propagated in hyphal form. Growth tests starting with point inoculation of defined amounts of vegetative hyphae on CM2 plates confirmed that both $\Delta brlA$ and $\Delta stuA$ strains are completely blocked in conidiation, as evident from visual (Fig. 1A) and light microscopic inspection (data not shown). In

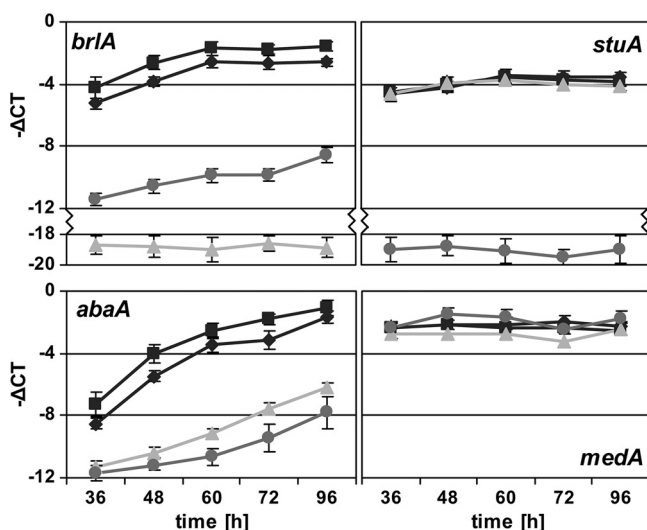


FIG. 2. Expression of *brlA*, *stuA*, *abaA*, and *medA* in *niaD18*, $\Delta ku70$, $\Delta brlA$, and $\Delta stuA$ strains in a time course experiment measured by qRT-PCR. The results are given as means $\pm$ standard deviations of results for independent biological triplicates with technical triplicates. Black diamonds, *niaD18*; black squares, $\Delta ku70$; light gray triangles, $\Delta brlA$; medium gray circles, $\Delta stuA$.

contrast, *stuA* deficiency in both *A. nidulans* and *A. fumigatus* results in the production of extremely shortened conidiophores that lack metulae and phialides but produce conidia directly from buds formed on the conidiophore vesicle (2, 58).

The complemented *brlA-kom* and *stuA-kom* strains showed restored conidiation, demonstrating that the conidiation defect was indeed caused by the deletion of the respective gene. Interestingly, the $\Delta brlA$ strain formed wild-type-like white mycelia whereas the $\Delta stuA$ mycelia were brown pigmented, indicating deregulation of pigment biosynthesis (see below).

Scanning electron microscopy of 48-h surface cultures visualized formation of conidiophores and conidia in the $\Delta ku70$ mutant, while the $\Delta brlA$ and $\Delta stuA$ mutants lacked these structures (Fig. 1B). Moreover, hyphae of the $\Delta brlA$ mutant showed zigzag growth and hyperbranching; hyphae of the $\Delta stuA$ mutant congregated to thick bundles and displayed numerous stumps, possibly indicating increased susceptibility to fragmentation (Fig. 1B). Measurement of the biomass production after 144 h in submerged culture indicated that inactivation of either *brlA* or *stuA* has no significant effect on the vegetative growth rate (see Fig. S4 in the supplemental material).

Inactivation of either *brlA* or *stuA* decreases expression of developmental regulators. In a time course experiment, samples were taken after 36 h to 96 h of growth on solid medium. qRT-PCR measurements revealed an increase of *brlA* and *abaA* transcript levels, whereas *stuA* and *medA* levels remained largely constant in both $\Delta ku70$ and *niaD18* strains (Fig. 2). A difference in threshold cycle values ($-\Delta C_T$) of <-18 (i.e., a decrease of <18 in C_T) confirmed the deletion of the respective genes in $\Delta brlA$ and $\Delta stuA$ mutants (Fig. 2). Remarkably, *brlA* was severely downregulated in the $\Delta stuA$ mutant ($-\Delta C_T$ of -8 , equaling about 250-fold), while *stuA* transcript levels remained unaltered in the $\Delta brlA$ mutant, which indicates that StuA regulates the *brlA* transcript level but not vice versa.

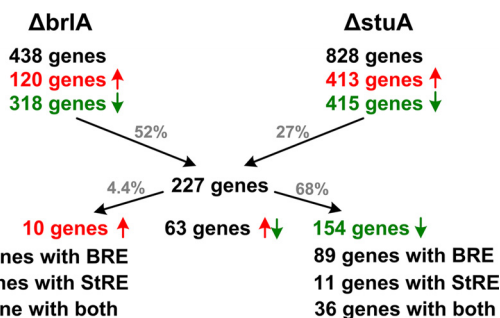


FIG. 3. Schematic summary of the genome-wide comparative expression profiling of $\Delta brlA$ and $\Delta stuA$ strains compared to the $\Delta ku70$ strain. Genes were scored as deregulated when their expression was ≥ 3 -fold changed at ≥ 3 consecutive time points compared to the $\Delta ku70$ strain. BRE and StRE elements in forward, reverse, or both orientations within 1,000 bp upstream of the start codons were considered.

Moreover, inactivation of either *brlA* or *stuA* decreased expression of *abaA* ($-\Delta C_T$ of up to -6 , equaling about 60-fold) but did not affect the *medA* transcript level. Interestingly, the *brlA* transcript level was still upregulated during the time course in the $\Delta stuA$ mutant and the *abaA* transcript level was still upregulated during the time course in the $\Delta stuA$ and $\Delta brlA$ mutants, demonstrating that expression of both *brlA* and *abaA* is responsive to more than one regulatory mechanism. Taken together, these data reveal a regulatory cascade with BrlA activating expression of *abaA* and StuA activating expression of *brlA* and *abaA* during conidiogenesis, whereby StuA might influence *abaA* indirectly via BrlA.

Microarray analysis of $\Delta brlA$ and $\Delta stuA$ mutants reveals a complex regulatory network. In order to gain more insight into molecular changes associated with the gene deletions of *brlA* and *stuA*, the $\Delta ku70$, $\Delta brlA$ and $\Delta stuA$ mutant strains were compared by genome-wide transcriptome analysis. For a time course study, RNA was isolated from all strains grown on solid CM2 for 36, 48, 60, 72 and 96 h. Formation of conidia was observed after 48 h of growth for the $\Delta ku70$ mutant but not the $\Delta brlA$ and $\Delta stuA$ mutants. All strains showed similar radial growth rates, and therefore differences in the expression profiles of the two mutant strains are not expected to be caused by differences in growth rate.

In the $\Delta ku70$ strain, orthologs of 17 genes previously found to encode regulatory and structural genes upregulated during conidiogenesis in *A. nidulans* or *A. fumigatus* (2, 51, 68) were upregulated ≥ 3 -fold at three or more time points compared to their status at the 36-h time point, e.g., the hydrophobin-encoding genes and the pigment biosynthetic genes (Table 1; see Fig. 4A to D). In contrast, transcript levels of *stuA* and *medA* did not significantly change during the time course. The microarray-based expression profiles of *brlA*, *abaA*, *stuA*, and *medA* nicely fit to the qRT-PCR analyses shown here (Fig. 2). A schematic summary of the transcription profiling of the $\Delta ku70$, $\Delta brlA$, and $\Delta stuA$ mutants is shown in Fig. 3. Compared to the results for the $\Delta ku70$ mutant, 438 genes were ≥ 3 -fold deregulated at three or more consecutive time points in the $\Delta brlA$ mutant, with 27% of these genes being upregulated and 73% being downregulated. In the $\Delta stuA$ mutant, 828 genes were deregulated, with 50% being upregulated and 50% being downregulated. A total of 227 deregulated genes are common

to both $\Delta brlA$ and $\Delta stuA$ mutants, a majority, 68%, of which were downregulated (see Table S4 in the supplemental material). Within 1 kb of the 5' upstream region, 58% of these genes contain at least one BrlA response element (BRE) (M RAGGGR) (13), 7% contain at least one StuA response element (StRE) (WCGCGWNM) (17), and 23% contain both elements. Taking into account the downregulation of *brlA* by inactivation of *stuA* (see above), these data indicate that StuA might regulate the majority of these genes rather indirectly via BrlA. The genes downregulated in both $\Delta brlA$ and $\Delta stuA$ mutants enclosed orthologs of nine genes, including *abaA*, previously found to be involved in conidiogenesis in *A. nidulans* (Table 1; Fig. 4B). Gene expression correlation analysis indicated that 93 genes are coregulated with *abaA* (see Table S5 in the supplemental material), 85 of which are downregulated in both $\Delta brlA$ and $\Delta stuA$ mutants, including seven of the nine orthologs previously identified to be involved in conidiogenesis in *A. nidulans* (Fig. 4B). Of the 154 genes downregulated in both $\Delta brlA$ and $\Delta stuA$ mutants and of the 93 genes coregulated with *abaA*, 93.5% and 94.6%, respectively, contain at least one AbaA-responsive element (CATTCY, identified in *A. nidulans* [5]) within 1 kb of the 5' upstream region. These data indicate that the majority of genes downregulated in the $\Delta brlA$ and $\Delta stuA$ mutants and coregulated with *abaA* are regulated via AbaA. Interestingly, this gene set also includes an ortholog of Asp F4 (Table 1), an allergen of *A. fumigatus* (16), which indicates developmental regulation of allergen formation. Orthologs of seven other genes previously found to be involved in conidiogenesis in *A. nidulans*, including *wetA*, were downregulated in the $\Delta brlA$ mutant but upregulated at early time points in the $\Delta stuA$ mutant (Table 1, Fig. 4C). Analysis of *wetA* coregulated genes identified six of these seven genes and additional 38 genes (Fig. 4C; see Table S6 in the supplemental material). This suggests that these genes are activated by BrlA (possibly via AbaA) but are temporally repressed by StuA, in both cases most likely via WetA. A proposed regulatory scheme is summarized in Fig. S5 in the supplemental material.

The derepression of genes encoding early steps of conidial melanin synthesis (*wA*, *arpA*, *arpB*, and *aygA*) combined with the repression of genes encoding late steps (*abrA* and *yA*) might explain the brownish pigmentation of the $\Delta stuA$ hyphae (Fig. 1A and 4B and C), in particular as deletion of *abrA* and *yA* changes the conidial color from green to brown in *A. fumigatus* (34, 68). In agreement, in the $\Delta brlA$ mutant, all these pigment genes were repressed, resulting in white hyphae (Fig. 1A and 4B and C).

The 212 genes downregulated in the $\Delta stuA$ mutant but not the $\Delta brlA$ mutant included *pclA1* and *ppoC* (Fig. 4D). Interestingly, *P. chrysogenum* possesses three genes (*pclA1*, *pclA2*, and *pclA3*) homologous to the single *pcl*-like cyclin-encoding *pclA* of *A. nidulans* (55), whereas the three paralogs display different expression patterns (Fig. 4B and D). *ppoC* encodes a fatty acid oxygenase, contributing to the production of a secreted lipogenic signal molecule, that together with other factors (*ppoA*, *ppoB*) governs timing and balance of sexual and asexual development in *A. nidulans* (69).

Other genes implicated in conidiogenesis in *A. nidulans* were largely unaffected or affected ≤ 3 -fold by inactivation of *brlA* and *stuA*, e.g., *medA*, *phiA*, *sfgA*, *pga1*, *velA*, *laeA*, and the

"fluffy" genes *fluG*, *tmpA*, *flbA*, *flbB*, *flbC*, *flbD*, and *flbE* (Table 1 and data not shown).

Similarly to *P. chrysogenum*, a complex regulatory network involving BrlA, StuA, AbaA, and WetA with several feedback loops controlling conidiogenesis has been characterized previously in *A. nidulans* and, in a more fragmentary form, in other fungal species (1, 8, 49, 58). The numerous genes identified in this study to be affected by deletion of *brlA*, *stuA*, or both, as well as the genes found to be coregulated with *abaA* and *wetA*, will now aid further elucidation of conidiogenesis of *P. chrysogenum* and other fungal species.

Inactivation of *stuA* or *brlA* affects expression of secondary metabolite-encoding genes. van den Berg et al. (71) identified 51 genes encoding putative nonribosomal peptide synthases (NRPS), polyketide synthases (PKS), or hybrid enzymes postulated to be part of 39 gene clusters. This indicates that *P. chrysogenum* has the potential to produce a variety of secondary metabolites. The genome-wide expression profiling revealed that inactivation of either BrlA or StuA deregulates expression ≥ 3 -fold of six of these putative secondary metabolite-encoding clusters (for the cluster definition, see the legend to Fig. 4) and nine single genes encoding NRPS or PKS (Fig. 4E and F). Of these gene clusters, only one has been functionally characterized and shown to be responsible for biosynthesis of the β -lactam antibiotic penicillin. The cluster containing the putative NRPS Pc21g15480 and the tryptophan dimethylallyl transferase Pc21g15430 (*dmaW*) most likely encodes the biosynthesis of the mycotoxin roquefortine C (33, 71). Roquefortine C, a prenylated indole derivative containing tryptophan and histidine moieties, was isolated first from *Penicillium roqueforti* and identified later in many *Penicillium* strains, including *P. chrysogenum*. Roquefortine C is also the precursor for further prenylated indole alkaloids such as meleagrine, oxaline, and glandicolines (79). Among all StuA- or BrlA-regulated gene clusters, the penicillin and roquefortine C biosynthetic gene clusters were affected most significantly. Expression of both clusters was largely unaffected in the $\Delta brlA$ strain but drastically downregulated in the $\Delta stuA$ strain (Fig. 4E). qRT-PCR analysis confirmed downregulation of the penicillin biosynthetic genes *pcbAB* ($-\Delta C_T$ of -7 , equaling about 130-fold), *pcbC* ($-\Delta C_T$ of -7 , equaling about 130-fold), and *penDE* ($-\Delta C_T$ of -9 , equaling about 500-fold) in the $\Delta stuA$ mutant but not the $\Delta brlA$ mutant at all time points (Fig. 5A).

***stuA* but not *brlA* is essential for PENV production.** To further analyze the involvement of *stuA* and *brlA* in secondary metabolism, penicillin V (PENV) production was measured in liquid cultures. In agreement with expression patterns of PENV biosynthesis genes scored on solid medium, PENV production was wild-type-like in the $\Delta brlA$ mutant and 99% reduced in the $\Delta stuA$ mutant (Fig. 5B). Complementation of $\Delta stuA$ with a functional *stuA* copy restored productivity to wild-type levels, reinforcing the crucial role of *stuA* in penicillin biosynthesis. qRT-PCR analysis confirmed that inactivation of *stuA* but not *brlA* blocks PENV production by transcriptional downregulation of the penicillin biosynthetic genes *pcbAB*, *pcbC*, and *penDE* in liquid medium cultures (Fig. 5C) as well as in solid medium cultures as shown above (Fig. 5A). These data also show that the developmental regulator StuA, which is essential for conidiation, also plays a role during submerged

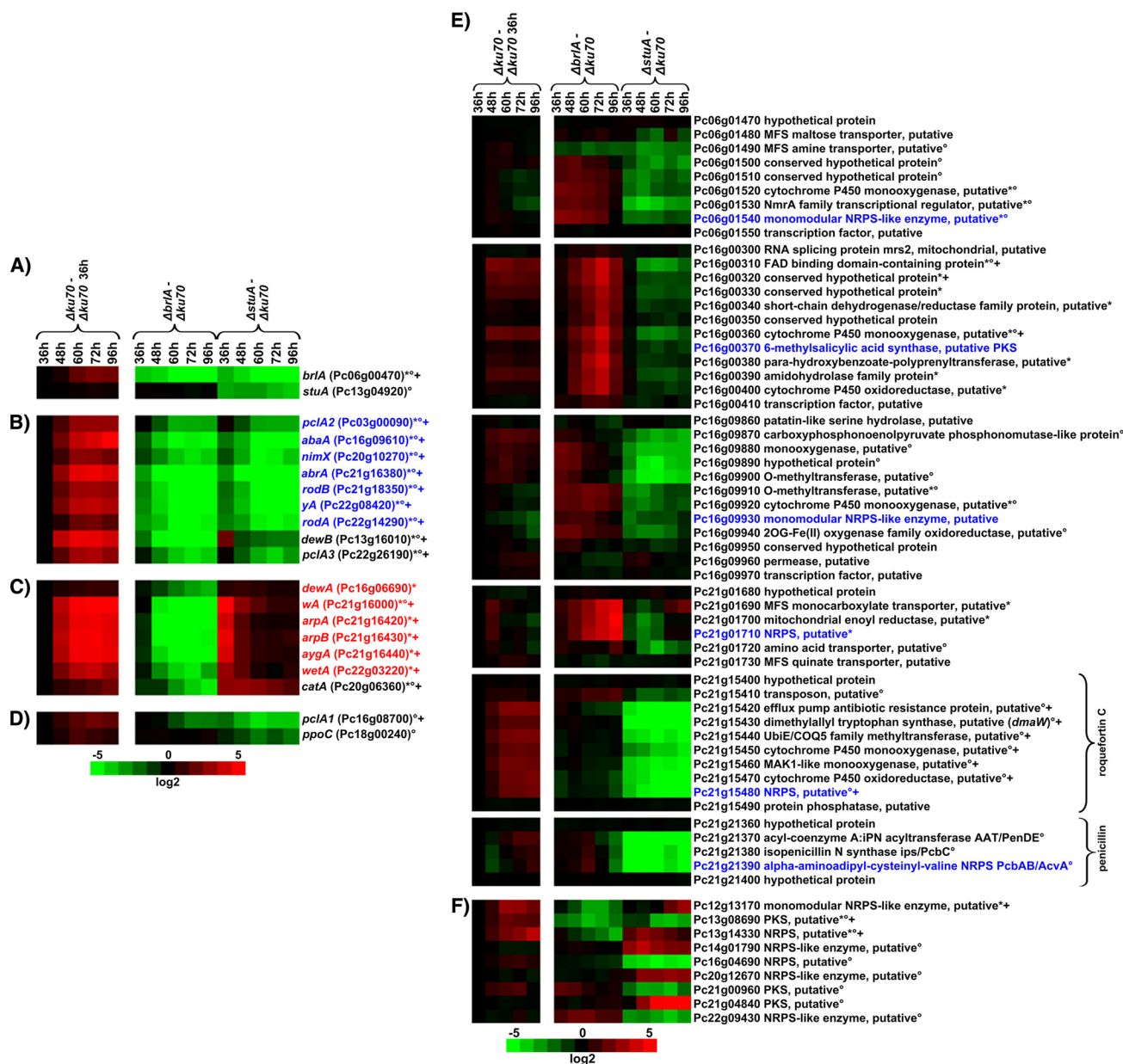


FIG. 4. Heat map representation of the microarray analysis of $\Delta brlA$ and $\Delta stuA$ mutants compared to the $\Delta ku70$ mutant, including orthologs of genes previously implicated in conidiogenesis in *A. nidulans* or *A. fumigatus* and genes potentially involved in secondary metabolism. (A) *brlA* and *stuA* mutants. (B) Conidiogenesis-related genes downregulated in both $\Delta brlA$ and $\Delta stuA$ mutants. (C) Conidiogenesis-related genes downregulated in the $\Delta brlA$ mutant. (D) Conidiogenesis-related genes downregulated in the $\Delta stuA$ mutant. (E) Gene clusters containing NRPS- or PKS-encoding genes deregulated in either the $\Delta brlA$ or the $\Delta stuA$ mutant or both. (F) Individual NRPS- or PKS-encoding genes deregulated in either the $\Delta brlA$ or $\Delta stuA$ mutant. The left column displays the time course expression in the $\Delta ku70$ mutant, with all values normalized to the respective 36-h value; +, genes upregulated ≥ 3 -fold at three or more consecutive time points compared to results at the 36-h time. The right column displays the change in transcript levels in $\Delta brlA$ and $\Delta stuA$ mutants, respectively, normalized to the same time point as the $\Delta ku70$ mutant. Values are shown in log₂ scale in green for downregulation and red for upregulation. * and °, genes deregulated ≥ 3 -fold at three or more consecutive time points compared to $\Delta ku70$ in $\Delta brlA$ and $\Delta stuA$ strains, respectively. *abaA* coregulated and *wetA* coregulated genes are shown in blue and red, respectively. Gene clusters were regarded as deregulated when three or more genomically neighboring genes were ≥ 3 -fold deregulated at three or more consecutive time points; gaps of single less-regulated genes were allowed for three or more neighboring genes; the heat map representation of regulated gene clusters includes the flanking unregulated genes. NRPS and PKS in panel E are shown in blue.

growth. StuA has the most severe influence on PENV production among all identified regulators yet. Recently, deficiency in LaeA and VelA was shown to decrease PENV production in the same *P. chrysogenum* strain grown under identical conditions by approximately 80% (27). In *Aspergillus fumigatus*, 6 of

22 putative secondary metabolite-encoding gene clusters were found to be StuA dependent (15, 70), including those for synthesis of gliotoxin, pseurotin A, and the alkaloids fumigaclavin and fumitremogin. Moreover, the StuA ortholog was found to play a crucial role in production of the mycotoxin

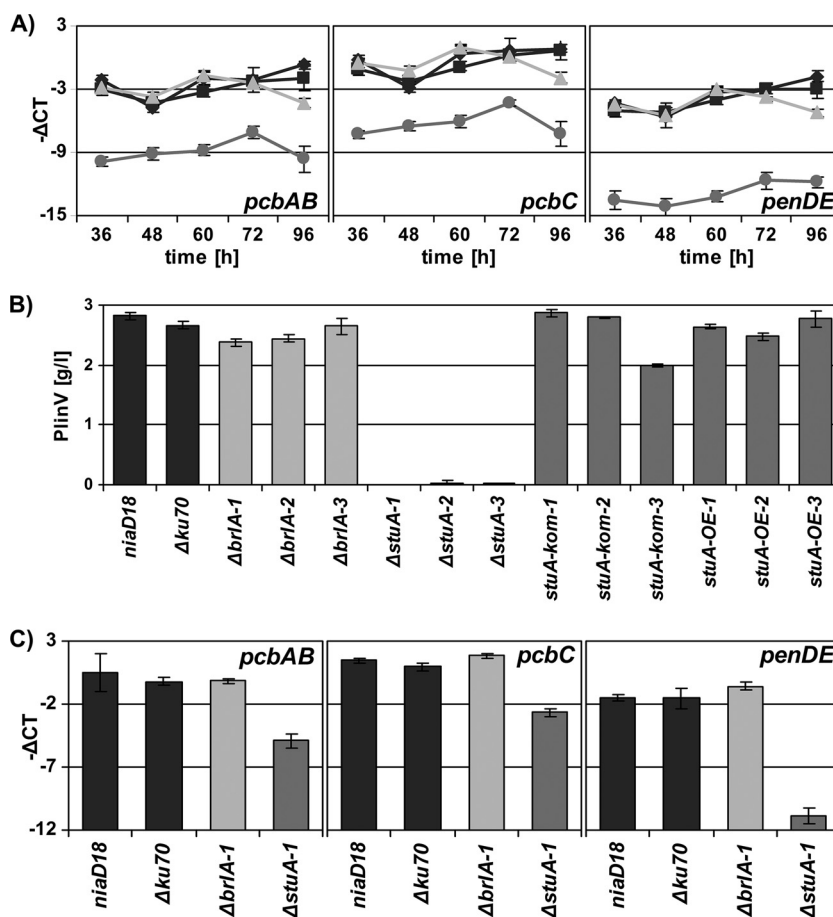


FIG. 5. Penicillin biosynthesis by $\Delta brlA$ or $\Delta stuA$. (A) qRT-PCR analysis of the expression of penicillin biosynthetic genes in a time course experiment. (B) Quantification of PENV production by HPLC analysis. Mean values $\pm$ standard deviations are derived from biological sextuplicates. (C) qRT-PCR analysis of the expression of the penicillin biosynthetic genes in panel B. The results are given as means $\pm$ standard deviations of three independent biological with three technical triplicates. Black diamonds, *niaD18*; black squares, $\Delta ku70$; light gray triangles, $\Delta brlA$; medium gray circles, $\Delta stuA$.

alternariol in *Stagnospora nodorum* (30), underlining the impact of StuA orthologs on secondary metabolism.

Overexpression of *stuA* derepresses conidiation during submerged growth but does not affect PENV production. To further analyze the role of *stuA* in conidiogenesis and PENV production, *stuA* was overexpressed by replacement of its native promoter by the *Pc22g-P* promoter, which drives the constitutively highly expressed gene *Pc22g10010* (28) in the $\Delta ku70$ strain as described in "Generation of transformation fragments" in the supplemental material. Six of 13 transformants, termed *stuA-OE*, were proved, by single-spore reisolation and Southern blot analysis, to carry the promoter replacement (see Fig. S3E in the supplemental material). *stuA-OE* colonies appeared phenotypically identical on CM2 plates compared to the recipient strain but displayed an approximately doubled production of conidia (Fig. 1A). Remarkably, overexpression of *stuA* resulted in heavy conidiation during submerged growth (Fig. 6A), causing dark green staining of the culture broth. qRT-PCR demonstrated that the promoter replacement indeed caused overexpression of *stuA*, up to 4 $-\Delta C_T$ units, equaling about 16-fold (Fig. 6B). Moreover, qRT-PCR analysis proved that *stuA* overexpression causes upregulation of

brlA ($-\Delta C_T$ of up to 3, equaling about 8-fold) and *abaA* ($-\Delta C_T$ of up to 6, equaling about 60-fold), which underlines the positive role of *stuA* in regulation of *brlA* and *abaA* identified in the *stuA* deletion analysis (see above). Consequently, the induction of conidiation during submerged growth might be due to upregulation of *stuA*, *brlA*, *abaA*, or a combination. In *A. nidulans*, overexpression of either *fluG*, *flbA*, *tmpA*, *flbC*, or *flbD* but not *flbB* was shown to induce *brlA* expression and conidiation in submerged cultures (2, 18, 38). Moreover, overexpression of *brlA* induces the expression of *abaA* and *wetA* and conidiation during submerged growth in *A. nidulans* (1, 24), while overexpression of *abaA*, *stuA*, and *wetA* does not result in conidiation (17, 24, 39). In further contrast to results with *P. chrysogenum*, forced expression of *stuA* in submerged cultures leads to repression of *abaA* in *A. nidulans*, indicating differences despite the general conservation of the regulatory system in these two species.

In contrast to conidiation, *stuA* overexpression did not influence PENV production (Fig. 5B). In agreement, the transcript levels of the penicillin biosynthesis genes *pcbAB*, *pcbC*, and *penDE* were similar in *stuA-OE* and $\Delta ku70$ strains (Fig. 6B).

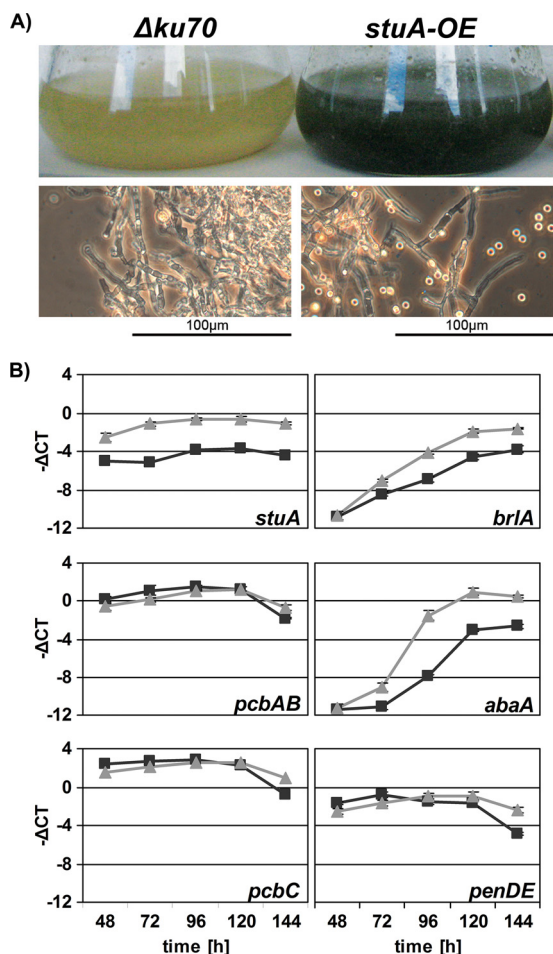


FIG. 6. (A) *stuA* overexpression derepresses conidiation in submerged growth in minimal medium. MM1-containing flasks were inoculated with $\Delta ku70$ and *stuA*-OE-1 strains and incubated for 120 h at 25°C. (B) Expression of penicillin biosynthetic genes, *stuA*, *brlA*, and *abaA* in *stuA*-OE-1 and $\Delta ku70$ strains, measured by qRT-PCR. The results are given as means $\pm$ standard deviations of independent biological triplicates with technical triplicates. Black squares, $\Delta ku70$; light gray triangles, *stuA*-OE-1.

Analysis of a putative StuA-binding site in the *penDE* promoter. A search in the promoter regions of *pcbAB*, *pcbC*, and *penDE* strains for a putative StuA response element (StRE) (WCGCGWNM) identified in *A. nidulans* (17) revealed a single putative StRE 50 bp upstream of the *penDE* start codon. In order to analyze its functionality, StuA-binding activity was inactivated by mutation of two nucleotides within the core sequence, as described previously in *A. nidulans* (17). Native and mutated *penDE* promoters were fused to the *E. coli uidA* reporter gene and integrated at the *Pcku70* locus using the terbinafine resistance gene *ergA* as a selection marker, driven by the *acnP* promoter, as described in “Generation of transformation fragments” in the supplemental material. After single-spore reisolation, Southern blot analysis demonstrated that 3 out of 9 transformants carried the reporter constructs with the native promoter (strain *GUS*) and 9 out of 27 transformants with the mutated promoter (strain *GUS-m*) (see Fig. S3F in the supplemental material). Notably, growth rate,

conidiation, and penicillin production were wild-type-like in all reporter strains, indicating that the transformation procedure did not result in side effects (data not shown). qRT-PCR analyses did not reveal differences in the expression of *uidA* in the reporter strains carrying the native and mutated *penDE* promoter (see Fig. S6 in the supplemental material), indicating either that the putative StuA-binding site is not functional or that the performed mutation did not inactivate StuA binding.

Conclusion. Taken together, these data indicate an intimate but not exclusive link between regulation of conidiation and secondary metabolism in *P. chrysogenum*. Inactivation of *stuA* was demonstrated to block both conidiation and PENV production. Consistently, deficiency in *LaeA* and *VelA* was recently shown to decrease conidiation and PENV production in the same *P. chrysogenum* strain grown under identical conditions by 80% (27), underlining the link between development and secondary metabolism. On the other hand, inactivation of the $G\alpha$ subunit of a heterotrimeric G protein, *Pga1*, increases conidiation but decreases production of penicillin and roquefortine C (19), and inactivation of *brlA* blocks conidiation but does not affect PENV biosynthesis in *P. chrysogenum* (see above).

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Supplementary Data: Generation of transformation fragments

Plasmid px-ergA (5) was used as basis for the generation of the marker split fragments needed for disruption of *brlA* using the bipartite marker technique (4). Firstly the two flanking regions were amplified from genomic DNA using primers brlA-3-for and -rev and brlA-5-for and -rev. The 2.2kb 3'-flanking region was inserted into the 6.7kb *NotI-XbaI* vector fragment of plasmid px-ergA and the 2.1kb 5'-flanking region into the 8.5kb *EcoRI-SalI* vector fragment of plasmid px-ergA using the In-FusionTM Dry-Down PCR Cloning Kit (Clontech, No. 32.53.720.211), resulting in plasmids p4.103 and p4.104. For generation of the marker split fragments a 4.7kb PCR fragment containing the 3'-flanking region and part of the *ergA* selection marker was amplified from plasmid p4.103 with primers brlA-3-rev-1 and pergA-rev-1, and a 3.6kb PCR fragment containing the 5'-flanking region and part of the *ergA* gene was amplified from plasmid p4.104 with primers brlA-5-for-2 and pergA-for-11. The two PCR fragments shared a 517bp overlap within the selection marker gene. During transformation, homologous integration of each fragment allows recombination of the *ergA* fragments and generation of the intact resistance gene at the site of recombination.

Disruption of *stuA* was achieved as described for *brlA* deletion using primers stuA-C-for and -rev and stuA-N-for and -rev to amplify the 3'- and 5'-flanking regions from genomic DNA, which were integrated into the above described vector fragments of px-ergA resulting in plasmids p4.128 and p4.127. With primers stuA-C-rev-1 and pergA-rev-1 the generation of the 4.0kb marker split fragment containing the 3'-flanking region and part of the *ergA* selection marker from plasmid p4.128 was performed, and with primers stuA-N-for-1 and pergA-for-11 the 3.1kb fragment containing the 5'-flanking region and part of *ergA* from plasmid p4.127. The two PCR fragments shared a 517bp overlap within the selection marker gene.

For reconstitution of the *brlA* deletion strain $\Delta brlA$ with a functional *brlA* copy, the 1.7kb *NotI*-*MscI* fragment of plasmid px-ergA containing the xylanase promoter *xylP* was replaced by the 0.6kb *NotI*-*MscI* digested *acnP* promoter fragment of *P. chrysogenum* which was amplified using primers acnP-Prom-5' and acnP-Prom-3', resulting in plasmid pa-ergA. This promoter replacement allows a second selection on terbinafine by adding 2.5% glucose instead of xylose to the selection medium MM2. A 3.7kb PCR fragment, amplified with primers pbrlA-for-k and -rev-k from genomic DNA, was then subcloned into the *NotI* linearised vector pa-ergA using infusion cloning. The resulting 9.4kb plasmid p4.122 was used circular to transform $\Delta brlA$ protoplasts. For reconstitution of the *stuA* deletion strain $\Delta stuA$ with a functional *stuA* copy, the *stuA* gene including 5'- and 3'-regions was amplified in a first step from genomic DNA with primers stuA-kom-for and -rev. This 4.95kb fragment was then subcloned into the 5.7kb *NotI* vector fragment of plasmid pa-ergA. In the resulting plasmid p4.183 the terbinafine selection marker was replaced by a phleomycin selection marker. This was done by amplifying a 1.9kb PCR fragment containing the Tn5 phleomycin gene under the control of the *ipnA* promoter of *P. chrysogenum* and the *cytI* terminator of *Saccharomyces cerevisiae* with primers ipns-P-for and cyt1-T-rev from plasmid pBC1003 (2) and inserting it into the 8.2kb *NotI*-*SfuI* vector fragment of 4.183. The resulting plasmid p4.196 was used circular to transform $\Delta stuA$ protoplasts.

For targeted over-expression of *stuA* at the respective gene locus the native promoter was replaced by the bidirectional *P. chrysogenum* promoter region situated between ORF Pc22g10000 (coding for a permease of the major facilitator superfamily with a strong similarity to NTG1, the acetylglucosamine transporter in *Candida albicans* (1)) and ORF Pc22g10010 (coding for a protein with strong similarity to the N-acetylglucosamine-6-phosphate deacetylase, CaNAG2 of *C. albicans* (6)). In array analysis both genes have been shown to be expressed at very high levels during the life cycle of *P. chrysogenum* (3). In this study the bidirectional promoter region is termed *Pc22g-P*. For the construction of a *stuA*

promoter replacement cassette, first the *PciI*-restriction site in pBluescript II SK (+) was blunt-ended. Then a 1.04kb C-terminal truncated PCR product of *ergA*, amplified with primers *pergA(m)-for* and *pergA(m)-rev*, was inserted into the 2.9kb *XbaI-SacI* pBluescript-II-SK(+) vector fragment using the In-FusionTM Dry-Down PCR Cloning Kit. The resulting plasmid was digested with *PciI-EcoRI* and the isolated 3.9kb plasmid fragment fused with the 861bp PCR fragment of *Pc22g-P*, amplified with primers *Pc22g-for* and *Pc22g-rev*, creating a translational fusion between the *Pc22g-P* promoter and the truncated *ergA* gene. Finally, a 1.4kb PCR-fragment of a C-terminal truncated form of *stuA* amplified with primers *stuA-trunc-for* and *-rev* from genomic DNA was inserted into the 4.7kb *NcoI-HindIII* vector fragment, carrying the C-terminal truncated form of the selection marker gene *ergA* under the control of the *P. chrysogenum* promoter *Pc22g-P*. Within the resulting plasmid p4.177 the N-terminus of *stuA* was translationally fused to the *Pc22g-P* promoter. For transformation of the *P. chrysogenum* *Aku70* (3) strain using the bipartite marker technique the 3.2kb *PmeI* fragment of p4.177 was combined with a 3.1kb PCR fragment containing the *stuA* 5'-flanking region and an N-terminal truncated part of the *ergA* gene including the *P. chrysogenum* *acnP* terminator, amplified with primers *pergA-for-11* and *stuA-N-for-1* from plasmid p4.127. The fragments shared a 530bp overlap within the selection marker gene *ergA*.

For the analysis of the putative StuA-binding-site in the *penDE* promoter, the promoter with a mutated and an unmutated binding site was fused to the *uidA* reporter gene of *E. coli*. For this purpose in a first step a 1.7kb PCR fragment was amplified with primers *pactP-for-k-1* and *pergA-rev-k-1* from plasmid *pa-ergA*, containing a C-terminal truncated form of the *ergA* selection marker gene and the *P. chrysogenum* *acnP* promoter, and then inserted into the 2.9kb *SacII-NotI* vector fragment of plasmid pBluescript-II-SK+ using the In-FusionTM Dry-Down PCR Cloning Kit. The resulting plasmid was digested with *BamHI-NotI* and the isolated 4.6kb vector fragment fused with the *P. chrysogenum* *acnP* terminator containing 654bp PCR fragment, amplified with primers *acnP-T5B* and *acnP-T3B*. In a next step the

1.3kb *Pcku70* 5' flanking region, amplified with primers musA-N-for-2 and -rev-2, was inserted into the *HindIII/PstI* digested 5.2kb vector fragment. Subsequently, the 1.9kb *uidA* gene, amplified with primers uidA-5GB and -3GB, was cloned into the 6.4kb *SmaI-NarI* vector fragment. The resulting plasmid was digested with *SphI* and *PstI* and the isolated 8.3kb vector fragment was fused with the 1.3kb *P. chrysogenum penDE* promoter, amplified with primers penDE-P-for and -rev from genomic DNA, resulting in plasmid p4.181 (9.6kb).

To mutate the putative StuA-binding-site 58bp upstream of ATG two PCR fragments were generated, overlapping within the binding site. Using plasmid p4.181 as a template PCR-A (448bp) was performed with primers penDE-P-for-1 and -rev-1, PCR-B (147bp) with primers penDE-P-for-2 and uidA-rev, with primers penDE-P-rev-1 and penDE-P-for-2 both bearing the point mutation of GC to AT within the putative binding site. A *PstI-BsmI* digested 535bp PCR fusion of PCR-A and -B with primers penDE-P-for-1 and uidA-rev was ligated with the 9.0kb *PstI-BsmI* vector fragment of p4.181 resulting in plasmid p4.182. For transformation of the *P. chrysogenum Δku70* (3) strain using the bipartite marker technique, the 6.6kb *PmeI* fragments of both p4.181 and p4.182 were each combined with a 3.8kb PCR fragment containing the *Pcku70* 3'-flanking region and a N-terminal truncated part of the *ergA* gene, amplified with primers pergA-for-11 and musA-C-rev-1 from plasmid p4.180. The fragments shared a 530bp overlap within the selection marker gene. Plasmid p4.180 resulted from the fusion of the 6.7kb *HindIII* vector fragment of plasmid px-ergA with the 2.1kb 3'-flanking region of *Pcku70*, amplified with primers musA-c-for and -rev (primers see Supplementary Table S3).

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An_BrlA	S	P	T	T	P	I	R	S	C	S	L	G	T	G	S	G	A	D	T	P	L	S	R	L	S	G	G	P	C	E	Y	M	D	D	F	Q	P	S	P	V	Y	R	D	G	-	F	Q	R	P	H	
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Pc_BrlA	R	Q	P	S	R	K	S	S	K	K	Q	L	L	R	S	N	L	S	L	E	N	L	P	S	I	I	K	Q	V	Q	F	K	C	K	E	P	G	C	K	G	R	F	K	R	Q	E	H	L	K	R	
Af_BrlA	R	Q	P	S	R	K	M	A	R	K	Q	P	S	K	Q	S	L	S	L	E	N	L	P	S	I	I	K	Q	V	Q	F	K	C	K	E	P	G	C	K	G	R	F	K	R	Q	E	H	L	K	R	
An_BrlA	R	V	A	S	R	K	M	L	R	R	Q	T	S	K	Q	N	L	M	L	E	N	L	P	Q	V	I	K	Q	V	Q	F	K	C	K	E	P	G	C	N	G	R	F	K	R	Q	E	H	L	K	R	
		360		370		380		390		400																																									
Pc_BrlA	H	M	K	S	H	S	K	E	K	P	H	V	C	W	I	P	G	C	H	R	A	F	S	R	S	D	N	L	N	A	H	Y	T	K	T	H	S	K	R	G	G	R	N	R	Y	V	A	T	L	D	
Af_BrlA	H	M	K	S	H	S	K	E	K	P	H	V	C	W	V	P	G	C	H	R	A	F	S	R	S	D	N	L	N	A	H	Y	T	K	T	H	S	K	R	G	G	R	N	R	Y	V	A	T	L	D	
An_BrlA	H	M	K	S	H	S	K	E	K	P	H	V	C	W	V	P	G	C	H	R	A	F	S	R	S	D	N	L	N	A	H	Y	T	K	T	H	S	K	R	G	G	R	N	R	Y	V	A	T	L	D	
		410		420		430		440		450																																									
Pc_BrlA	E	T	S	Q	D	F	D	P	D	F	R	G	Q	L	T	P	D	G	R	P	I	Y	G	S	K	L	E	D	T	M	P	D	C	G	E	L	S	V	D	G	W	D	D	D							
Af_BrlA	E	T	S	P	D	Y	N	P	D	Y	R	G	P	L	T	A	D	G	R	P	M	P	G	G	T	L	D	E	S	M	P	-	S	R	E	I	S	M	E	-	W	D	E	E							
An_BrlA	E	N	S	P	D	Y	D	P	E	F	R	G	Q	L	T	P	D	G	R	P	I	Y	G	S	K	L	D	D	P	I	P	G	A	G	D	M	S	L	D	G	W	D	E	E							

FIG. S1. Protein sequence alignment of *P. chrysogenum* BrlA with the corresponding amino acid sequences of *A.nidulans* AnBrlA and *A. fumigatus* AfBrlA.

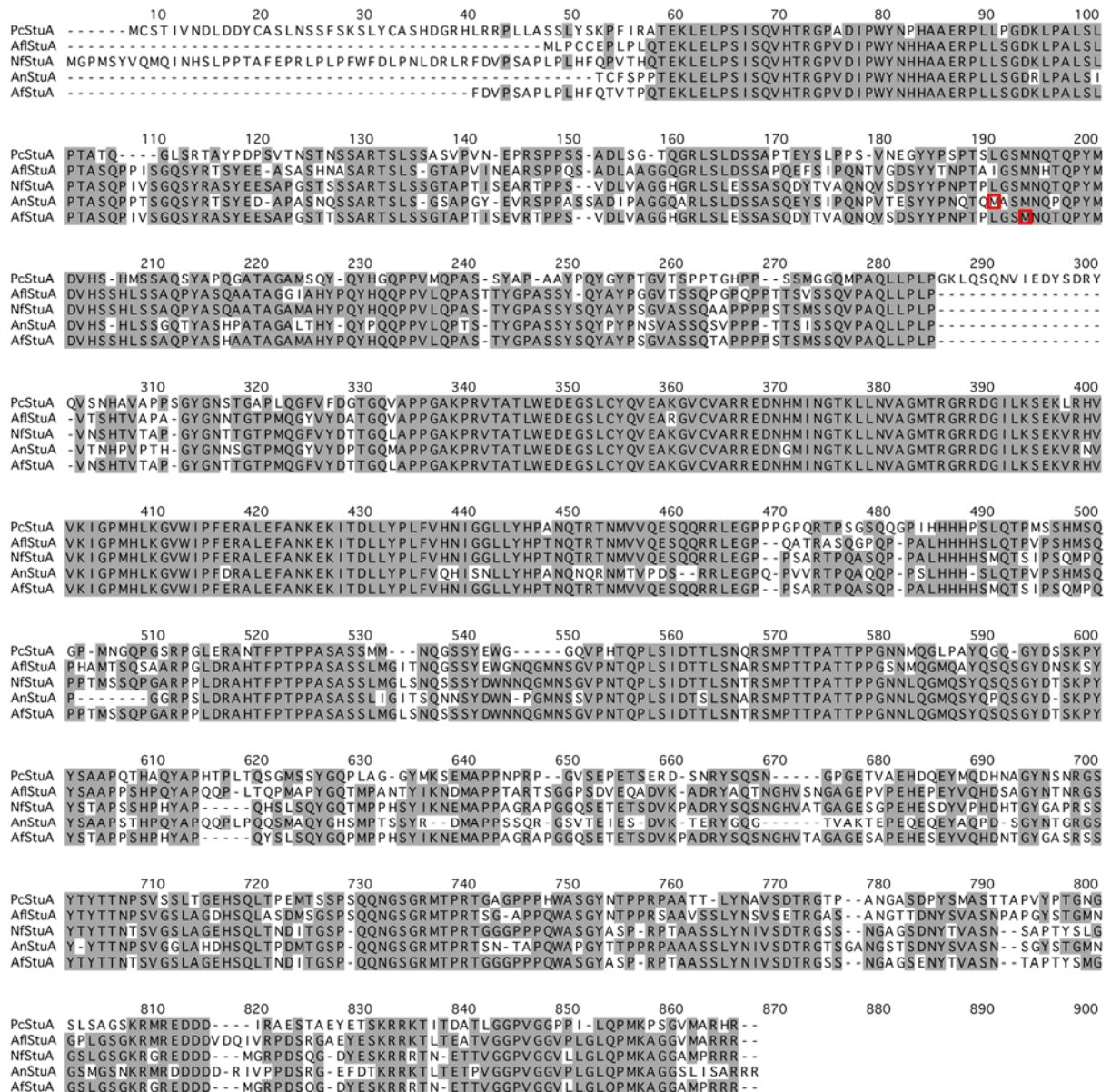


FIG. S2. Protein sequence alignment of *P. chrysogenum* StuA with the corresponding amino acid sequences of *A. flavus* AflStuA, *Neosartorya fischeri* NfStuA, *A. nidulans* AnStuA and *A. fumigatus* AfStuA. The published translation starts (1,2) of *A. nidulans* and *A. fumigatus* orthologs are indicated by red squares.

1. Miller, K. Y., J. Wu, and B. L. Miller. 1992. StuA is required for cell pattern formation in *Aspergillus*. *Genes Dev.* 6:1770-1782.
2. Sheppard, D. C., T. Doedt, L. Y. Chiang, H. S. Kim, D. Chen, W. C. Nierman, and S. G. Filler. 2005. The *Aspergillus fumigatus* StuA protein governs the up-regulation of a discrete transcriptional program during the acquisition of developmental competence. *Mol. Biol. Cell* 16:5866-5879.

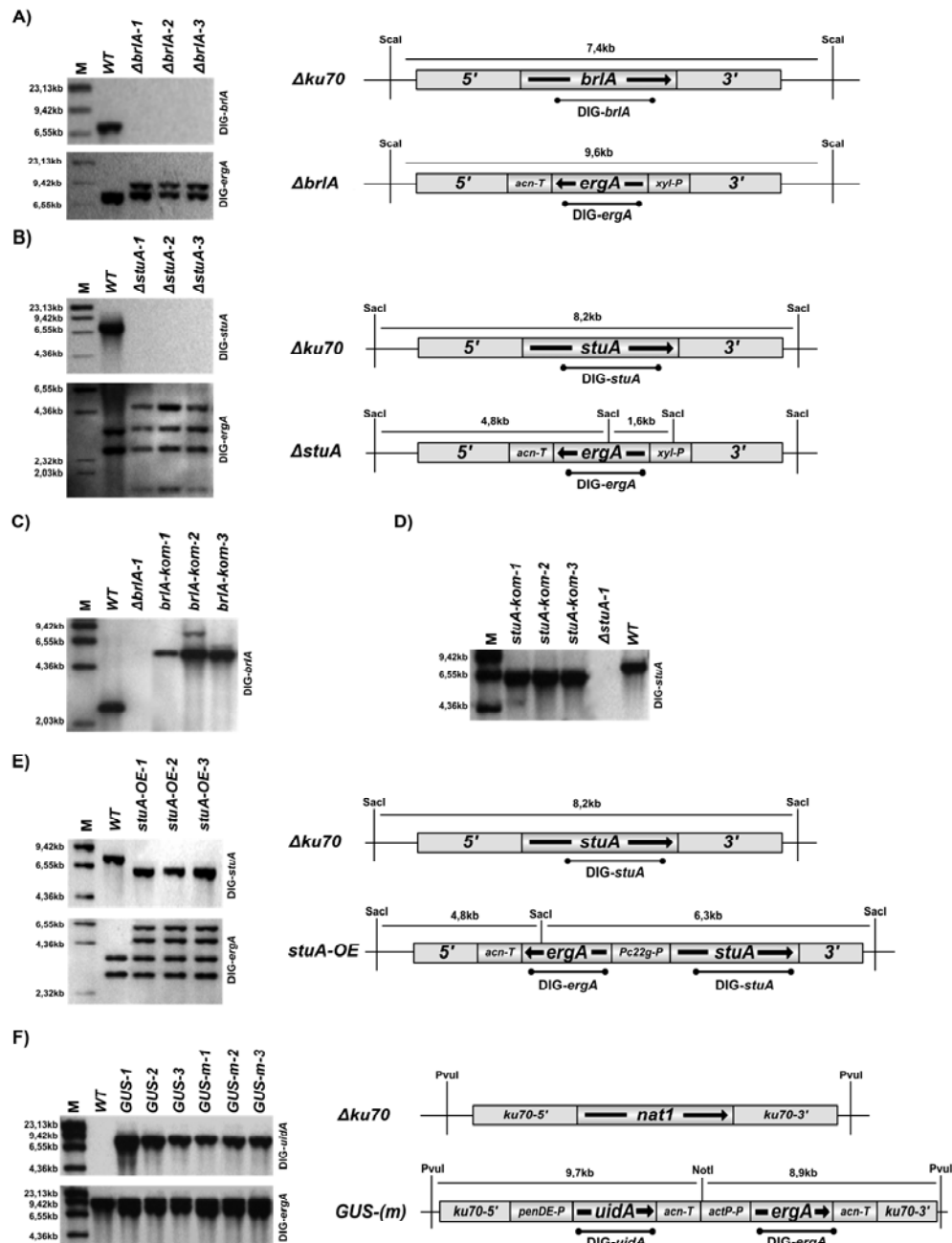


FIG. S3. Generation of the *P. chrysogenum* gene deletion strains $\Delta briA$ (**A**) and $\Delta stuA$ (**B**), the respective complemented strains *briA-kom* (**C**) and *stuA-kom* (**D**), the *stuA*-overexpressing strain *stuA-OE* (**E**), and the promoter-reporter-gene strains *GUS* and *GUS-m* (**F**). The DIG-labelled probes and restriction enzymes used are indicated at the right margin of the Southern blots. The *ergA* probe also visualises the endogenous *ergA* gene as 7.9kb signal after *ScaI* digestion, as 2.6 and 3.3kb signals after *SacI* digestion and as 12.5kb signal after *PvuI* digestion.

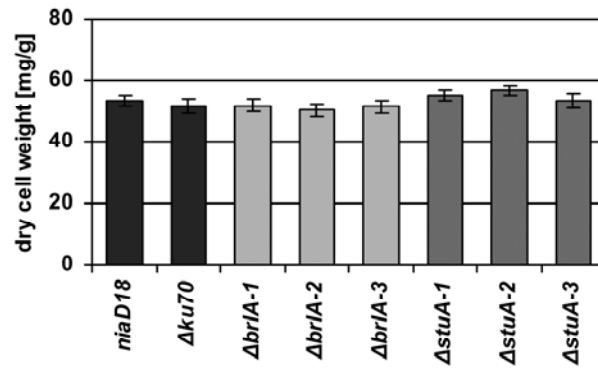


FIG. S4. Dry weight of strains $\Delta brlA$ and $\Delta stuA$ grown in production medium for 96h compared to *niaD18* and $\Delta ku70$. The dry weight was determined as mean values with $\pm$ STD of three independent experiments.

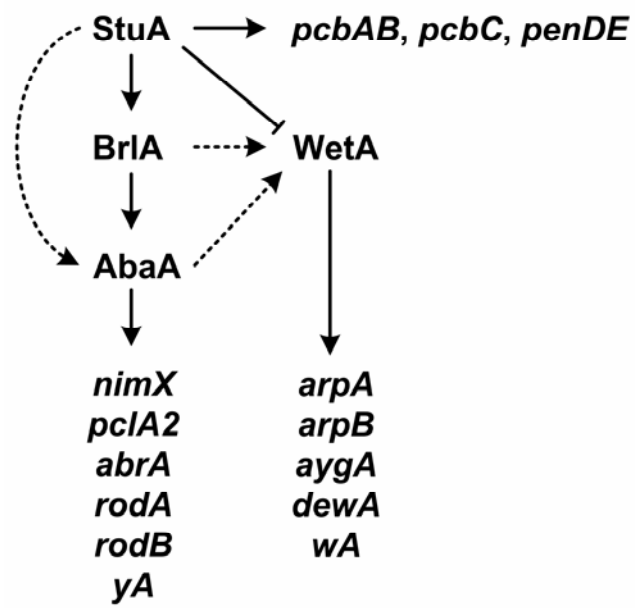


FIG. S5. Regulatory network controlling conidiogenesis in *P. chrysogenum*.

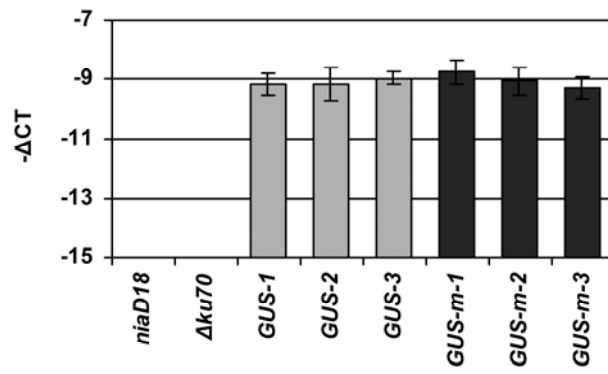


FIG. S6. Expression of the reporter gene *uidA* in the *GUS* and *GUS-m* strains measured by qRT-PCR. Strains were grown in production medium at 25°C for 96h. The results are given as mean with $\pm$ STD of independent biological triplicates with technical triplicates.

Supplementary Table S1. Fungal strains used in this study

Strains	Genotype	Reference
P2niaD18	<i>niaD</i> ⁻	(2)
ΔPcku70	<i>ku70Δ::nat1; niaD</i> ⁻	(1)
P2ΔbrlA	<i>brlAΔ::ergA; ku70Δ::nat1; niaD</i> ⁻	This study
P2ΔstuA	<i>stuAΔ::ergA; ku70Δ::nat1; niaD</i> ⁻	This study
P2brlA-kom	<i>brlAΔ::ergA; ku70Δ::nat1; PbrlA::brlA::TbrlA; ergA; niaD</i> ⁻	This study
P2stuA-kom	<i>stuAΔ::ergA; ku70Δ::nat1; PstuA::stuA::TstuA; phleo; niaD</i> ⁻	This study
P2stuA-OE	<i>ku70Δ::nat1; PPc22g::stuA::TstuA; ergA; niaD</i> ⁻	This study
P2GUS	<i>ku70Δ::nat1; PpenDE::uidA::TacnP; ergA; niaD</i> ⁻	This study
P2GUS-m	<i>ku70Δ::nat1; PpenDE(m)::uidA::TacnP; ergA; niaD</i> ⁻	This study

Reference List

1. **Hoff, B., J. Kamerewerd, C. Sigl, I. Zadra, and U. Kück.** 2010. Homologous recombination in the antibiotic producer *Penicillium chrysogenum*: strain Delta Pcku70 shows up-regulation of genes from the HOG pathway. *Appl. Microbiol. Biotechnol.* **85**:1081-1094.
2. **Hoff, B., S. Pöggeler, and U. Kück.** 2008. Eighty years after its discovery, Fleming's *Penicillium* strain discloses the secret of its sex. *Eukaryot. Cell* **7**:465-470.

Supplementary Table S2. Plasmid constructs employed in this study

Plasmid	Characteristics	Reference
px-ergA	<i>xylP</i> promoter of <i>P. chrysogenum</i> , <i>ergA</i> ORF, <i>acnP</i> terminator of <i>P. chrysogenum</i> ; pBluescript II SK (+) sequences	(2)
pa-ergA	<i>acnP</i> promoter of <i>P. chrysogenum</i> , <i>ergA</i> ORF, <i>acnP</i> terminator of <i>P. chrysogenum</i> ; pBluescript II SK (+) sequences	This study
p4.103	3' <i>brlA</i> flanking region in px-ergA	This study
p4.104	5' <i>brlA</i> flanking region in px-ergA	This study
p4.127	5' <i>stuA</i> flanking region in px-ergA	This study
p4.128	3' <i>stuA</i> flanking region in px-ergA	This study
p4.122	5' <i>brlA</i> flanking region, <i>brlA</i> ORF, 3' <i>brlA</i> flanking region in pa-ergA	This study
p4.183	5' <i>stuA</i> flanking region, <i>stuA</i> ORF, 3' <i>stuA</i> flanking region in pa-ergA	This study
pBC1003	<i>ipnA</i> promoter of <i>P. chrysogenum</i> , <i>Tn5-phleo</i> ORF, <i>cyt1</i> terminator of <i>S. cerevisiae</i>	(1)
p4.196	5' <i>stuA</i> flanking region, <i>stuA</i> ORF, 3' <i>stuA</i> flanking region; <i>ipnA</i> promoter of <i>P. chrysogenum</i> , <i>Tn5-phleo</i> ORF, <i>cyt1</i> terminator of <i>S. cerevisiae</i> ; pBluescript II SK (+) sequences	This study
p4.177	C-terminal truncated <i>stuA</i> ORF, <i>Pc22g</i> promoter of <i>P. chrysogenum</i> ORFs <i>Pc22g10000</i> and <i>Pc22g10010</i> , C-terminal truncated <i>ergA</i> ORF; pBluescript II SK (+) sequences	This study
p4.181	5' <i>ku70</i> flanking region, <i>penDE</i> promoter of <i>P. chrysogenum</i> , <i>uidA</i> ORF of <i>E. coli</i> , <i>acnP</i> terminator of <i>P. chrysogenum</i> ; <i>acnP</i> promoter of <i>P. chrysogenum</i> , C-terminal truncated <i>ergA</i> ORF, pBluescript II SK (+) sequences	This study
p4.182	5' <i>ku70</i> flanking region, <i>penDE</i> promoter of <i>P. chrysogenum</i> (m), <i>uidA</i> ORF of <i>E. coli</i> , <i>acnP</i> terminator of <i>P. chrysogenum</i> ; <i>acnP</i> promoter of <i>P. chrysogenum</i> , C-terminal truncated <i>ergA</i> ORF, pBluescript II SK (+) sequences	This study
p4.180	3' <i>ku70</i> flanking region in px-ergA	This study

Reference List

1. **Haas, H., K. Albermann, I. Zadra, and Stöffler G.** 1997. Overexpression of *nreB*, a new GATA factor-encoding gene of *Penicillium chrysogenum*, leads to repression of the nitrate assimilatory gene cluster. *J. Biol. Chem.* **272**:22576-22582.
2. **Zadra, I., B. Abt, W. Parson, and H. Haas.** 2000. *xylP* promoter-based expression system and its use for antisense downregulation of the *Penicillium chrysogenum* nitrogen regulator NRE. *Appl. Environ. Microbiol.* **66**:4810-4816.

Supplementary Table S3. Oligonucleotides used in this work

Primer	Sequence (5'-3')	Specificity
brlA-3-for	CGACACATGTCCTTGCTCAATGGCCAC	3' flanking region of <i>brlA</i>
brlA-3-rev	ACTAGGATCCTCAGTACCAGATCTCGGTC	3' flanking region of <i>brlA</i>
brlA-5-for	CCCCCTCGAGGTCGAGGCCGATCGCTATC	5' flanking region of <i>brlA</i>
brlA-5-rev	TGCAGCCGCGGAATTGTATTGTTTTGTGTGTGAAGAAC	5' flanking region of <i>brlA</i>
brlA-3-rev-1	CAGGAAGTCCTTGGAGTCGAGTG	3' flanking region of <i>brlA</i>
brlA-5-for-2	GGTCTCACCTGACGCGTGATGG	5' flanking region of <i>brlA</i>
pergA-rev-1	TCGCAGACCGATTACTAGGGTGAC	<i>ergA</i> gene
pergA-for-11	GTC AACCTTGAAGCAGTGGGC	<i>ergA</i> gene
stuA-C-for	ATCTGGTCAGGGGCTGCAGGACCGGATTCAACAACCTC	3' flanking region of <i>stuA</i>
stuA-C-rev	TGTTGAATCCGGTCCAGTAGAAGACTGAACCTCTTCG	3' flanking region of <i>stuA</i>
stuA-N-for	GAGATGACAACTAGTCCTGGGGATGCATCATGCAGG	5' flanking region of <i>stuA</i>
stuA-N-rev	CATCCCCAGGACTAGTTTGTCTATCTCCGTCAGCC	5' flanking region of <i>stuA</i>
stuA-C-rev-1	CTACCACGGTTTGTGACTCTCCTC	3' flanking region of <i>stuA</i>
stuA-N-for-1	CGGTATCGATAAGCTTCGCTGTCTCACGAGAGC	5' flanking region of <i>stuA</i>
acnP-Prom-3'	GGCGTGGCCATTGAGCAAGGACATCCATGGTGACTGATTAAACAAG	<i>acnP</i> promoter
acnP-Prom-5'	CACCTGCGGCCGCCCGTATTATCCCCATC	<i>acnP</i> promoter
pbrlA-for-k	ACCGCGGTGGCGGCCGCACAGACAATTCAACGTCTGACC	5' flanking region of <i>brlA</i>
pbrlA-rev-k	ATAATACGGGCGGCCGCTGAAGAGATGATGACATGGCG	3' flanking region of <i>brlA</i>
stuA-kom-for	TTCTACAGGACTATGCATTCTGCTGCGGGTCGGAAG	3' flanking region of <i>stuA</i>
stuA-kom-rev	GGCACTAGCCTGGGCTGCAG	3' flanking region of <i>stuA</i>
ipns-P-for	GGGACAGCGGATTCTGAAGCCTATCGGCGTGTTCCG	<i>ipnA</i> promoter
cyt1-T-rev	CTGTGAGCAGCGGCCGCCAAGCTTGAGCTCGAATTCCG	<i>cyt1</i> terminator
pergA(m)-for	ATCCACTAGTTCTAGACATGTCCTTGCTCAATGGCCACG	<i>ergA</i> gene
pergA(m)-rev	GGGAACAAAAGCTGGCGGCCGCGTTTAAACATCGTTGAGCGCCACAGTCATG	<i>ergA</i> gene
Pc22g-for	TTGAGCAAGGACATGTGTTTATAGGAAATATTCCTTATC	<i>Pc22g-P</i> promoter
Pc22g-rev	CTTGATATCGAATTCCATGGTTGTAGGATAACAAGATCTAC	<i>Pc22g-P</i> promoter
stuA-trunc-for	TATCCTACAACCATGTGTTTCGACGATTGTTAATGATC	<i>stuA</i> gene
stuA-trunc-rev	CGGTATCGATAAGCTTGTTTAAACAAGTCAGTGAACAGTTCTCAAATTG	<i>stuA</i> gene
pactP-for-k-1	AGTTCTAGAGCGGCCGCCGCTATTATCCC	<i>acnP</i> promoter
pergA-rev-k-1	AGCTGGAGCTCCACCGTTTAAACATCGTTGAGCGCCACAGTCATG	<i>ergA</i> gene
acnP-T5B	GCAGCCCGGGGATCCGGCGCCATTTAAATCAGCACTTTACTACTCG	<i>acnP</i> terminator
acnP-T3B	ATAATACGGGCGGCCGCTAGACTTTCCTCAGAG	<i>acnP</i> terminator
musA-N-for-2	CGGTATCGATAAGCTTGTTTAAACGCAATGGGCACTAGAATCAAG	5' flanking region of <i>ku70</i>
musA-N-rev-2	CCGGATCCCCCGGGCTGCAGGACCGGATTCAACAACCTC	5' flanking region of <i>ku70</i>
uidA-5GB	CCGGTCTGTCAGCCCGGGCATGCATAGTCTGTAGAAACCCCA	<i>uidA</i> gene
uidA-3GB	TGCTGATTTAAATGGCGCCGCGCATCCTCTAGAGTC	<i>uidA</i> gene
penDE-P-for	TGTTGAATCCGGTCCAGTAGAAGACTGAACCTCTTCG	<i>penDE</i> promoter
penDE-P-rev	TTCTACAGGACTATGCATTTCTGCTGCGGGTCGGAAG	<i>penDE</i> promoter
penDE-P-for-1	CCCCCTCGAGGTCGATACCCTAGTCATCCATTACAGGGC	<i>penDE</i> promoter
penDE-P-rev-1	ACCGCGGTGGCGGCCCTACCAATTTGTGCTAACTAGGG	<i>penDE</i> promoter
penDE-P-for-2	TGCAGCCGCGGAATTTCTCGAAGGCATATTGAGATATTGAG	<i>penDE</i> promoter
uidA-rev	GATCCAGACTGAATGCCACAGG	<i>uidA</i> gene
musA-C-rev-1	GAACCTCATCACATACTGCATGGCC	3' flanking region of <i>ku70</i>
musA-c-for	ATTTCGATATCAAGCTTGCATGCACATGGCCGTG	3' flanking region of <i>ku70</i>
musA-c-rev	CGGTATCGATAAGCTTCGCTGTCTCACGAGAGC	3' flanking region of <i>ku70</i>
Pc_stuA_U1	GACGAGAGGACAACCACATGATC	<i>stuA</i> gene
Pc_stuA_L1	CACAACGTGGCGAAGCTTTT	<i>stuA</i> gene
Pc_abaA_U2	CGCGATACTGGGAAAGATGG	<i>abaA</i> gene
Pc_abaA_L2	CCGTTCCGACCACTTTCTCC	<i>abaA</i> gene
Pc_br1A_U2	GCCTCGAGAACTTGCCATCA	<i>br1A</i> gene
Pc_br1A_L2	GCTCTTCATGTGTCGCTTGAGA	<i>br1A</i> gene
pc_pcbC_U1	CAGGTAGGCGTTGTGCTCC	<i>pcbC</i> gene
pc_pcbC_L1	GCGCTCTCTCGGTTGTTC	<i>pcbC</i> gene
pc_penDE_U2	ATTTGCATACGGGCTCAAG	<i>penDE</i> gene
pc_penDE_L2	CAGGTTCTCTTGGTGCGAG	<i>penDE</i> gene
Pc_medA_U2	TCCAGAAGCCCGTTGCC	<i>medA</i> gene
Pc_medA_L2	TCGGGGTCGTCACATAAGGT	<i>medA</i> gene
Pc_actA_U1	GGTGATGAGGCACAGTCGAAG	<i>actA</i> gene
Pc_actA_L1	AGCTCGTTGTAGAAGGTGTGGTG	<i>actA</i> gene
Pc_pcbAB_L1	CCTCGCTTTCCTCAACCAGA	<i>pcbAB</i> gene
Pc_pcbAB_U1	CCGCTTACGGACGATACACAC	<i>pcbAB</i> gene

Supplementary Table S4. Genes de-regulated ≥ 3 -fold at ≥ 3 consecutive time points in both *AbrlA* and *AstuA* compared to *Aku70* (green: down-regulated in both mutants; red: up-regulated in both mutants; white: de-regulated in both mutants)

ORF code	description of putative <i>P. chrysogenum</i> ORF	proposed function
Pc01g00030	weak similarity to hypothetical protein An12g08820 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc03g00090	strong similarity to Pcl-like cyclin pclA - <i>Aspergillus nidulans</i>	Pcl-like cyclin, putative
Pc03g00180	strong similarity to hypothetical Ydr124wp-like protein - <i>Pneumocystis carinii</i>	conserved hypothetical protein
Pc03g00300	similarity to hypothetical transcriptional regulator FCR1 - <i>Candida albicans</i>	transcriptional regulator, putative
Pc04g00130	hypothetical protein	hypothetical protein
Pc06g00210	strong similarity to secretory component IDGF - <i>Sarcophaga peregrina</i>	secretory component, putative
Pc06g00470	strong similarity to developmental regulatory protein brlA - <i>Aspergillus nidulans</i>	C2H2 transcription factor BrlA
Pc06g00660	similarity to negative regulatory protein Hex2 - <i>Saccharomyces cerevisiae</i>	putative regulatory protein
Pc06g01530	similarity to hypothetical protein 4 - <i>Streptomyces coelicolor</i>	conserved hypothetical protein
Pc06g02050	strong similarity to hypothetical protein An08g11990 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc06g02080	weak similarity to hypothetical protein An06g02320 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc07g00100	hypothetical protein	hypothetical protein
Pc09g00380	hypothetical protein	hypothetical protein
Pc12g00010	strong similarity to glucose oxidase GOX - <i>Penicillium amagasakiense</i>	choline dehydrogenase, putative
Pc12g01540	strong similarity to sulfate transporter - <i>Neosartorya fischeri</i>	sulfate transporter, putative
Pc12g01890	weak similarity to hypothetical protein C29F5.1 - <i>Caenorhabditis elegans</i>	conserved hypothetical protein
Pc12g02490	similarity to hypothetical protein SPAC23H3.04 - <i>Schizosaccharomyces pombe</i>	conserved hypothetical protein
Pc12g03260	similarity to hypothetical protein An16g07330 - <i>Aspergillus niger</i>	extracellular matrix protein, putative
Pc12g07520	similarity to cofilin Cof1 - <i>Saccharomyces cerevisiae</i>	cofilin, putative
Pc12g08720	strong similarity to hypothetical protein HI0753 - <i>Synechocystis</i> sp.	conserved hypothetical protein
Pc12g08750	strong similarity to polyamine oxidase PAO - <i>Zea mays</i>	flavin containing polyamine oxidase, putative
Pc12g08800	similarity to developmentally regulated spherulin 4 - <i>Physarum polycephalum</i>	spherulin 4-like cell surface protein, putative
Pc12g09930	similarity to protein involved in nonactin biosynthesis NonF - <i>Streptomyces griseus</i>	nonactin biosynthesis protein, putative
Pc12g10360	strong similarity to hypothetical membrane transport protein SPAC3H1.06c - <i>Schizosaccharomyces pombe</i>	membrane transport protein, putative
Pc12g10800	strong similarity to endo 1,5-alpha-arabinanase abnA - <i>Aspergillus niger</i>	endo 1,5-alpha-arabinanase, putative
Pc12g12350	hypothetical protein	hypothetical protein
Pc12g13160	strong similarity to mixed-linked glucanase precursor MLG1 - <i>Cochliobolus carbonum</i>	mixed-linked glucanase precursor, putative
Pc12g13190	strong similarity to cDNA O-methyltransferase mt-I - <i>Aspergillus parasiticus</i>	cDNA O-methyltransferase, putative
Pc12g13430	strong similarity to hypothetical protein B2J23.120 - <i>Neurospora crassa</i>	cell wall glycosyl hydrolase Dfg5, putative
Pc12g13490	strong similarity to hypothetical protein An08g03650 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc12g13750	strong similarity to hypothetical oxidoreductase - <i>Streptomyces coelicolor</i>	oxidoreductase, putative
Pc12g14590	strong similarity to aquaporin water channel protein Aqp2 - <i>Saccharomyces cerevisiae</i>	aquaporin water channel protein, putative
Pc12g14960	similarity to hypothetical protein An08g06730 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc12g15510	strong similarity to iron and manganese transporter Ccc1 - <i>Saccharomyces cerevisiae</i>	iron and manganese transporter, putative
Pc13g01210	similarity to transcription activator thi1p - <i>Schizosaccharomyces pombe</i>	transcription activator, putative
Pc13g02450	strong similarity to hypothetical protein contig1471_1.tfa_1040cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc13g05850	strong similarity to hypothetical protein 1217_scaffold_3.tfa_70cg - <i>Fusarium graminearum</i>	conserved hypothetical protein
Pc13g06200	similarity to pyoverdine biosynthesis protein PvcA - <i>Pseudomonas aeruginosa</i>	glutamyl-tRNA(Gln) amidotransferase, subunit A, putative
Pc13g06290	strong similarity to heavy metal ion resistance protein Zrc1 - <i>Saccharomyces cerevisiae</i>	cation diffusion facilitator family metal ion transporter, putative
Pc13g07110	strong similarity to alpha-l-arabinofuranosidase a precursor abfA - <i>Aspergillus niger</i>	alpha-l-arabinofuranosidase A precursor, putative
Pc13g07280	strong similarity to hypothetical protein 1158caffold_2.tfa_100wg - <i>Fusarium graminearum</i>	sulfatase domain protein, putative
Pc13g08370	strong similarity to hypothetical protein An02g07830 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc13g08540	strong similarity to membrane protein involved in arsenite transport like protein An18g03550 - <i>Aspergillus niger</i>	membrane protein, putative
Pc13g08690	strong similarity to lovastatin diketide synthase lovF - <i>Aspergillus terreus</i>	lovastatin diketide synthase, putative
Pc13g08700	weak similarity to hypothetical protein BAB11562.1 - <i>Arabidopsis thaliana</i>	conserved hypothetical protein

Pc13g11090	hypothetical protein	hypothetical protein
Pc13g11510	similarity to Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase YLL057c - <i>Saccharomyces cerevisiae</i> [putative sequencing error]	NACHT and WD domain protein, putative
Pc13g11710	strong similarity to hypothetical protein An11g01710 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc13g13210	strong similarity to hypothetical protein contig46_part_i.tfa_2960cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc13g13450	strong similarity to purine-cytosine permease pcpl3 - <i>Kluyveromyces marxianus</i>	purine-cytosine permease, putative
Pc13g13920	hypothetical protein	hypothetical protein
Pc13g15850	strong similarity to heat shock protein hsp30 - <i>Aspergillus nidulans</i>	heat shock protein, putative
Pc13g15970	strong similarity to hypothetical protein An01g09900 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc13g16010	weak similarity to spore-wall fungal hydrophobin dewA - <i>Aspergillus nidulans</i>	spore-wall fungal hydrophobin dewB
Pc14g00110	weak similarity to hypothetical protein An03g00160 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc14g00160	similarity to hypothetical monocarboxylate permease Esbp6 - <i>Saccharomyces cerevisiae</i>	MFS monocarboxylate transporter, putative
Pc14g00510	similarity to hypothetical protein contig46_part_ii.tfa_180wg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc15g00470	weak similarity to hypothetical protein UNC-89 - <i>Caenorhabditis elegans</i>	NTP binding protein, putative
Pc16g00180	strong similarity to hypothetical protein contig10.tfa_220cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc16g00310	strong similarity to hypothetical 6-hydroxy-D-nicotine oxidase related protein 13E11.250 - <i>Neurospora crassa</i>	6-hydroxy-D-nicotine oxidase related protein, putative
Pc16g00360	strong similarity to cytochrome P450 trichodiene oxygenase TRI4 - <i>Fusarium sporotrichioides</i>	cytochrome P450 trichodiene oxygenase, putative
Pc16g00510	strong similarity to arylsulfatase ars-1 - <i>Neurospora crassa</i>	arylsulfatase, putative
Pc16g00640	strong similarity to triacylglycerol lipase Lip4 - <i>Candida rugosa</i>	extracellular lipase, putative
Pc16g02410	strong similarity to plasma membrane glycoprotein Axl2 - <i>Saccharomyces cerevisiae</i>	transmembrane glycoprotein, putative
Pc16g02800	similarity to hypothetical protein contig46_part_i.tfa_580wg - <i>Aspergillus fumigatus</i>	GPI anchored serine-threonine rich protein, putative
Pc16g03630	strong similarity to choline permease Hnm1 - <i>Saccharomyces cerevisiae</i>	choline permease, putative
Pc16g04060	strong similarity to hypothetical membrane transport protein SPAC3H1.06c - <i>Schizosaccharomyces pombe</i>	membrane transport protein, putative
Pc16g04080	strong similarity to member of the major facilitator superfamily Las21 - <i>Saccharomyces cerevisiae</i> [putative sequencing error]	major facilitator superfamily member, putative
Pc16g04550	hypothetical protein	hypothetical protein
Pc16g04840	hypothetical protein	hypothetical protein
Pc16g05710	strong similarity to beta-fructofuranosidase precursor suc1 - <i>Aspergillus niger</i>	glycosyl hydrolase, putative
Pc16g06660	weak similarity to hypothetical protein encoded by CG4090 - <i>Drosophila melanogaster</i>	chitin binding peritrophin-A domain protein, putative
Pc16g07080	strong similarity to hypothetical protein An07g09780 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc16g07110	similarity to hypothetical protein YCR010c - <i>Saccharomyces cerevisiae</i>	acetate transporter, putative
Pc16g08460	strong similarity to sorbitol dehydrogenase gutB - <i>Bacillus subtilis</i> , strong similarity to NADP-dependent leukotriene B4 12-hydroxydehydrogenase like protein An13g01120 - <i>Aspergillus niger</i>	alcohol dehydrogenase, zinc-containing, putative
Pc16g08570	strong similarity to hypothetical protein An01g02040 - <i>Aspergillus niger</i> [putative sequencing error]	conserved hypothetical protein
Pc16g09170	similarity to hypothetical serine-rich zinc-finger protein RZF - <i>Schizosaccharomyces pombe</i>	serine-rich zinc-finger protein, putative
Pc16g09610	strong similarity to protein abaA - <i>Aspergillus nidulans</i> (Identities = 471/807 (58%), Positives = 574/807 (71%), Gaps = 23/807 (2%)	transcription factor AbaA
Pc16g10900	strong similarity to fructosyltransferase (inulosucrase) sft - <i>Aspergillus sydowii</i>	fructosyltransferase, putative
Pc16g11190	weak similarity to hypothetical protein mg09829.1 - <i>Magnaporthe grisea</i>	conserved hypothetical protein
Pc16g11300	strong similarity to hypothetical protein An07g00330 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc16g11310	strong similarity to allergen rAsp f 4 - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc16g11470	strong similarity to ATP-binding cassette multidrug transport protein atrB - <i>Aspergillus nidulans</i>	ATP-binding cassette multidrug transport protein, putative
Pc16g12000	weak similarity to beta transducin-like protein het-e1 - <i>Podospora anserina</i>	beta transducin-like protein, putative
Pc16g12210	weak similarity to hypothetical Ydr124wp-like protein - <i>Pneumocystis carinii</i>	conserved hypothetical protein
Pc16g13260	similarity to hypothetical protein An01g10940 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc16g14270	similarity to heterokaryon incompatibility protein het-6 - <i>Neurospora crassa</i>	heterokaryon incompatibility protein, putative
Pc16g15470	strong similarity to P-type ATPase ENA1 - <i>Schwanniomyces occidentalis</i>	P-type ATPase, putative
Pc17g00020	weak similarity to hypothetical protein An04g07890 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc17g00490	hypothetical protein	hypothetical protein
Pc17g00530	strong similarity to polyamine transport protein Tpo1 - <i>Saccharomyces cerevisiae</i>	polyamine transport protein, putative
Pc17g00940	hypothetical protein	hypothetical protein
Pc18g01150	strong similarity to polyamine transport protein Tpo1 - <i>Saccharomyces cerevisiae</i>	MFS multidrug transporter, putative

Pc18g01410	strong similarity to high affinity hexose transporter Hxt1 - <i>Saccharomyces cerevisiae</i>	hexose transporter, putative
Pc18g01580	hypothetical protein	hypothetical protein
Pc18g01790	hypothetical protein	hypothetical protein
Pc18g02600	strong similarity to glucan 1,3-beta-glucosidase precursor exg1 - <i>Aspergillus oryzae</i>	exo-beta-1,3-glucanase (Exg1), putative
Pc18g02640	strong similarity to beta-galactosidase like protein An02g00610 - <i>Aspergillus niger</i>	beta-galactosidase, putative
Pc18g03030	weak similarity to integral membrane protein PTH11 - <i>Magnaporthe grisea</i>	integral membrane protein, putative
Pc18g04570	weak similarity to hypothetical protein C0390w - <i>Plasmodium falciparum</i>	GPI anchored protein, putative
Pc18g04580	weak similarity to hypothetical protein An04g07540 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc19g00400	strong similarity to mannose-6-phosphate isomerase manA - <i>Candida albicans</i>	mannose-6-phosphate isomerase, putative
Pc19g00410	strong similarity to decarboxylating phosphogluconate dehydrogenase like protein An11g06120 - <i>Aspergillus niger</i>	decarboxylating phosphogluconate dehydrogenase like protein, putative
Pc19g00420	strong similarity to hypothetical protein nc09821.1 - <i>Neurospora crassa</i>	conserved hypothetical protein
Pc19g00450	strong similarity to glutathione-dependent formaldehyde dehydrogenase FDH - <i>Methylobacter marinus</i>	glutathione-dependent formaldehyde dehydrogenase, putative
Pc20g01290	strong similarity to Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase YLL057c - <i>Saccharomyces cerevisiae</i>	Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase, putative
Pc20g01320	strong similarity to hypothetical protein YMR200w - <i>Saccharomyces cerevisiae</i>	conserved hypothetical protein
Pc20g02080	strong similarity to hypothetical protein contig1487_1.tfa_1460cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc20g02250	similarity to chitinase - <i>Chelonus</i> sp.	chitinase, putative
Pc20g02640	strong similarity to glutaminase A gtaA - <i>Aspergillus oryzae</i>	glutaminase A, putative
Pc20g03190	strong similarity to hypothetical protein An11g09750 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g04430	strong similarity to hypothetical protein An09g02520 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g04440	hypothetical protein	hypothetical protein
Pc20g04560	similarity to cyanovirin-N CV-N - <i>Nostoc ellipsosporum</i>	cyanovirin-N, putative
Pc20g04580	similarity to ribonuclease III like protein An03g00790 - <i>Aspergillus niger</i>	ribonuclease III like protein, putative
Pc20g04630	strong similarity to hypothetical protein contig_1_110_scaffold_8.tfa_760cg - <i>Aspergillus nidulans</i>	conserved hypothetical protein
Pc20g05740	strong similarity to superoxide dismutase (Cu-Zn) 4-2 - <i>Caenorhabditis elegans</i>	superoxide dismutase, putative
Pc20g06250	strong similarity to 123K chain alpha,alpha-trehalose-phosphate synthase (UDP-forming) Tsl1 - <i>Saccharomyces cerevisiae</i>	trehalose-phosphate synthase, putative
Pc20g06330	strong similarity to NADPH-dependent beta-ketoacyl reductase RhlG - <i>Pseudomonas aeruginosa</i>	NADPH-dependent beta-ketoacyl reductase, putative
Pc20g06360	strong similarity to catalase A catA - <i>Aspergillus nidulans</i>	catalase A, catA
Pc20g06370	strong similarity to glycerol-3-phosphate dehydrogenase (35) precursor Gpd1 - <i>Saccharomyces cerevisiae</i>	glycerol-3-phosphate dehydrogenase (35) precursor, putative
Pc20g07010	strong similarity to ferulic acid esterase A faeA - <i>Aspergillus niger</i>	ferulic acid esterase A, putative
Pc20g09100	strong similarity to hexose transporter Hxt2 - <i>Saccharomyces cerevisiae</i>	hexose transporter, putative
Pc20g09230	strong similarity to hypothetical protein T6K22.50 - <i>Arabidopsis thaliana</i>	conserved hypothetical protein
Pc20g09570	strong similarity to mutarotase enzyme like protein An02g09090 - <i>Aspergillus niger</i>	mutarotase enzyme like protein, putative
Pc20g10170	strong similarity to beta-glucosidase 1 bg11 - <i>Aspergillus aculeatus</i>	beta-D-glucoside glucohydrolase, putative
Pc20g10210	similarity to hypothetical monocarboxylate permease Esbp6 - <i>Saccharomyces cerevisiae</i>	MFS monocarboxylate transporter, putative
Pc20g10270	strong similarity to protein kinase functional homolog of cdc2 nimX - <i>Aspergillus nidulans</i>	cell division control protein 2 kinase, putative
Pc20g11170	similarity to hypothetical protein BAA10688.1 - <i>Synechocystis</i> sp.	L-PSP endoribonuclease family protein, putative
Pc20g11700	strong similarity to hypothetical protein contig1496_0.tfa_30cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc20g12790	strong similarity to urease URE - <i>Coccidioides immitis</i>	urease, putative
Pc20g12820	strong similarity to hypothetical protein 18_scaffold_1.tfa_360wg - <i>Fusarium graminearum</i>	conserved hypothetical protein
Pc20g12890	similarity to hypothetical protein An08g12140 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g12930	strong similarity to hypothetical protein An08g11390 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g13710	weak similarity to hypothetical protein - <i>lasmodium falciparum</i>	conserved hypothetical protein
Pc20g14200	strong similarity to beta-glucan synthesis associated protein Kre6 - <i>Saccharomyces cerevisiae</i>	beta-1,6 glucan synthetase (Kre6), putative
Pc21g00130	strong similarity to transposase - <i>Fusarium oxysporum</i>	transposase, putative
Pc21g00390	strong similarity to hypothetical related to spore coat protein SP96 B15I20.140 - <i>Neurospora crassa</i>	spore coat protein, putative
Pc21g01270	weak similarity to hard surface induced protein 3 chip3 - <i>Glomerella cingulata</i>	conserved hypothetical protein
Pc21g01390	strong similarity to hypothetical protein SPAC869.06c - <i>Schizosaccharomyces pombe</i>	conserved hypothetical protein
Pc21g01400	strong similarity to butanediol dehydrogenase Fun49 - <i>Saccharomyces cerevisiae</i>	butanediol dehydrogenase, putative
Pc21g02480	weak similarity to cyanovirin-N CV-N - <i>Nostoc ellipsosporum</i>	cyanovirin-N, putative

Pc21g02590	weak similarity to nitrate reductase (NADPH) niaD - <i>Aspergillus niger</i>	nitrate reductase, putative
Pc21g02690	strong similarity to phosphate-repressible phosphate permease pho-4 - <i>Neurospora crassa</i>	phosphate-repressible phosphate permease, putative
Pc21g05380	hypothetical protein	hypothetical protein
Pc21g05610	strong similarity to hypothetical amine transporter SPCC18.02 - <i>Schizosaccharomyces pombe</i>	amine transporter, putative
Pc21g06360	strong similarity to hypothetical protein B2O8.250 - <i>Neurospora crassa</i>	conserved hypothetical protein
Pc21g08220	similarity to hypothetical protein B10H18.060 - <i>Neurospora crassa</i>	conserved hypothetical protein
Pc21g08640	weak similarity to hypothetical transcription regulator SPCC4F11.01 - <i>Schizosaccharomyces pombe</i>	GATA transcription factor (Ams2), putative
Pc21g09520	similarity to hypothetical protein Dcw1 - <i>Saccharomyces cerevisiae</i>	conserved hypothetical protein
Pc21g09760	hypothetical protein	hypothetical protein
Pc21g09810	strong similarity to hypothetical membrane transport protein SPAC3H1.06c - <i>Schizosaccharomyces pombe</i>	MFS multidrug transporter, putative
Pc21g09920	hypothetical protein	hypothetical protein
Pc21g10540	similarity to hypothetical protein An15g07790 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc21g11170	strong similarity to triacylglycerol lipase lipI - <i>Geotrichum candidum</i>	triacylglycerol lipase, putative
Pc21g12840	strong similarity to hypothetical protein contig_1_153_scaffold_12.tfa_490wg - <i>Aspergillus nidulans</i>	conserved hypothetical protein
Pc21g13140	strong similarity to cholinesterase 1 ChE1 - <i>Branchiostoma floridae</i>	cholinesterase, putative
Pc21g13290	strong similarity to hypothetical protein An11g08860 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc21g13950	strong similarity to aspergillopepsin II - <i>Aspergillus niger</i>	aspergillopepsin, putative
Pc21g15540	similarity to hypothetical protein CAD11786.1 - <i>Neurospora crassa</i>	conserved hypothetical protein
Pc21g15780	strong similarity to chitinase precursor chit33 - <i>Trichoderma harzianum</i> [putative sequencing error]	chitinase precursor, putative
Pc21g16130	similarity to hypothetical protein contig1495_1.tfa_1320wg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc21g16380	strong similarity to cell surface ferroxidase precursor Fet3 - <i>Saccharomyces cerevisiae</i>	conidial pigment biosynthesis oxidase Abr1
Pc21g18350	strong similarity to rodletless protein rodA - <i>Aspergillus nidulans</i>	rodletless protein rodB
Pc21g18530	strong similarity to hypothetical protein An07g03590 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc21g20790	strong similarity to hypothetical precursor of spore coat protein sp96 - <i>Neurospora crassa</i>	extracellular protein, putative
Pc21g22630	hypothetical protein	hypothetical protein
Pc22g01840	similarity to hypothetical protein An09g03310 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc22g04080	weak similarity to glucan 1,3-beta-glucosidase Bgl2 - <i>Saccharomyces cerevisiae</i>	cell wall glucanase (Scw4), putative
Pc22g04640	strong similarity to high affinity glucose transporter HGT1 - <i>Kluyveromyces lactis</i>	hexose transporter, putative
Pc22g05480	strong similarity to hypothetical oxidoreductase PA0147 - <i>Pseudomonas aeruginosa</i>	oxidoreductase, putative
Pc22g05490	weak similarity to 2-haloacid halido-hydrolyase IVa - <i>Burkholderia cepacia</i>	2-haloacid halido-hydrolyase, putative
Pc22g06520	weak similarity to potassium transport protein Trk2 - <i>Saccharomyces cerevisiae</i>	potassium transport protein, putative
Pc22g06670	similarity to hypothetical protein YER080w - <i>Saccharomyces cerevisiae</i>	conserved hypothetical protein
Pc22g07130	strong similarity to 4-hydroxyphenylpyruvate dioxygenase HPPD - <i>Mycosphaerella graminicola</i>	4-hydroxyphenylpyruvate dioxygenase, putative
Pc22g07450	strong similarity to hypothetical protein An11g09420 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc22g07470	strong similarity to hexose transporter Hxt13 - <i>Saccharomyces cerevisiae</i>	MFS sugar transporter, putative
Pc22g07480	strong similarity to hypothetical protein An03g02880 - <i>Aspergillus niger</i>	LPS glycosyltransferase, putative
Pc22g07490	weak similarity to zeaxanthin epoxidase ABA2 - <i>Nicotiana plumbaginifolia</i>	zeaxanthin epoxidase, putative
Pc22g07760	strong similarity to NADP-dependent leukotriene B4 12-hydroxydehydrogenase like protein An13g01120 - <i>Aspergillus niger</i>	beta-lactamase, putative
Pc22g08420	strong similarity to brown 2 protein abr2 - <i>Aspergillus fumigatus</i>	conidial pigment biosynthesis oxidase Abr2
Pc22g08820	hypothetical protein	hypothetical protein
Pc22g12210	strong similarity to integral membrane protein like protein An04g06980 - <i>Aspergillus niger</i>	integral membrane protein, putative
Pc22g12240	strong similarity to choline permease Hnm1 - <i>Saccharomyces cerevisiae</i>	amino acid permease, putative
Pc22g13780	strong similarity to hypothetical protein brt1 - <i>Schizophyllum commune</i>	conserved hypothetical protein
Pc22g13830	strong similarity to hypothetical protein An09g06360 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc22g14280	hypothetical protein	hypothetical protein
Pc22g14290	strong similarity to rodletless protein rodA - <i>Aspergillus nidulans</i>	rodletless protein rodA
Pc22g14300	similarity to hypothetical protein An02g09010 - <i>Aspergillus niger</i>	extracellular serine-threonine rich protein, putative
Pc22g14570	strong similarity to protein disulfide isomerase A pdiA - <i>Aspergillus niger</i>	protein disulfide isomerase A, putative
Pc22g14640	strong similarity to hexose transporter Hxt2 - <i>Saccharomyces cerevisiae</i>	MFS monosaccharide transporter, putative
Pc22g16030	strong similarity to hypothetical protein contig5_part_ii.tfa_1430wg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc22g16660	similarity to hypothetical protein F12A12.10 - <i>Arabidopsis thaliana</i>	oxidoreductase, 2OG-Fe(II) oxygenase family, putative
Pc22g17040	hypothetical protein	hypothetical protein
Pc22g17430	hypothetical protein	hypothetical protein
Pc22g19880	strong similarity to hypothetical protein 1468_scaffold_9.tfa_10wg -	conserved hypothetical protein

	Fusarium graminearum	
Pc22g20780	weak similarity to hypothetical protein F3E22.6 - Arabidopsis thaliana	conserved hypothetical protein
Pc22g22810	strong similarity to sulphhydryl oxidase Sox from patent EP565172-A1 - Aspergillus niger	sulphydryl oxidase, putative
Pc22g22820	strong similarity to cytochrome P450 monooxygenase P450I - Gibberella fujikuroi	cytochrome P450 monooxygenase, putative
Pc22g22860	similarity to cinnamyl-alcohol dehydrogenase CAD1 - Eucalyptus gunnii	cinnamyl-alcohol dehydrogenase, putative
Pc22g22870	strong similarity to levodione reductase lvr - Corynebacterium aquaticum	levodione reductase, putative
Pc22g22880	similarity to geranyl diphosphate:4-hydroxybenzoate geranyltransferase lepgt-2 - Lithospermum erythrorhizon	geranyl diphosphate:4-hydroxybenzoate geranyltransferase, putative
Pc22g22900	strong similarity to hypothetical protein contig_1_172_scaffold_16.tfa_140wg - Aspergillus nidulans	conserved hypothetical protein
Pc22g23520	strong similarity to cercosporin transporter CFP - Cercospora kikuchii	cercosporin transporter, putative
Pc22g23660	strong similarity to Swe1 regulating protein kinase Hsl1 - Saccharomyces cerevisiae	serine/threonine protein kinase (Kcc4), putative
Pc22g24580	strong similarity to thermostable alcohol dehydrogenase adhT - Bacillus stearothermophilus	alcohol dehydrogenase, putative
Pc22g24750	similarity to hypothetical protein B8J22.030 - Neurospora crassa	conserved hypothetical protein
Pc22g26040	hypothetical protein	hypothetical protein
Pc22g26190	strong similarity to Pcl-like cyclin pclA - Aspergillus nidulans	Pcl-like cyclin pclA, putative
Pc22g26820	similarity to transcription factor atf21p - Schizosaccharomyces pombe	transcription factor, putative
Pc23g00040	weak similarity to cell division protein kinase - Encephalitozoon cuniculi	cell division protein kinase, putative
Pc23g00270	weak similarity to hypothetical protein T21J18.40 - Arabidopsis thaliana	conserved hypothetical protein
Pc23g00450	similarity to hypothetical protein contig17.tfa_160cg - Aspergillus fumigatus	conserved hypothetical protein
Pc23g00460	strong similarity to hypothetical protein An02g10610 - Aspergillus niger	conserved hypothetical protein
Pc24g00450	hypothetical protein	hypothetical protein
Pc24g00830	hypothetical protein	hypothetical protein
Pc24g00850	hypothetical protein	hypothetical protein
Pc24g01380	hypothetical protein	hypothetical protein
Pc24g01390	hypothetical protein	hypothetical protein
Pc24g01510	weak similarity to ferric/cupric reductase Fre2 - Saccharomyces cerevisiae	ferric/cupric reductase, putative
Pc24g01550	hypothetical protein	hypothetical protein
Pc24g02010	hypothetical protein	hypothetical protein
Pc24g02140	strong similarity to NAD-dependent D-arabinitol dehydrogenase ard - Candida tropicalis	NAD-dependent D-arabinitol dehydrogenase, putative
Pc24g02480	similarity to 35.6K acid phosphatase - Aspergillus nidulans	acid phosphatase, putative
Pc24g02890	similarity to hypothetical protein An14g04480 - Aspergillus niger	conserved hypothetical protein
Pc24g02900	strong similarity to plasma membrane H(+)-ATPase pmaA - Aspergillus nidulans	plasma membrane H(+)-ATPase pmaA, putative
Pc24g03090	hypothetical protein	hypothetical protein

Supplementary Table S5. 93 genes similarly regulated to *abaA* in both mutant strains compared to *Δku70* (analysis done with the “Find Similar Genes” tool in GeneSpring® 7.2 (Silicon Genetics) and a correlation coefficient of ≥ 0.9).

ORF code	description of putative <i>P. chrysogenum</i> ORF	proposed function
Pc03g00090	strong similarity to Pcl-like cyclin pclA - <i>Aspergillus nidulans</i>	Pcl-like cyclin, putative
Pc03g00180	strong similarity to hypothetical Ydr124wp-like protein - <i>Pneumocystis carinii</i>	conserved hypothetical protein
Pc06g00470	strong similarity to developmental regulatory protein brlA - <i>Aspergillus nidulans</i>	C2H2 transcription factor BrlA
Pc06g00660	similarity to negative regulatory protein Hex2 - <i>Saccharomyces cerevisiae</i>	putative regulatory protein
Pc12g00010	strong similarity to glucose oxidase GOX - <i>Penicillium amagasakiense</i>	choline dehydrogenase, putative
Pc12g01900	strong similarity to nuclear migration factor nudF - <i>Aspergillus nidulans</i>	nuclear migration protein NudF, putative
Pc12g03260	similarity to hypothetical protein An16g07330 - <i>Aspergillus niger</i>	extracellular matrix protein, putative
Pc12g08750	strong similarity to polyamine oxidase PAO - <i>Zea mays</i>	flavin containing polyamine oxidase, putative
Pc12g08800	similarity to developmentally regulated spherulin 4 - <i>Physarum polycephalum</i>	spherulin 4-like cell surface protein, putative
Pc12g09190	strong similarity to GTP:alpha-D-mannose-1-phosphate guanylyltransferase like protein An11g02380 - <i>Aspergillus niger</i>	GDP-mannose pyrophosphorylase A, putative
Pc12g12350	hypothetical protein	hypothetical protein
Pc12g13430	strong similarity to hypothetical protein B2J23.120 - <i>Neurospora crassa</i>	cell wall glycosyl hydrolase Dfg5, putative
Pc12g14960	similarity to hypothetical protein An08g06730 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc13g06200	similarity to pyoverdine biosynthesis protein PvcA - <i>Pseudomonas aeruginosa</i>	glutamyl-tRNA(Gln) amidotransferase, subunit A, putative
Pc13g06290	strong similarity to heavy metal ion resistance protein Zrc1 - <i>Saccharomyces cerevisiae</i>	metal ion transporter, putative
Pc13g07280	strong similarity to hypothetical protein 1158caffold_2.tfa_100wg - <i>Fusarium graminearum</i>	sulfatase domain protein, putative
Pc13g08370	strong similarity to hypothetical protein An02g07830 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc13g08700	weak similarity to hypothetical protein BAB11562.1 - <i>Arabidopsis thaliana</i>	conserved hypothetical protein
Pc13g11090	hypothetical protein	hypothetical protein
Pc13g11510	similarity to Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase YLL057c - <i>Saccharomyces cerevisiae</i>	NACHT and WD domain protein, putative
Pc13g13450	strong similarity to purine-cytosine permease pcpl3 - <i>Kluyveromyces marxianus</i>	purine-cytosine permease, putative
Pc13g13920	hypothetical protein	hypothetical protein
Pc14g00110	weak similarity to hypothetical protein An03g00160 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc14g00160	similarity to hypothetical monocarboxylate permease Esbp6 - <i>Saccharomyces cerevisiae</i>	MFS monocarboxylate transporter, putative
Pc15g00470	weak similarity to hypothetical protein UNC-89 - <i>Caenorhabditis elegans</i>	NTP binding protein, putative
Pc16g00180	strong similarity to hypothetical protein contig10.tfa_220cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc16g00640	strong similarity to triacylglycerol lipase Lip4 - <i>Candida rugosa</i>	extracellular lipase, putative
Pc16g02410	strong similarity to plasma membrane glycoprotein Axl2 - <i>Saccharomyces cerevisiae</i>	transmembrane glycoprotein, putative
Pc16g02800	similarity to hypothetical protein contig46_part_i.tfa_580wg - <i>Aspergillus fumigatus</i>	GPI anchored serine-threonine rich protein, putative
Pc16g04550	hypothetical protein	hypothetical protein
Pc16g04840	hypothetical protein	hypothetical protein
Pc16g05710	strong similarity to beta-fructofuranosidase precursor suc1 - <i>Aspergillus niger</i>	glycosyl hydrolase, putative
Pc16g06660	weak similarity to hypothetical protein encoded by CG4090 - <i>Drosophila melanogaster</i>	chitin binding peritrophin-A domain protein, putative
Pc16g07080	strong similarity to hypothetical protein An07g09780 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc16g07110	similarity to hypothetical protein YCR010c - <i>Saccharomyces cerevisiae</i>	acetate transporter, putative
Pc16g08460	strong similarity to sorbitol dehydrogenase gutB - <i>Bacillus subtilis</i>	alcohol dehydrogenase, zinc-containing, putative
Pc16g08570	strong similarity to hypothetical protein An01g02040 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc16g09610	strong similarity to protein abaA - <i>Aspergillus nidulans</i>	transcription factor AbaA
Pc16g10900	strong similarity to fructosyltransferase (inulosucrase) sft - <i>Aspergillus sydowii</i>	fructosyltransferase, putative
Pc16g11310	strong similarity to allergen rAsp f 4 - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc16g13260	similarity to hypothetical protein An01g10940 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc18g01150	strong similarity to polyamine transport protein Tpo1 - <i>Saccharomyces cerevisiae</i>	MFS multidrug transporter, putative
Pc18g01410	strong similarity to high affinity hexose transporter Hxt1 - <i>Saccharomyces cerevisiae</i>	hexose transporter, putative
Pc18g02600	strong similarity to glucan 1,3-beta-glucosidase precursor exg1 - <i>Aspergillus oryzae</i>	exo-beta-1,3-glucanase (Exg1), putative
Pc18g02640	strong similarity to beta-galactosidase like protein An02g00610 - <i>Aspergillus niger</i>	beta-galactosidase, putative
Pc18g04570	weak similarity to hypothetical protein C0390w - <i>Plasmodium falciparum</i>	GPI anchored protein, putative

Pc18g04580	weak similarity to hypothetical protein An04g07540 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g01320	strong similarity to hypothetical protein YMR200w - <i>Saccharomyces cerevisiae</i>	conserved hypothetical protein
Pc20g02080	strong similarity to hypothetical protein contig1487_1.tfa_1460cg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc20g03190	strong similarity to hypothetical protein An11g09750 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g09240	weak similarity to hypothetical integral membrane protein SC10B7.28 - <i>Streptomyces coelicolor</i>	GPI anchored protein, putative
Pc20g10170	strong similarity to beta-glucosidase 1 bgl1 - <i>Aspergillus aculeatus</i>	beta-D-glucoside glucohydrolase, putative
Pc20g10190	similarity to hypothetical protein contig_1_168caffold_14.tfa_600cg - <i>Aspergillus nidulans</i>	conserved hypothetical protein
Pc20g10210	similarity to hypothetical monocarboxylate permease Esbp6 - <i>Saccharomyces cerevisiae</i>	MFS monocarboxylate transporter, putative
Pc20g10270	strong similarity to protein kinase functional homolog of cdc2 nimX - <i>Aspergillus nidulans</i>	cell division control protein 2 kinase, putative
Pc20g10780	weak similarity to hypothetical protein An04g07410 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc20g11170	similarity to hypothetical protein BAA10688.1 - <i>Synechocystis</i> sp.	L-PSP endoribonuclease family protein, putative
Pc20g13710	weak similarity to hypothetical protein - <i>Plasmodium falciparum</i>	conserved hypothetical protein
Pc20g14200	strong similarity to beta-glucan synthesis associated protein Kre6 - <i>Saccharomyces cerevisiae</i>	beta-1,6 glucan synthetase (Kre6), putative
Pc21g01270	weak similarity to hard surface induced protein 3 chip3 - <i>Glomerella cingulata</i>	conserved hypothetical protein
Pc21g02590	weak similarity to nitrate reductase (NADPH) niaD - <i>Aspergillus niger</i>	nitrate reductase, putative
Pc21g08640	weak similarity to hypothetical transcription regulator SPCC4F11.01 - <i>Schizosaccharomyces pombe</i>	GATA transcription factor (Ams2), putative
Pc21g09810	strong similarity to hypothetical membrane transport protein SPAC3H1.06c - <i>Schizosaccharomyces pombe</i>	MFS multidrug transporter, putative
Pc21g13210	similarity to hypothetical protein An08g09040 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc21g13950	strong similarity to aspergillopepsin II - <i>Aspergillus niger</i>	aspergillopepsin, putative
Pc21g15540	similarity to hypothetical protein CAD11786.1 - <i>Neurospora crassa</i>	conserved hypothetical protein
Pc21g16130	similarity to hypothetical protein contig1495_1.tfa_1320wg - <i>Aspergillus fumigatus</i>	conserved hypothetical protein
Pc21g16380	strong similarity to cell surface ferroxidase precursor Fet3 - <i>Saccharomyces cerevisiae</i>	conidial pigment biosynthesis oxidase Abr1
Pc21g18350	strong similarity to rodletless protein rodA - <i>Aspergillus nidulans</i>	rodletless protein rodB
Pc21g18530	strong similarity to hypothetical protein An07g03590 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc21g20790	strong similarity to hypothetical precursor of spore coat protein sp96 - <i>Neurospora crassa</i>	extracellular protein, putative
Pc22g01840	similarity to hypothetical protein An09g03310 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc22g04080	weak similarity to glucan 1,3-beta-glucosidase Bgl2 - <i>Saccharomyces cerevisiae</i>	cell wall glucanase (Scw4), putative
Pc22g04640	strong similarity to high affinity glucose transporter HGT1 - <i>Kluyveromyces lactis</i>	hexose transporter, putative
Pc22g07130	strong similarity to 4-hydroxyphenylpyruvate dioxygenase HPPD - <i>Mycosphaerella graminicola</i>	4-hydroxyphenylpyruvate dioxygenase, putative
Pc22g07470	strong similarity to hexose transporter Hxt13 - <i>Saccharomyces cerevisiae</i>	MFS sugar transporter, putative
Pc22g07480	strong similarity to hypothetical protein An03g02880 - <i>Aspergillus niger</i>	LPS glycosyltransferase, putative
Pc22g07760	strong similarity to NADP-dependent leukotriene B4 12-hydroxydehydrogenase like protein An13g01120 - <i>Aspergillus niger</i>	beta-lactamase, putative
Pc22g08420	strong similarity to brown 2 protein abr2 - <i>Aspergillus fumigatus</i>	conidial pigment biosynthesis oxidase Abr2
Pc22g08820	hypothetical protein	hypothetical protein
Pc22g12210	strong similarity to integral membrane protein like protein An04g06980 - <i>Aspergillus niger</i>	integral membrane protein, putative
Pc22g12240	strong similarity to choline permease Hnm1 - <i>Saccharomyces cerevisiae</i>	amino acid permease, putative
Pc22g14290	strong similarity to rodletless protein rodA - <i>Aspergillus nidulans</i>	rodletless protein rodA
Pc22g14300	similarity to hypothetical protein An02g09010 - <i>Aspergillus niger</i>	extracellular serine-threonine rich protein, putative
Pc22g14640	strong similarity to hexose transporter Hxt2 - <i>Saccharomyces cerevisiae</i>	MFS monosaccharide transporter, putative
Pc22g16660	similarity to hypothetical protein F12A12.10 - <i>Arabidopsis thaliana</i>	oxidoreductase, 2OG-Fe(II) oxygenase family, putative
Pc22g17040	hypothetical protein	hypothetical protein
Pc22g22050	strong similarity to hypothetical protein contig1497_4.tfa_1190cg - <i>Aspergillus fumigatus</i>	F-box domain protein, putative
Pc22g23660	strong similarity to Swe1 regulating protein kinase Hsl1 - <i>Saccharomyces cerevisiae</i>	serine/threonine protein kinase (Kcc4), putative
Pc22g26040	hypothetical protein	hypothetical protein
Pc24g01980	similarity to hypothetical protein mg07660.1 - <i>Magnaporthe grisea</i> [putative pseudogene]	conserved hypothetical protein
Pc24g02010	hypothetical protein	hypothetical protein
Pc24g02480	similarity to 35.6K acid phosphatase - <i>Aspergillus nidulans</i>	acid phosphatase, putative

Supplementary Table S6. 38 genes similarly regulated to *wetA* in both mutant strains compared to *Δku70* (analysis done with the “Find Similar Genes” tool in GeneSpring® 7.2 (Silicon Genetics) and a correlation coefficient of ≥ 0.9).

ORF code	description of putative <i>P. chrysogenum</i> ORF	proposed function
Pc12g00920	strong similarity to oxalate decarboxylase of patent CA2350328-A1 - <i>Aspergillus phoenices</i>	oxalate decarboxylase, putative
Pc12g01280	similarity to RNA helicase GLH-2 - <i>Caenorhabditis elegans</i>	RNA helicase, putative
Pc12g02250	strong similarity to glucose dehydrogenase like protein An07g08190 - <i>Aspergillus niger</i>	oxidoreductase, putative
Pc12g14760	strong similarity to nitrosoguanidine resistance factor Sng1 - <i>Saccharomyces cerevisiae</i>	nitrosoguanidine resistance factor, putative
Pc13g14950	strong similarity to catalase kat - <i>Methanosarcina barker</i>	catalase, putative
Pc13g15970	strong similarity to hypothetical protein An01g09900 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc16g06690	similarity to spore-wall fungal hydrophobin dewA - <i>Aspergillus nidulans</i>	spore-wall fungal hydrophobin dewA
Pc16g11980	strong similarity to hypothetical transmembrane protein L2185.03 - <i>Leishmania major</i>	transporter protein, putative
Pc18g01100	weak similarity to hypothetical protein gi_19075870 - <i>Schizosaccharomyces pombe</i>	conserved hypothetical protein
Pc19g00400	strong similarity to mannose-6-phosphate isomerase manA - <i>Candida albicans</i>	mannose-6-phosphate isomerase, putative
Pc19g00410	strong similarity to decarboxylating phosphogluconate dehydrogenase like protein An11g06120 - <i>Aspergillus niger</i>	6-phosphogluconate dehydrogenase, putative
Pc19g00420	strong similarity to mitochondrial proton-pumping NADH:ubiquinone reductase 9.6 kD acyl-carrier subunit precursor ACP - <i>Neurospora crassa</i>	oxidoreductase, putative
Pc20g04060	similarity to DNA-directed RNA polymerase II largest chain - <i>Mastigamoeba invertens</i>	DNA-directed RNA polymerase, putative
Pc20g06330	strong similarity to NADPH-dependent beta-ketoacyl reductase RhlG - <i>Pseudomonas aeruginosa</i>	short-chain dehydrogenase, putative
Pc20g10220	strong similarity to hypothetical protein contig_1_5_scaffold_1.tfa_810cg - <i>Aspergillus nidulans</i>	conserved hypothetical protein
Pc20g12790	strong similarity to urease URE - <i>Coccidioides immitis</i>	urease, putative
Pc21g06360	strong similarity to hypothetical protein B2O8.250 - <i>Neurospora crassa</i>	histidine acid phosphatase, putative
Pc21g10040	similarity to protein kinase STY - <i>Mus musculus</i>	serine-threonine protein kinase, putative
Pc21g12140	weak similarity to hypothetical galactosyltransferase spdbp - <i>Schizosaccharomyces pombe</i>	galactosyl transferase protein, putative
Pc21g13070	similarity to phosphatidylserine decarboxylase Psd2 - <i>Saccharomyces cerevisiae</i>	phosphatidylserine decarboxylase family protein, putative
Pc21g15150	strong similarity to hypothetical protein contig_1_139_scaffold_11.tfa_60cg - <i>Aspergillus nidulans</i>	auxin efflux carrier superfamily protein, putative
Pc21g16000	strong similarity to polyketide synthase PKS1 - <i>Colletotrichum lagenarium</i>	conidial pigment polyketide synthase PksP/Alb1(wA)
Pc21g16420	strong similarity to scytalone dehydratase - <i>Pyricularia oryzae</i>	conidial pigment biosynthesis scytalone dehydratase Arp1
Pc21g16430	strong similarity to 1,3,6,8-tetrahydroxynaphthalene reductase arp2 - <i>Aspergillus fumigatus</i>	conidial pigment biosynthesis 1,3,6,8-THN reductase Arp2
Pc21g16440	strong similarity to hypothetical yellowish-green 1 ayg1	conidial pigment biosynthesis protein Ayg1
Pc22g03220	regulator protein wetA- <i>Penicillium chrysogenum</i>	regulator protein wetA
Pc22g06890	weak similarity to hypothetical protein related to VeA - <i>Neurospora crassa</i>	developmental regulator VosA
Pc22g17350	strong similarity to hypothetical protein contig_1_115_scaffold_8.tfa_20wg - <i>Aspergillus nidulans</i>	short-chain dehydrogenase, putative
Pc22g22320	weak similarity to hypothetical protein related to VeA - <i>Neurospora crassa</i>	developmental regulator VelB
Pc22g22440	strong similarity to hypothetical protein An01g06460 - <i>Aspergillus niger</i>	integral membrane protein, putative
Pc22g26550	weak similarity to hypothetical protein An12g06780 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc22g26780	strong similarity to hypothetical protein An16g09280 - <i>Aspergillus niger</i>	conserved hypothetical protein
Pc22g26950	hypothetical protein	hypothetical protein
Pc22g26960	similarity to nuclear polyadenylated RNA-binding protein Nab3 - <i>Saccharomyces cerevisiae</i>	arginine/serine-rich splicing factor, putative
Pc24g02140	strong similarity to NAD-dependent D-arabinitol dehydrogenase ard - <i>Candida tropicalis</i>	short-chain dehydrogenase, putative
Pc24g02160	strong similarity to retrotransposon Tto1 - <i>Nicotiana tabacum</i>	retrotransposon, putative
Pc24g02170	similarity to hypothetical protein 176_scaffold_1.tfa_30wg - <i>Fusarium graminearum</i>	conserved hypothetical protein
Pc24g02900	strong similarity to plasma membrane H(+)-ATPase pmaA - <i>Aspergillus nidulans</i>	plasma membrane H(+)-ATPase, putative