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CHAPTER 2

Systems approaches to elucidate the functions of glycoside hydrolases during the life cycle of *Aspergillus niger* using developmental mutants $\Delta brlA$ and $\Delta flbA$

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Abstract

The filamentous fungus *Aspergillus niger* encounters carbon starvation in nature as well as during industrial fermentations. In response, different but partially overlapping regulatory networks initiate and control autolysis and sporulation. Carbohydrate active enzymes play an important role in these processes, for example by modifying cell walls. In this study we provide the first system-wide overview of the impact of the developmental transcriptional regulators FlbA and BrlA on the expression of genes encoding carbohydrate active enzymes during carbon starvation. We systematically investigated and compared the transcriptomes, proteomes, enzyme activities and the fungal cell wall compositions of the wild type and mutant strains. During exponential growth, a strain carrying an *flbA* deletion had a reduced specific growth rate, an increased chitin content of the cell wall and its transcriptome showed signs of cell wall stress. In addition, transcription of genes encoding growth related cell wall modifying enzymes was reduced during both exponential growth and carbon starvation, and glycogen metabolism appeared to be negatively affected. During carbon starvation, expression of *nagA*, *engA* and two alpha-mannosidases was *flbA* dependent, while that of autolysis-related chitinase *cfcA* and a number of mannan degrading enzymes was not. The variation in expression of autolytic hydrolases suggests that a fine-tuned regulatory mechanism may underpin their expression pattern, indicating the importance of regulation as well as giving clues for potential industrial strain engineering. The combination of the developmental mutants lacking *brlA* and *flbA* resulted in the identification of sporulation specific chitinases *cfcI* and *ctcB*, and the β -glucan acting enzymes encoded by *bgtD*, An02g00850 and *gelG*. In addition, we found sporulation specific high expression levels for An15g02350, a member of the recently identified auxiliary activity family AA11. Importantly, we demonstrate here for the first time a physiological role for a member of this family of copper-dependent lytic polysaccharide monooxygenases. The identification of this set of sporulation specific enzymes may assist in understanding asexual development.

Introduction

The filamentous fungus *Aspergillus niger* is used for the industrial production of enzymes and organic acids, and has been granted a Generally Regarded As Safe status by the US Food and Drug Administration (Bennett, 2009; Ward *et al.*, 2006). Saprophytic fungi such as *A. niger* may encounter nutrient starvation both during industrial fermentations as well as in nature. These conditions induce the expression of hydrolytic enzymes, hyphal fragmentation and loss of biomass, generally referred to as autolysis (White *et al.*, 2002). Analysis of the transcriptome and proteome of *A. niger* and *A. nidulans* indicated that carbon starvation activates recycling of cell components and initiates asexual sporulation. Furthermore, these studies identified the up-regulated glycoside hydrolases and proteases (Nitsche *et al.*, 2012; Szilagyi *et al.*, 2013).

During carbon starvation, glycoside hydrolases are thought to degrade the fungal cell wall (White *et al.*, 2002) by acting on its carbohydrate network of β -glucans, chitin, α -glucans, galactomannan and galactosaminogalactan (Latgé *et al.*, 2005). The role of individual enzymes has been established in a number of cases. For example β -glucanase EngA and chitinase ChiB are responsible for fragmentation of mycelial pellets and a decrease in biomass in carbon-starved cultures of *A. nidulans*, and thereby play a role in autolysis (Shin *et al.*, 2009; Szilagyi *et al.*, 2010; Yamazaki *et al.*, 2007). Glycoside hydrolases are thus important effectors of cell wall recycling during carbon starvation, thereby generating energy and building blocks that may be used for maintenance and sporulation.

Carbon starvation induces the formation of spore forming structures and spores in submerged cultures of aspergilli (Adams *et al.*, 1998; Broderick & Greenshields, 1981; Jorgensen *et al.*, 2010; Nitsche *et al.*, 2012). These morphological changes require extensive remodeling of the fungal cell wall, that can be observed by changes in appearance (Adams *et al.*, 1998), as well as by the differences in carbohydrate composition of vegetative mycelium and spores (Maubon *et al.*, 2006). Correspondingly, sporulation specific chitin synthases (Fujiwara *et al.*, 2000; Lee *et al.*, 2004) and chitinases (Chapter 4) have been identified that contribute to these cell wall changes. Thus, glycoside hydrolases and other Carbohydrate-Active enZymes (CAZymes) are also of importance in cell wall remodeling during sporulation.

Understanding the processes initiated by filamentous fungi in response to carbon

starvation, such as autolysis and sporulation, is key to understanding an important part of the fungal life cycle, but is also of commercial relevance. Autolytic phenomena may cause problems during industrial fermentations, such as proteolytic product degradation, and in downstream processing (White *et al.*, 2002). Sporulation affects the accumulation of secondary metabolites in submerged fermentations and has a detrimental influence on the mycelial protein secretion (Jorgensen *et al.*, 2011a; Krijgsheld *et al.*, 2013b). Changes in the fungal cell wall play a role in this reduction in protein secretion (Krijgsheld *et al.*, 2013b). Thus, understanding the regulation of the carbon starvation response and the accompanied expression of carbohydrate-active enzymes as key effectors of cell wall remodeling, is a starting point for manipulation of the carbon starvation response to improve industrial fermentations.

Our understanding of the regulatory mechanisms behind the responses to carbon starvation is incomplete. It involves a complex network of interacting pathways. Asexual sporulation is controlled by the FluG-BrlA regulatory pathway, which has been best investigated in *A. nidulans* and is likely to be conserved in *A. niger* (Adams *et al.*, 1998; Krijgsheld *et al.*, 2013a). The cytoplasmic protein FluG synthesizes a small, extracellular, diffusible product that activates the FlbB-E (fluffy low *brlA* expression) protein cascade and subsequently the BrlA transcriptional activator, resulting in asexual sporulation (Fig. 1)(Lee & Adams, 1994b). Importantly, in *A. nidulans*, a FluG loss of function mutant did not form conidiophores and showed decreased autolysis (Emri *et al.*, 2005), demonstrating the regulatory connection between asexual sporulation and autolysis. The FlbA protein is a central player in the regulatory pathway, as it connects the cascade of FlbB-E proteins and BrlA to the G-protein coupled receptor signaling that is required to halt vegetative growth during conidiation. FadA is the α -subunit of this heterotrimeric G-protein complex that is further composed of SfaD and GpgA. GTP-bound, activated FadA stimulates growth and blocks sporulation. FlbA activates the GTPase activity of FadA, thus promoting the return of the protein to an inactive state, halting vegetative growth and allowing asexual sporulation (Yu *et al.*, 1996; Yu *et al.*, 1999). Deletion of *flbA* in *A. nidulans* results in strongly reduced sporulation and excessive growth of aerial or submerged hyphae followed by autolytic collapse of the mycelium (Lee & Adams, 1994a; Wieser *et al.*, 1994). Interestingly, no autolytic phenotype was observed in *A. fumigatus* (Mah & Yu, 2006). Very recently, a non-conidiating phenotype has been shown for *flbA* and *brlA* gene deletion strains of *A. niger* (Krijgsheld *et al.*, 2013b).

Fig. 1. Regulatory network underpinning autolysis and conidiation. FlbA, a regulator of G-protein signaling, activates GTPase activity of FadA resulting in a halt of vegetative growth. The FlbA, FluG and FlbB-E-proteins activate BrIA, the regulator of sporulation. Reprinted with permission from Krijghsheld et al., J Proteome Res 12, 1808-1819. Copyright 2013 American Chemical Society.

but so far the effect of *flbA* or *brlA* deletion on autolysis has not been determined.

The expression of glycoside hydrolases and proteases during carbon starvation is one of the factors affecting the autolytic phenotype. In the *A. nidulans flbA* deletion strain, complete hyphal disintegration is observed after 3 days of growth in a submerged culture. The chitinase ChiB is required for the observed disintegration, as no such phenotype is observed in a strain carrying both *flbA* and *chiB* gene deletions (Shin *et al.*, 2009). Furthermore, an *A. nidulans* strain carrying a deletion of *brlA* was found to have lower expression or delayed up-regulation of *chiB* and *engA* during carbon starvation (Pocsi *et al.*, 2009; Szilagyi *et al.*, 2010). Similar effects on selected hydrolases or proteases have been found following deletion of other regulators from this pathway (Emri *et al.*, 2008). However, a systematic overview of the influence of this regulatory network on the expression of hydrolytic enzymes during carbon starvation is lacking.

Here, we describe the role of glycoside hydrolases during carbon starvation in *A. niger* using transcriptomics, proteomics, measurements of enzyme activities and

an analysis of their effect on the fungal cell wall. We uncover the set of glycoside hydrolases regulated by the developmental regulators FlbA and BrIA by applying this system-wide analysis to *A. niger* wild-type and strains carrying *flbA* or *brIA* gene deletions.

Methods

Media and strains. *Aspergillus niger* strains were grown on solidified (2 % agar) minimal medium (MM) (Bennett *et al.*, 1991) or complete medium (CM) containing, in addition to MM, 1% yeast extract and 0.5% casamino acids. Minimal medium for bioreactor cultivations was composed as previously described (Jorgensen *et al.*, 2010) with 8 % (w/v) maltose-monohydrate as the sole growth limiting nutrient. The generation of *A. niger* strains carrying a *brIA* (An01g10540) or *flbA* (An02g03160) gene deletion in the N402 (Bos *et al.*, 1988) background, has been described previously (Krijgsheld *et al.*, 2013b).

Bioreactor cultivation and sampling. Except minor modifications regarding the inoculation as described below, duplicate bioreactor batch cultivations were performed as previously described (Jorgensen *et al.*, 2010) in a 6.6 L Bioflo 3000 bioreactor (New Brunswick Scientific) with a volume of 5 L. Cultures of the non-co-nidiating $\Delta brIA$ and $\Delta flbA$ mutants were inoculated with mycelial biomass obtained from shaking flask pre-cultures grown for 16 h at 30 °C in CM at 250 RPM. Prior to inoculation, mycelial biomass was blended and washed twice with minimal medium used for bioreactor cultivation. Cultures were started directly with 750 RPM, sparger aeration (1 L min⁻¹) and 0.01 % polypropylene glycol as antifoam agent. Throughout cultivation, the pH was maintained at 3.0 by addition of titrants (2 M NaOH or 1 M HCl) and the dissolved oxygen tension (DOT) remained above 40 %. Cultivations were sampled at regular intervals as described by (Nitsche *et al.*, 2012). Cultivation and sampling of the wild-type strain N402 has been reported previously (Nitsche *et al.*, 2012).

Transcriptome and proteome analysis. Isolation of RNA, the hybridization to microarrays and data analysis were performed as described by (Nitsche *et al.*, 2012). In short, samples were taken during the exponential growth phase, and after 16 h (day 1), 60 h (day 3) and 140 h (day 6) of carbon depletion of batch fermentations of strains $\Delta brIA$ and $\Delta flbA$. RNA was isolated with the Trizol reagent, cleaned

using spin columns and hybridized to Affymetrix whole genome gene chips (Platform GPL6758). Data was analyzed together with those obtained previously for *A. niger* wild-type strain N402 (Nitsche *et al.*, 2012). Data normalization was done as described (Nitsche *et al.*, 2012), using a False Discovery Rate (FDR) cut-off of 0.005 to determine statistical significance of transcriptional differences. Microarray data of genes encoding CAZymes are available at the GEO database (Barrett *et al.*, 2011) under accession numbers GSE21752 (strain N402, exponential growth phase) and GSE39559 (strain N402, carbon starvation time points). Data for CAZyme expression in strains $\Delta brlA$ and $\Delta flbA$ is available in Supplementary Table S1. R/Bioconductor (Gentleman *et al.*, 2004) (<http://bioconductor.org>) was used for the analysis of Affymetrix microarray data. CEL files were processed using the Robust Multi Array Average algorithm (Irizarry *et al.*, 2003) as implemented in the affy package (Gautier *et al.*, 2004). Quality of microarray raw data was tested using various functions provided with the affycoretools package (MacDonald, 2008). Differential gene expression analysis was performed using the limma package (Smyth, 2004). Identification of genes with similar expression profiles was guided by hierarchical clustering of genes encoding CAZymes with a minimum normalized expression value of 0.2 % of *actA* (An15g00560) and a significant change in expression levels in one or more conditions. Hierarchical clustering analysis was done using McQuitty's and Ward's methods as implemented in the hclust function. Heatmaps were generated with a modified version of the aspectHeatmap function of the classdiscovery package (Wang *et al.*, 2009).

Proteome analysis was performed by LC-MS/MS as described (Nitsche *et al.*, 2012) for a single fermentation of the mutant strain $\Delta flbA$ and compared to the values obtained in duplicate for the wild-type strain (Nitsche *et al.*, 2012).

Detection of enzyme activities. Protease activity in the culture filtrate was measured using N,N-dimethylated BSA as substrate as described previously (Nitsche *et al.*, 2012). Carbohydrate-active enzyme activities in culture filtrates were quantified using isolated fungal cell walls as a model substrate. Cell walls were obtained from *A. niger* strain N402, grown in bioreactor batch cultures until the end of exponential growth phase as described above. Mycelium was disrupted using a bead-beater with 425-600 μm glass beads and fragmentation was verified by microscopy. Cell walls were collected by centrifugation, washed and cell wall associated enzyme activity was inactivated by incubating for 10 min at 100 °C. Culture filtrates were incubated with 0.1% (w/v) cell walls while shaking at 30°C for 1 h. Reactions were

terminated by incubation at 95 °C for 15 min. Released carbohydrate monomers were quantified with HPAEC-PAD at least in duplicate for each biological replicate. Monomers were separated on a CarboPac PA1 analytical column at 20°C, using either 100 mM sodium hydroxide as a mobile phase for the separation of N-acetylglucosamine and mannose or 12 mM sodium hydroxide to separate glucose and galactose. Detection was performed with a standard quadruple waveform (Rocklin *et al.*, 1998).

Exo-acting enzyme activity was separately quantified by incubating 30 µl culture filtrate in 50 µl volume with 0.5 or 1 mM GlcNAc-β-pNP, GalNAc-β-pNP or Glu-β-pNP at 30 °C. Reactions were stopped by addition of 0.1 M Na₂CO₃, and released pNP was detected at 405 nm. For each biological replicate, at least triplicate measurements were performed for each time point. Statistical analysis was performed using a 2-sided t-test with n=2.

Cell wall carbohydrate composition. Mycelium was obtained at 7 time-points during the bioreactor cultivation of wild-type strain N402 and mutant strains $\Delta flbA$ and $\Delta brlA$. Mycelium (0.7 g fresh weight) was mixed with 0.5 ml acid washed glass beads (425-600 µm) and disrupted using a mini-bead-beater (Biospec Products). Cell walls were isolated and carbohydrates were hydrolyzed to monomers and quantified by HPAEC-PAD as described in Chapter 4. Changes in the cell wall composition were tested for statistical significance using a two sample t-test.

Results

Phenotypic analysis of *brlA* and *flbA* deletion mutants in *A. niger*. The *A. niger* genes An01g10540 and An02g03160 were identified as orthologs of the *A. nidulans* developmental regulators *brlA* (AN0973) and *flbA* (AN5893), respectively, and their open reading frames were replaced with the hygromycin resistance cassette in the *A. niger* wild-type strain N402 (Krijgsheld *et al.*, 2013b). Both mutants formed aconidial fluffy colonies. Colonies of the $\Delta brlA$ strain kept developing aerial hyphae that eventually touched the lid of the Petri dish. Aerial hyphae of the $\Delta flbA$ strain began collapsing in the center of the colony after three days of incubation leading to disintegration of the complete aerial hyphae (Fig. 2). The phenotypes of both deletion strains are in agreement with those described for the *A. nidulans* and *A. niger* deletion strains (Adams *et al.*, 1998; Krijgsheld *et al.*, 2013b).

Fig. 2. Morphology of N402 (A), $\Delta flbA$ (B) and $\Delta brlA$ (C) during growth on agar plates. The wild-type strain N402 forms aerial hyphae and conidiophores with black conidia, clearly visible from the top view (top) and schematic representation (bottom). The mutant strain $\Delta flbA$ forms only aerial hyphae that collapse in the colony center. Mutant strain $\Delta brlA$ lacks sporulation and forms conidial stalks that extend into the air, as clearly visible in the side view (middle).

Submerged growth in bioreactor batch cultures. To investigate the properties of the developmental mutants $\Delta flbA$ and $\Delta brlA$ during nutrient limitation, the strains were grown in liquid batch cultures in maltose limited minimal medium in a bioreactor and compared to bioreactor cultures of the wild-type strain which have been described previously (Nitsche *et al.*, 2012). Tight control of culture conditions, where the pH was maintained at 3.0, resulted in reproducible biomass profiles and a dispersed mycelial morphology. During exponential growth, biomass accumulation in strains N402 and $\Delta brlA$ was comparable, with maximum specific growth rates of 0.242 ± 0.001 and $0.218 \pm 0.003 \text{ h}^{-1}$, respectively. Strain $\Delta flbA$ grew slower, with a maximum specific growth rate of $0.162 \pm 0.006 \text{ h}^{-1}$. Growth curves were synchronized at time point $t = 0 \text{ h}$, defined as the end of the exponential growth phase, which was recognized by an increase in dissolved oxygen (Nitsche *et al.*, 2012). At the end of the exponential growth phase, the accumulation of biomass in strain $\Delta flbA$ was with $3.4 \pm 0.02 \text{ g (kg culture broth)}^{-1}$ less than for the wild-type and $\Delta brlA$ strains (4.9 ± 0.02 and $4.8 \pm 0.01 \text{ g (kg culture broth)}^{-1}$, respectively) (Fig. 3A). Upon carbon depletion, a continuous decrease in biomass was observed for strains N402 and $\Delta brlA$. During the first 20 h of starvation, biomass decrease was more pronounced for strain $\Delta flbA$ than for strains N402 and $\Delta brlA$, whereas after that only a minimal decrease in biomass was observed for $\Delta flbA$ (Fig. 3A).

Fig. 3. Growth of strains N402 (squares), $\Delta flbA$ (triangles) and $\Delta brlA$ (dots) in batch cultures. The biomass (A) in dry weight and protein content (B) (Bradford assay), given as mean $\pm$ SE of measurements performed for two biological duplicates.

The protein content of culture filtrates (Fig. 3B) increased during exponential growth (up to 0 h) to a concentration of approximately 10 mg l⁻¹ for all 3 strains. The protein concentration increased further during the early starvation phase (0 – 16 h) and remained constant thereafter.

Transcriptional profiling of CAZymes. To identify the CAZymes that are regulated by BlrA and FlbA, transcriptional profiles of genes encoding CAZymes were established for the $\Delta brlA$ and $\Delta flbA$ strains at four time points during the batch cultivation (Supplementary Table S1). Samples were taken during exponential growth, and 16 h (day 1), 60 h (day 3) and 140 h (day 6) post carbon depletion, corresponding to time points of the previously published DNA microarray data set for the N402 wild-type strain cultivated under identical conditions (Nitsche *et al.*, 2012). Together, the expression data set consists of 12 different conditions; three strains (wild-type, $\Delta brlA$ and $\Delta flbA$) and four time points. When comparing the transcriptome of genes encoding CAZymes, we observed notable differences between strain $\Delta flbA$ compared with the wild-type and $\Delta brlA$ strain at all time-points. In addition, we identified conidiation-related genes, which had different expression profiles at day 3 and day 6 of carbon starvation between the wild-type compared with $\Delta flbA$ and $\Delta brlA$. These findings are presented and discussed in detail in the following paragraphs.

Strain $\Delta flbA$ during the exponential growth phase: signs of cell wall stress. During the exponential growth phase transcription of α -glucan synthase encoding

agsA was increased 3-fold in $\Delta flbA$ compared to the wild-type and $\Delta brlA$ strains (Table 1A). Gene *agsA* is a target of the cell wall integrity (CWI) pathway (Damveld *et al.*, 2005) and this may suggest that strain $\Delta flbA$ experiences cell wall stress during exponential growth. Accordingly, expression of *crhB* and *crhC*, which encode putative GH16 chitin- β -glucan transferases, increased 5- and 6-fold respectively during exponential growth phase in the $\Delta flbA$ strain compared to the wild-type strain, and 4- and 5-fold compared to strain $\Delta brlA$. Their *S. cerevisiae* homologs *crh1* and *crh2* are up-regulated during cell wall stress (Bermejo *et al.*, 2008; Garcia *et al.*, 2004). These enzymes cross-link chitin to β -glucan, an activity that is part of the mechanism by which the cell counters cell wall stress (Cabib *et al.*, 2007).

Surprisingly, expression of genes encoding plant cell wall degrading enzymes was increased in $\Delta flbA$ during exponential growth. These included putative endo- β -1,4-glucanases/cellulases belonging to GH12 (An03g05530, 5-fold increase) and GH5 (An16g06800, 10-fold increase) as well as the GH28 endo-polygalacturonase II *pgalI* (23-fold increase). Expression of the gene encoding endo-polygalacturonidase PgaB increased 3- and 4-fold in $\Delta flbA$ and $\Delta brlA$ respectively. Together these results indicate that FlbA not only affects gene expression during developmental stage, but that this regulator also has a role during vegetative growth.

Effect of $\Delta flbA$ mutation of glycogen metabolism and cell wall modification.

Other genes were affected in strain $\Delta flbA$ both during exponential growth and during carbon starvation (Table 1B). Transcriptional changes were observed in genes responsible for glycogen metabolism, which may result in altered turnover of this storage compound. Transcription of the glycogen synthase encoding gene An02g10310 decreased 6-fold during exponential growth of $\Delta flbA$ compared to the wild-type. Transcription of gene An14g04190, encoding glycogen branching enzyme GbeA, decreased 5- and 6-fold during the exponential growth phase and carbon starvation day 1. Comparable to the regulatory mechanism in higher eukaryotes, the activity of fungal glycogen synthase is regulated by its phosphorylation state (Freitas *et al.*, 2010). The protein phosphatase-1, which is responsible for dephosphorylating - and thereby activating - glycogen synthase, consists of a catalytic subunit that can interact with different regulatory subunits that modify its function. During dephosphorylation of glycogen synthase in yeast, the regulatory subunit Gac1p interacts with the catalytic subunit Glc7p and through its CBM21 also with glycogen synthase (Wu *et al.*, 2001). The *A. niger* An02g05260 gene is a homolog of *gac1*. The transcription

Table 1. Normalized gene expression levels of genes discussed in the manuscript.

ORF	Name	wild-type		$\Delta lfbA$			$\Delta brlA$			SP ^B	GPI ^C	CAZy family	Function				
		Exp ^a	Day 1	Day 3	Day 6	Exp	Day 1	Day 3	Day 6								
A																	
An04g09890	AgsA	1.6	1.3	0.8	0.6	5.5	1.3	0.7	0.6	1.7	0.7	0.5	0.5	1	0	GH13-GT5	α -glucan synthase
An07g07530	ChnB	4.4	3.3	3.6	4.3	21.1	8.1	7.7	7.3	4.8	2.3	6.8	5.9	1	1	CBM18-GH16	putative chitin- β -1,3-glucan transferase
An07g01160	ChnC	2.4	0.9	2.0	2.4	13.6	7.2	4.3	9.0	2.9	1.9	5.6	7.2	1	1	CBM18-GH16	putative chitin- β -1,3-glucan transferase
An03g05530		3.8	2.8	3.2	2.0	18.6	8.2	4.4	3.6	5.1	1.8	1.4	1.0	1	0	GH12	putative endo- β -1,4-glucanase
An16g06800		2.8	1.8	2.6	2.6	27.9	5.5	2.3	2.2	2.7	1.2	1.7	2.0	1	0	GH5_5-CBM1	endo- β -1,4-glucanase
An15g05370	PgalI	0.3	0.6	0.7	0.8	6.7	3.6	0.9	0.5	0.3	0.7	0.4	0.3	1	0	GH28	endo-polygalacturonase
An02g04900	PgaB	2.5	0.7	0.5	0.5	8.5	1.1	0.6	0.6	10.4	1.3	0.6	0.4	1	0	GH28	endo-polygalacturonase
B																	
An02g10310		13.1	12.2	12.9	14.2	2.0	5.8	6.5	5.6	8.0	7.9	6.2	5.6	0	0	GT3	glycogen synthase
An14g04190	GbeA	10.9	42.6	23.7	22.1	2.2	7.2	9.5	8.4	4.1	23.2	21.6	13.3	0	0	CBM48-GH13	glycogen branching enzyme
An02g05260		5.3	17.3	28.8	25.3	0.7	0.7	1.4	1.1	1.8	5.1	10.7	6.1	1	0	CBM21	putative carbohydrate binding module
An03g06400	CicA	33.1	9.5	6.8	5.5	3.4	0.9	0.4	0.5	16.3	3.8	1.6	0.6	1	0	GH18	endo-chitinase, group B
An08g09610	AgnD	22.4	21.0	21.8	16.2	7.4	4.6	4.7	1.9	38.4	31.9	24.2	12.4	1	0	GH71	putative endo- α -1,3-glucanase
An18g00730		57.2	20.9	3.6	1.6	3.7	1.4	0.9	1.0	59.0	20.2	2.1	0.8	1	0	GT69	
C																	
An02g07020	CicA	1.3	69.6	75.9	83.9	2.6	79.2	91.0	57.7	2.0	37.9	73.2	73.6	0	0	GH18	chitinobiosidase, group A
An02g11720		2.1	26.0	20.7	22.0	2.0	20.1	18.9	19.5	2.1	14.7	16.4	16.0	0	0	GH38	class 2 ER α -mannosidase
An07g12550		0.8	95.8	85.8	55.5	0.5	101.3	108.5	65.9	0.6	50.3	62.4	42.4	1	0	GH47	α -1,2-mannosidase
An01g06500	DfgD	0.4	2.5	3.6	4.8	0.4	0.7	2.2	3.9	0.4	4.1	7.9	8.7	1	A	GH76	putative α -1,6-mannanase
An07g07700		1.3	27.3	32.6	24.4	1.3	29.1	27.7	20.7	1.1	17.2	16.0	12.7	1	0	GH76	putative α -1,6-mannanase
An04g08730		4.9	18.7	18.2	16.2	2.7	19.4	12.5	16.1	2.0	8.4	9.3	7.3	1	0	GH125	putative exo- α -1,6-mannanase
An14g04240		0.2	4.9	3.1	1.9	0.3	4.3	1.7	0.6	0.2	1.9	2.3	1.5	1	0	GH92	putative α -mannosidase
An11g03120		0.6	10.5	8.6	5.6	0.5	11.6	12.7	9.3	0.5	11.4	8.2	4.8	1	0	GH43	putative endo- β -1,4-xylosidases
An02g10550	AbnC	0.7	48.4	119.5	95.0	0.8	67.5	51.0	36.3	1.7	34.9	26.5	12.4	1	0	GH43	putative endo- α -1,5-arabinanase
An12g01850		0.9	6.2	3.9	5.0	0.8	3.7	3.4	4.7	1.0	6.8	5.7	6.4	0	0	GH2	β -mannosidase
D																	
An03g02240	NagA	0.8	63.6	50.7	49.7	1.5	8.0	10.2	3.7	1.4	38.3	34.9	17.7	1	0	GH20	β -N-acetylhexosaminidase
An01g03090	EngA	0.9	29.9	51.0	52.7	1.2	19.5	18.9	9.3	1.0	16.6	39.8	40.1	1	0	GH81	endo- β -1,3-glucanase
An08g08370		0.3	34.1	31.3	40.0	0.3	8.3	3.2	5.1	0.3	9.0	20.5	31.0	1	0	GH92	putative α -mannosidase
An16g02910		0.5	16.7	9.1	6.6	0.5	2.6	0.5	0.4	0.4	13.3	5.0	2.5	1	0	GH92	putative α -mannosidase
E																	
An09g02160	RgaeA	0.3	5.5	3.3	2.8	0.3	20.5	15.3	8.3	0.3	2.3	1.2	1.7	1	0	CE12	riemannogalacturonan acetyl esterase
An09g01190	AbnA	0.8	2.5	1.5	1.1	0.8	19.9	21.4	13.2	0.7	1.4	1.1	2.3	1	0	GH43	endo- α -1,5-L-arabinase
An14g01770		1.1	1.7	1.8	1.7	1.0	10.3	4.1	2.6	0.7	1.3	1.3	1.2	1	0	GH3	putative β -glucosidase
An07g09330	ChhA	0.3	0.5	2.0	0.7	0.3	2.5	0.4	0.4	0.3	0.7	0.7	0.7	1	0	GH7	cellobiohydrolase A
An07g11660	ChhB	0.3	0.3	0.4	0.4	0.4	3.2	1.3	0.3	0.3	0.3	0.4	0.3	1	0	GH7-CBM1	cellobiohydrolase B
An15g03350		0.4	0.4	0.8	0.8	0.4	2.0	0.7	0.5	0.4	0.6	0.5	0.3	1	0	GH43	putative α -L-arabinofuranosidase
An03g05260		0.4	0.4	0.4	0.4	0.5	2.8	2.1	1.3	0.4	0.3	0.4	0.3	1	0	GH75	chitosanase
An01g11670		0.2	0.2	0.2	0.2	0.2	1.0	2.6	0.5	0.2	0.2	0.2	0.2	1	0	GH5_5-CBM1	endo-glucanase
An03g00500		1.9	1.9	2.9	2.9	4.4	24.4	15.4	19.7	3.9	2.1	2.9	3.4	1	0	GH30	putative β -1,6-glucanase
An07g08640	AgnB	0.4	29.5	25.9	6.0	0.3	52.0	36.3	51.2	0.3	8.3	10.3	7.6	1	A	GH71	α -1,3-glucanase

ORF	Name	wild-type			ΔflbA			ΔbriA			SP ^b	GPI ^c	CAZY family	Function				
		Exp ^a	Day 1	Day 3	Day 6	Exp	Day 1	Day 3	Day 6	Exp					Day 1	Day 3	Day 6	
F	An01g10540	BriA	0.3	0.3	11.7	16.4	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0	0	N/A	transcription factor, role in sporulation	
	An01g03750	AbaA	0.6	0.5	8.8	20.4	0.6	0.4	0.4	0.3	0.5	0.4	0.6	0.8	0	0	N/A	transcription factor, role in sporulation
	An07g03340	Hvp1	1.9	2.6	29.2	72.8	2.0	2.3	3.0	3.3	1.7	2.5	2.8	3.4	1	0	N/A	hydropodin
	An09g05730	FwnA	1.4	0.9	11.4	23.7	0.8	0.7	0.7	0.6	0.7	0.8	0.9	0.7	0	0	N/A	polyketide synthase
	An14g05370	BnA	0.9	0.7	44.1	55.0	0.8	0.6	0.6	0.6	0.5	0.5	0.5	0.5	1	0	N/A	multicopper oxidase
	An02g13580	CfcI	0.4	0.8	19.4	38.9	0.3	1.1	0.8	0.6	0.2	0.9	0.3	0.2	1	0	GH18-CBM18	exo-chitinase, group A/C
	An09g05920	CtcB	0.3	0.4	11.0	37.0	0.3	0.4	0.4	0.4	0.3	0.5	0.5	0.6	1	0	GH18	endo-chitinase, group B
	An15g07370		0.3	0.3	2.2	7.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	CBM14	putative chitin binding module
	An06g01530	BglD	0.8	0.6	2.0	3.9	0.8	1.0	0.9	1.2	0.6	0.5	1.0	1.2	1	0	GH17	putative β-1,3-glucanotransferase, generating β-1,6-linkages
	An02g00850		1.2	2.2	5.6	9.8	1.3	1.4	1.0	0.9	1.1	1.2	1.1	1.0	1	1	GH16	endo-β-1,3-glucanase
	An02g09050	GelG	0.6	0.6	2.3	5.5	0.8	0.7	0.5	0.6	0.7	0.8	0.7	0.6	1	1	GH72	putative β-1,3-glucanotransferase
	An14g05340	UrhgB	0.2	0.3	7.0	8.7	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	GH105	putative rhamnogalacturonyl hydrolase
	An18g01410	DfgA	0.6	0.6	1.3	2.5	0.7	0.6	0.6	0.5	0.7	0.5	0.6	0.6	1	0	GH76	putative α-1,6-mannanase
	An06g01140		0.6	0.5	2.3	4.5	0.5	0.4	0.4	0.5	0.5	0.5	0.4	0.4	0	0	GT1	similarity to N-glycosyltransferase ngt - <i>Saccharothrix aerocolonigenes</i>
G	An15g02350		0.9	0.7	51.9	147.0	0.7	0.6	0.5	0.6	0.6	0.6	0.5	0.5	1	0	AA11	putative copper-dependent polysaccharide monooxygenases
	An14g00660	ChsC	5.4	4.6	4.0	4.5	3.4	1.6	0.5	0.5	4.6	2.2	1.3	1.0	0	0	GT2	chitin synthase
	An16g02850	ChnF	1.2	0.8	3.7	2.5	3.1	1.1	1.3	1.5	1.2	0.9	1.1	0.9	1	0	GH16	putative chitin-β-1,3-glucan transferase
	An10g00400	GelA	9.9	15.7	42.7	42.1	9.8	3.4	2.1	1.3	23.3	15.7	15.8	5.2	1	0	GH72	β-1,3-glucanotransferase
	An01g12450	BxgA	7.1	28.0	23.7	36.2	2.9	21.1	3.6	1.2	3.5	10.6	6.3	2.5	1	0	GH55	exo-β-1,3-glucanase
	An16g07040	BglE	48.7	36.1	39.9	30.7	59.9	30.6	11.2	8.8	48.0	24.7	14.2	4.1	1	0	GH17	putative β-1,3-glucanotransferase, generating β-1,6-linkages
	An14g02670		0.6	1.3	6.4	3.3	0.8	3.2	0.9	0.8	0.6	0.9	0.9	0.6	1	0	AA9-CBM1	putative copper-dependent polysaccharide monooxygenases
	An08g05230		0.5	2.0	12.1	9.3	0.6	5.2	1.1	0.6	0.4	1.2	1.3	0.6	1	0	AA9	putative copper-dependent polysaccharide monooxygenases
	An03g05170		0.9	10.0	27.9	32.2	2.2	23.9	12.7	8.4	1.0	8.1	6.1	1.8	1	0	AA11	putative copper-dependent polysaccharide monooxygenases
	H	An08g09030	CfcB	0.4	0.4	0.6	0.7	0.4	0.4	0.4	0.7	0.3	1.0	5.1	10.6	1	0	GH18
An14g02180		TpsC	0.5	0.8	1.3	3.5	0.7	1.1	1.6	1.8	0.7	2.1	9.1	21.4	0	0	GT20	putative trehalase-6-phosphate synthase
An02g07770			1.4	3.4	3.6	4.6	3.1	4.1	7.4	8.6	0.8	2.6	9.7	13.5	0	0	GT4	strong similarity to trehalose synthase TSase - <i>Grifola frondosa</i>
An13g00400		TppB	0.9	1.2	1.1	1.5	1.2	1.3	1.3	1.3	1.1	2.2	3.5	4.4	1	0	GH16	trehalose-6-phosphate phosphatase
An03g01050			0.4	1.3	0.8	0.7	0.4	0.6	0.6	0.6	0.3	0.9	1.1	4.6	1	0	GH5_16	putative endo-β-1,6-galactanase
An16g03720			1.4	3.0	3.5	5.0	1.2	1.4	2.5	2.3	1.4	3.2	7.5	9.3	0	0	CBM48	putative glycogen binding domain
An16g09090			0.6	0.8	1.7	3.1	0.8	1.1	1.6	2.0	0.7	1.4	3.4	5.3	0	0	GH3	putative β-hexosamidase

Mean gene expression levels given as % of *actA* expression during exponential growth.^a Exp: exponential growth, Day 1, 3 or 6; carbon starvation day 1, 3, 6^b SP: predicted signal peptide presence (1) or absence (0)^c GPI: predicted GPI-anchor presence (1), absence (0) or ambivalence (A)

of this gene strongly decreased in the $\Delta flbA$ strain, both in the exponential growth phase and during carbon starvation.

Several genes encoding fungal cell wall modifying glycoside hydrolases showed significant changes in expression between the wild-type strain and the $\Delta flbA$ strain during the exponential growth phase and during carbon starvation. Transcription of the *ctcA* gene decreased around 10-fold in strain $\Delta flbA$ during the exponential growth phase and during carbon starvation. The encoded chitinase CtcA is an ortholog of the cell wall anchored *A. nidulans* ChiA, which is located at hyphal branch sites and at hyphal tips and is thought to be involved in cell wall remodeling during growth (Yamazaki *et al.*, 2008). The gene An08g09610, encoding the putative α -1,3-glucanase AgnD, showed a 3- to 8-fold decrease in transcription over the time-course of the $\Delta flbA$ cultivation. During exponential growth of strain $\Delta flbA$, the glycoside transferase An18g00730 was the most repressed CAZyme encoding gene, showing a 16-fold decrease in transcription. It is similarly regulated during day 1 of carbon starvation. One of its homologs in *Cryptococcus neoformans*, Cmt1p, has α -1,3-mannosyltransferase activity (Sommer *et al.*, 2003) while its other homolog, CAP59, is essential for formation of the extracellular protective capsule (Garcia-Rivera *et al.*, 2004). This suggests a possible role for An18g00730 in FlbA-dependent synthesis of a cell wall component in *A. niger*.

CAZymes with increased transcription during carbon starvation. The expression of a set of CAZyme encoding genes increased at day 1 of carbon starvation compared to the exponential growth phase, both in the developmental mutants and in wild-type (Table 1C). In addition, a subset of genes showed increased transcription levels in wild-type but displayed altered expression in the $\Delta flbA$ and $\Delta brlA$ mutants (Table 1D). These two groups are discussed in more detail in the next two sections.

CAZymes expressed during carbon starvation in all 3 strains. As described previously (Nitsche *et al.*, 2012), *cfcA* was one of the genes with the highest increase in transcription after one day of carbon starvation (Table 1C). Expression of the orthologs of this chitinase in *A. fumigatus* (*chiB1*) and *A. nidulans* (*chiB*) increased during starvation (Erdei *et al.*, 2008; Jaques *et al.*, 2003) and in the latter species the ChiB protein has a role in autolysis and fragmentation (Shin *et al.*, 2009; Yamazaki *et al.*, 2007). Deletion of *flbA* or *brlA* did not appear to affect *cfcA* transcription. In contrast,

in *A.nidulans* the induction of *chiB* transcription by carbon starvation is delayed in the $\Delta brlA$ strain (Pocsi *et al.*, 2009).

In both wild-type and the $\Delta flbA$ and $\Delta brlA$ strains, carbon starvation resulted in increased transcription of the An02g11720 and An01g12550 genes, encoding intracellular α -mannosidase enzymes putatively involved in glycosylation. Also transcription of multiple genes encoding (putative) mannan-acting enzymes increased; the putative GH76 α -1,6-mannanase DfgD that has similarity to enzymes allowing cross-linking of proteins to the fungal cell wall (Kitagaki *et al.*, 2002; Maddi *et al.*, 2012), the GH76 putative α -1,6-mannanase An07g07700, the GH125 putative exo- α -1,6-mannanase An04g08730 and the GH92 putative α -mannosidase An14g04240. These enzymes form a complete set of activities needed for the break-down and modification of α -1,6-mannan, which is found in the *Aspergillus* cell wall (Fontaine *et al.*, 2000).

In all three fungal strains, starvation resulted in increased transcription levels of genes encoding enzymes predicted to be involved in degradation of plant derived carbohydrates. These included the GH43 putative xylosidase encoded by An11g03120, the putative endo- α -1,5-arabinanase encoded by *abnC* and the GH2 β -mannosidase encoded by An12g01850, which could act on substrates such as galacto(gluco)mannan. In view of the substrate specificity of these enzymes, a function in fungal cell wall remodeling during starvation appears unlikely. Alternatively, their expression may be induced by a de-repression mechanism for producing enzymes that scout for alternative carbon sources as proposed by (Delmas *et al.*, 2012; Martens-Uzunova & Schaap, 2009).

Carbon starvation up-regulated CAZymes that are dependent on FlbA. The expression of several genes encoding fungal cell wall acting enzymes is at least partly dependent on FlbA (Table 1D). During carbon starvation, transcription of the β -N-acetyl-hexosaminidase encoding gene *nagA* increased strongly (≥ 60 -fold) in wild-type. The increase in *nagA* expression was strongly reduced in the $\Delta flbA$ strain. This contrasts with the expression of *cfcA*, the other chitinolytic enzyme encoding gene with a strong increase in transcription during starvation. In the $\Delta brlA$ strain, the increase of *nagA* transcription was lower than in the wild-type on day 6 of carbon starvation, indicating a partial dependence on this regulator. The GH81 endo- β -1,3-glucanase EngA (Fontaine *et al.*, 1997), in *A. nidulans* involved in autolytic fragmen-

tation of mycelia pellets (Szilagyi *et al.*, 2010), appears to be partly regulated by FlbA, as transcription levels were strongly increased throughout carbon starvation in the wild-type and the $\Delta brlA$ strain, but less so on day 3 and 6 of carbon starvation in the $\Delta flbA$ strain.

The gene An08g08370 encodes an enzyme that belongs to GH92, a family of which only bacterial members have been biochemically characterized; most of them were identified as α -mannosidases (Maruyama *et al.*, 1994; Tiels *et al.*, 2012; Zhu *et al.*, 2010). Transcription of gene An08g08370 was increased > 90-fold during carbon starvation in the wild-type strain, but only $\geq$ 11-fold in the $\Delta flbA$ strain. This increase in transcription was delayed in $\Delta brlA$ but transcription eventually reached the same level as in the wild-type strain at day 6 of carbon starvation. The gene An16g02910 encodes another GH92 enzyme. Its transcription increased 32-fold in the wild-type strain at day 1 of carbon starvation compared with exponential growth, but only 5-fold in the $\Delta flbA$ strain.

CAZymes up-regulated in strain $\Delta flbA$ during carbon starvation. The transcription of 10 CAZyme-encoding genes differed between $\Delta flbA$ on the one hand and the wild-type or $\Delta brlA$ on the other hand after the transition from exponential growth to day 1 of carbon starvation (Table 1E). The majority of these genes encode CAZymes that degrade plant-derived polysaccharides, most notably the rhamnogalacturonan acetyl esterase RgaeA, endo-1,5- α -L-arabinosidase AbnA and a putative β -glucosidase encoded by An14g01770. RgaeA and AbnA were reported to be part of a set of pectin degrading enzymes that are up-regulated upon carbon source depletion in *A. niger* (Martens-Uzunova & Schaap, 2009). Also transcription of An03g00500, encoding the putative fungal cell wall-acting β -1,6-glucanase, was increased 3- to 6-fold during carbon starvation in $\Delta flbA$ but not in the wild-type or $\Delta brlA$ strains.

The gene AgnB, encoding an α -1,3-glucanase (Chapter 3), had high transcription levels during carbon starvation in the $\Delta flbA$ strain, while in the wild-type transcription was similarly increased at day 1 and 3 but was decreased at day 6 of carbon starvation.

Conidiation specific genes. During the carbon-limited batch fermentation of the wild-type strain N402 formation of asexual reproductive structures was observed from day 3 onwards (Nitsche *et al.*, 2012). Coherent with the phenotype of strains

$\Delta flbA$ and $\Delta brlA$ during growth on agar plates (Fig. 2), conidiation was not observed for these strains in liquid cultures. These differences were reflected in gene transcription levels (Table 1F). For example, transcription of the genes encoding the conidiation-specific regulators BrlA and AbaA (An01g03750), increased 43-fold and 15-fold in wild-type after day 3 of carbon starvation, compared to the exponential growth phase. This increase in transcription was lacking completely in the $\Delta flbA$ and $\Delta brlA$ strains. A similar pattern was observed for genes reported to be sporulation related, such as hydrophobin *hyp1/rodA* (An07g03340) (Parta *et al.*, 1994) as well as polyketide synthase *fwnA* (An09g05730) and multicopper oxidase *brnA* (An14g05370), which are required for the production of spore pigment (Jorgensen *et al.*, 2011b). Transcription of these genes was increased at least 16-fold, 8-fold and 51-fold respectively only in the wild-type from day 3 of carbon starvation onwards, compared to exponential growth phase. Thus, transcription of conidiation-specific genes was increased from day 3 onwards only in the wild-type.

Seven glycoside hydrolases, a glycoside transferase as well as a protein containing only a CBM14 domain, shared this conidiation-specific expression profile, indicating that their transcription is strictly dependent on the conidiation pathway (Table 1F). Six of these seven up-regulated hydrolases are predicted or known to be active on substrates that are located in the fungal cell wall, suggesting they may have a role in cell wall modification during conidiophore or spore formation. Transcription of the chitinases *cfcl* and *ctcB* was increased most strongly (at least 48-fold and 39-fold respectively) among the conidiation specific hydrolases. CtcB belongs to a phylogenetic group containing only endo-chitinases, and Cfcl is an exo-chitinase that hydrolyzes chitin oligosaccharides to monomers (Chapter 5). We recently showed that these chitinases are expressed in the conidiophores and that they play a role in modification of the spore wall by hydrolyzing cell wall chitin (Chapter 4). The gene An15g07370, encoding a protein consisting of a sole carbohydrate binding module of family 14 (CBM14), was expressed together with *cfcl* and *ctcB* in a *brlA* and *flbA* dependent manner, supporting a possible role for this protein in modifying CtcB and Cfcl activity during conidiation (Chapter 4). In addition, the expression of β -glucan acting BgtD, An02g00850 and GelG during day 3 and day 6 of carbon starvation was strictly dependent on *brlA* and *flbA*. The only glycoside transferase that shared this transcription pattern is the so far uncharacterized GT1 gene An06g01140. Comparative genomics using the *Aspergillus* Genome Database (Arnaud *et al.*, 2010) shows that the presence of this gene is restricted to six species, which all belong to the black aspergilli (Peterson, 2008).

By far the highest level of transcription for genes encoding conidiation-specific CAZymes was observed for An15g02350, with levels over 4-fold more abundant than subsequent genes. The transcription profile of this gene was clearly conidiation-specific: a 60- and 170-fold increase of transcription was observed at day 3 and 6 of carbon starvation in the wild-type strain when compared to exponential growth. No expression was detected in the $\Delta brlA$ and $\Delta flbA$ strains. This gene is a member of a CAZy family of copper-dependent lytic polysaccharide monooxygenases that has recently been established, auxiliary activity family AA11. A biochemical function in oxidative cleavage of chitin chains has been demonstrated for the first representative of this family, *A. nidulans* AoLpmo11 (Hemsworth *et al.*, 2014). Thus, An15g02350 also may be active on chitin, and a function for this enzyme in hydrolysis of cell wall chitin during conidiation would complement the role of the conidiation-specific chitinases CfcI and CtcB in cell wall modification. Importantly, we demonstrated here for the first time a physiological role for a member of this AA11 family.

Other genes down-regulated during lack of conidiation. A second group of genes had reduced transcription levels in the $\Delta brlA$ and $\Delta flbA$ strains on day 3 and day 6 of carbon starvation when compared to the wild-type, similar to the conidiation-specific genes described above. However, in contrast to the conidiation-specific genes, this second group of genes was also expressed during other time-points, such as exponential growth (Table 1G). This group included genes encoding cell wall acting enzymes such as chitin synthase ChsC, the putative chitin- β -glucanotransferase CrhF as well as the β -1,3-glucanotransferase GelA, the exo- β -glucanase BxgA and the putative β -glucanotransferase BgtE, which may generate β -1,6-linkages. It also included genes encoding AA9 (previously GH61) and AA11 putative lytic polysaccharide mono-oxygenases.

Genes up-regulated specifically in $\Delta brlA$. Specifically in the $\Delta brlA$ strain, six genes encoding CAZymes showed ≥ 3 -fold increase in transcription levels only after both 3 and 6 days of starvation compared to exponential growth (Table 1H). This increase in transcription levels was absent or much lower in the wild-type or the $\Delta flbA$ strain. Transcription of *cfcB*, which encodes an enzyme with high sequence similarity to GH18 phylogenetic group A exo-chitinases that release chitobiose, was increased 8- and 15-fold in the $\Delta brlA$ strain after 3 and 6 days of starvation, respectively.

The disaccharide trehalose (Glc- α -1,1- α -Glc) acts as stress protectant and reserve

carbohydrate in fungi. During its synthesis, trehalose-6-phosphate synthase (Tps) produces trehalose-6-phosphate from UDP-glucose and glucose-6-phosphate. Subsequently trehalose-6-phosphate phosphatase (Tpp) removes the phosphate to produce trehalose (Avonce *et al.*, 2006). Transcription of *tpsC* and gene An02g07770 with similarity to Tps was increased 6-fold and 3-fold respectively in the wild-type on day 6 but much more strongly in the $\Delta brlA$ strain (13- and 30-fold on day 3 and day 6 respectively for *tpsC* and 12- and 17-fold for An02g07770). Only in the $\Delta brlA$ strain, transcription of *tppB* was increased 3-fold after 3 and 6 days starvation compared to exponential growth. This suggests that trehalose synthesis may be increased in the $\Delta brlA$ strain, perhaps signifying a need for increased stress protection.

Proteome analysis of culture filtrates. Using semi-quantitative proteomics, proteins in the culture filtrate of the $\Delta flbA$ strain were identified and compared to the previously analyzed proteome of the N402 wild-type culture filtrates (Nitsche *et al.*, 2012). The $\Delta flbA$ strain was recently reported to have a more complex secretome than the wild-type during growth as sandwiched colony on xylose (Krijgsheld *et al.*, 2013b); at present it is unknown whether this is also the case during submerged fermentations and during carbon starvation.

During carbon starvation of the wild-type and $\Delta flbA$ strains, the percentage of CAZymes among total proteins was similar with regard to number (29 - 41 %) and amount (mass) (39 – 53 %) of protein. During exponential growth, CAZymes made up 51 % of the total amount of detected proteins (24 % of total number) in the wild-type strain, but only 22 % of the total amount (11 % of total number) in the $\Delta flbA$ strain. This difference was largely due to an increase in intracellular proteins from 15 % of the total amount in the wild-type to 41 % in the $\Delta flbA$ strain.

The most abundant CAZymes in the culture filtrates of the wild-type and $\Delta flbA$ strains were in general similar (Table 2). Glucoamylase GlbA, acid amylase AamA and α -glucosidase AgdA were abundantly present during exponential growth phase and carbon starvation. During carbon starvation in both strains, an increased amount was detected of enzymes that act on cell wall β -glucan, for example for the GH16 chitin- β -glucanotransferase CrhD, GH17 β -glucanotransferase BgtB that generates β -1,6 branches, the β -glucanotransferase GelD and exo- β -1,3-glucanase BxgA. Putative endo-arabinase AbnC and α -arabinofuranosidase AbfB were also secreted at high levels in both strains, despite the low gene expression levels of *abfB*.

Table 2. Protein levels detected in culture filtrates of the wild-type strain (2 biological replicates) and strain *Δf1bA*

ORF	Wild-type				Wild-type				<i>Δf1bA</i>	
	Exp ^a	Day 1	Day 3	Day 6	Exp	Day 1	Day 3	Day 6	Exp	Day 1
An03g06550	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
An11g03340	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
An02g10550	+++++	+++++	+++++	+++++	-	+++++	+++++	+++++	+++++	+++++
An04g06920	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
An01g11010	+++	++++	++++	++++	+++	++++	++++	+++++	++++	++++
An03g05290	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
An15g02300	++	++++	++++	++++	-	++++	++++	++++	++	++++
An18g03570	++	++++	++++	++++	+	++++	++++	++++	++	++++
An09g00670	++++	++++*	++++	++++*	+++	++++*	++++	++++*	++++	++++
An01g12450	++	++++	++++	++++	-	++++	++++	++++	++	++
An06g00170	++	++++	++++	++++	-	++++	++++	++++*	-	++++
An04g08730	+++	++++	++++	++++	++	++++	++++	++++	+++	++++
An09g02240	-	++++	++++	++++	-	++++	++++	++++	-	-
An03g00500	+++	++++*	++++	++++	++	++++	++++	++++	++++	++++
An01g12550	++	++++	++++	++	-	++++	++++	++*	-	++
An03g05530	++++	++++	++++*	++++*	++++	++++*	++++	++++	++++	++++
An16g06800	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++
An11g01540	+++	++++	++++	++++	.*	++++	++++*	++++	++++	++++
An01g10930	++	++++	++++	++++	++	++++	++++	++++	++++	++++
An01g04560	-	++++	++++	++++	-	-	++++	++++	-	-
An08g03060	++	++++	++++	++++	-	+++	++++	++++	++	-
An02g11150	++	++++*	++++*	++++	.*	+++	++++	+++	-	++++
An09g02160	-	++++	++++	+++	-	++++	++++	++++*	-	++++
An12g08280	-	++++	++++	++++	.*	++++	++++	++++	++	++
An14g04240	-	++++*	++++	++++	-	+++	++++	++++	-	-
An08g10780	-	+++	+++	-	.*	+++	++++	+++	-	.*
An03g04190	+++	++++	+++	-	++	++++	++++	-	+++	++++
An02g07020	-	++	-	-	-	++	-	-	-	-
An04g06930	++	+++	++++	++++*	++	+++	++++*	+++*	+++	+++
An01g01540	+++	+++	++++	++++	++	+++	++++	+++	+++*	-
An08g05230	-	++++	++++	-	-	++++*	++++	-	-	-
An03g00960	-	+++	++++	+++	-	-	++++	-	-	-
An15g05370	-	-	-	+++	-	-	+++	+++	++++	++++
An07g09330	-	-	++++	+++	-	-	++++	++++	-	-
An08g08370	-	++++	+++*	++	.*	+++	+++	-	-	-
An03g01050	-	++++*	++++	++++	-	++++*	+++	+++	-	-
An01g00330	.*	+++	++++	+++*	.*	++++*	++++	+++	-	-
An11g06540	++	+++	++++	++++	-	-	++++*	+++	++	++
An09g01190	-	++++	++++	++++	-	-	++++	-	-	++++
An14g01800	.*	+++	++++*	++++	-	+++	+++	+++	-	.*
An14g01790	-	+++*	+++	+++	.*	+++*	++++	+++	-	-
An01g09960	-	+++*	++++	++++*	-	++	+++	+++	-	-
An07g08640	-	+++	++	-	-	+++	+++	-	-	-
An14g02670	-	-	++++	+++	-	-	++++*	+++	-	-
An01g14600	-	-	+++	+++	-	-	++++	-	+++	-
An07g07700	-	+++	-	-	-	+++	-	-	-	-
An01g03340	-	-	-	-	-	-	++++	-	-	-
An10g00400	+++	++	-	-	+++	++	-	-	+++	-
An04g09650	-	+++	+++	+++	-	.*	+++*	++	-	-
An14g03520	-	+++	+++	-	-	-	-	-	-	-
An01g01920	-	-	+++*	+++	-	-	+++*	++	-	-
An02g13180	-	+++	+++	+++	-	++	+++	-	-	-
An01g11660	-	+++	+++	+++	-	-	-	-	.*	-
An08g07350	+++	+++	-	-	++	+++	-	-	+++	+++

<i>ΔfibA</i>		SP ^b	GPI ^c	CAZy family	Name	Function
Day 3	Day 6					
+++++	+++++	1	0	GH15-CBM20	GlaA	glucoamylase
+++++	+++++	1	0	GH13	AamA	acid α-amylase
+++++	+++++	1	0	GH43	AbnC	putative endo-α-1,5-arabinanase
+++++	+++++	1	0	GH31	AgdA	α-glucosidase
+++++	+++++	1	1	GH16	CrhD	putative chitin-glucan transferase
+++++	+++++	1	1	GH17	BgtB	β-1,3-glucanotransferase, β-1,6-branching
+++++	+++++	1	0	GH54-CBM42	AbfB	α-arabinofuranosidase
+++++	+++++	1	0	GH3	Bgl1	β-glucosidase
++++	+++++	1	1	GH72-CBM43	GelD	β-1,3-glucanotransferase
++++	+++++	1	0	GH55	BxgA	exo-β-1,3-glucanase
++++	+++++	1	0	GH27-CBM13	AglA	α-N-acetylgalactosaminidase
+++++	+++++	1	0	GH125		putative exo-α-1,6-mannanase
-	++++	1	0	GH20	NagA	β-N-acetylhexosaminidase
+++++	+++++	1	0	GH30		putative β-1,6-glucanase
-	-	1	0	GH47		α-1,2-mannosidase
++++	++++	1	0	GH12		putative endo-β-1,4-glucanase
++++	+++	1	0	GH5-CBM1	EglB	putative endo-glucanase
++++*	++++	1	1	GH16	CrhA	putative chitin-glucan transferase
++++	+++	1	0	GH31	AgdB	putative α-glucosidase
++++	-	1	0	GH16		putative β-1,3-glucanase
++	++	1	0	GH92		putative α-mannosidase
++++	++++	1	0	GH27	AglB	α-galactosidase
++++	++++	1	0	CE12	RgaeA	rhamnogalacturonan acetyl esterase
++++*	++++	1	0	GH32	InuE	exo-inulinase
++	-	1	0	GH92		putative α-mannosidase
+++	++++	1	0	GH43-CBM35	GbgA	putative galactan β-1,3-galactosidase
-	-	1	0	EXPN-CBM63		expansin
-	-*	0	0	GH18	CfcA	chitobiosidase, group A
+++*	+++	1	0	GH13	AmyC	amylase
+++	+++	1	0	GH65		α,α-trehalase
-	-*	1	0	AA9		putative copper-dependent lytic polysaccharide monooxygenase
-*	-	1	0	GH62	AxhA	β-1,4-arabinoxylan arabinofuranohydrolase
++++	++++	1	0	GH28	PgalI	endo-polygalacturonase
-	-	1	0	GH7	CbhA	cellobiohydrolase
-	-	1	0	GH92		putative α-mannosidase
-	-	1	0	GH5		endo-β-1,6-galactanase
+++	+++	1	0	GH51	AbfA	α-arabinofuranosidase
++++	++++	1	0	GH2	MndA	β-mannosidase
++++	-	1	0	GH43	AbnA	arabinan endo-α-1,5-arabinosidase
-	+++	1	0	GH27		putative α-galactosidase
+++	+++*	0	0	CBM35		putative galactan binding module
+++	-	1	0	GH3	XlnD	xylosidase
-	-	1	A	GH71	AgnB	α-1,3-glucanase
-	-	1	0	AA9-CBM1		putative copper-dependent lytic polysaccharide monooxygenase
-	-	1	0	GH11		putative endo-β-1,4-xylanase
-	-	1	0	GH76		putative α-1,6-mannanase
-	++++	1	0	GH12		xyloglucan-specific endo-β-1,4-glucanase
-	-*	1	1	GH72	GelA	β-1,3-glucanotransferase
-	++	1	1	GH76		putative α-1,6-mannanase
-	-	1	1	GH76	DfgC	putative α-1,6-mannanase
-	-	1	0	GH20		putative β-N-acetylhexosaminidase
-	-	1	0	GH55	BgxB	putative β-1,3-glucanase
++++	++++	1	0	GH7-CBM1	CbhB	cellobiohydrolase
-	-	1	1	GH72	GelB	β-1,3-glucanotransferase

Table 2. Protein levels detected in culture filtrates of the wild-type strain (2 biological replicates) and strain *ΔflbA*

ORF	Wild-type				Wild-type				<i>ΔflbA</i>	
	Exp ^a	Day 1	Day 3	Day 6	Exp	Day 1	Day 3	Day 6	Exp	Day 1
An02g04900	-	-	-	-	-	-	-	-	++++*	++++*
An11g00200	-	-	-	+++	-	-	-	+++	-	-
An07g07530	++	+++	-	-	-	+++	-	-	++++	-
An16g07040	+++	-	-	-	++	+++	-	*	++++	+++
An09g00260	-	-	-	-	-	-	-	-	-	-
An09g03260	-	-	-	+++	-	-	+++	-	-	-
An11g06080	-	+++	-	-	-	+++	-	-	-	-
An02g00850	-	-	-	++	-	-	-	++	-	-
An03g06310	-	-	-	-	-	-	-	-	++++*	*
An02g11890	-	++	++	+++	-	++	+++	+++	-	-
An13g02110	-	++	++	++	-	++	+++	-	-	-
An01g00780	+++	-	-	-	++	+++	-	-	+++	-
An14g02760	-	-	-	-	-	-	-	-	*	-
An03g02960	-	-	-	-	-	-	-	-	-	-
An09g06400	+++	-	-	-	++	-	-	-	+++	-
An14g04200	-	-	-	-	-	-	+++	-	+++	-
An14g01620	-	-	-	-	-	-	+++	-	-	-
An05g01320	-	-	-	-	-	-	-	-	++++*	++++*
An08g01900	-	-	+++	++	*	-	-	-	-	-
An16g06990	-	+++	-	-	++	-	-	-	++	-
An08g09610	+++	-	-	-	-	-	-	-	+++	-
An14g04370	-	-	-	-	-	-	-	-	++++	-
An04g09700	-	-	-	-	-	-	-	-	-	-
An07g01160	-	-	-	-	-	-	-	-	-	+++
An16g02730	-	-	-	-	++	-	-	-	+++	-
An01g06620	-	-	-	-	-	-	-	-	-	-
An16g08090	-	-	-	-	-	-	-	-	-	-
An07g04650	++	-	-	-	-	-	-	-	++	-
An03g05260	-	-	-	-	-	-	-	-	++	-
An09g03070	+	-	-	-	-	-	-	-	++	-
An16g03720	-	-	-	-	-	-	-	-	++	-

Protein levels are quantified as follows: - not detected + < 5 ng/ml ++ < 250 ng/ml +++ < 1000 ng/ml ++++ < 4000 ng/ml ++++++

Values marked with an asterisk (*) had a relative standard deviation of > 50 & between technical replicates

^a Exp; exponential growth, Day 1,3 or 6: carbon starvation day 1, 3, 6

^b SP: predicted signal peptide presence (1) or absence (0)

^c GPI: predicted GPI-anchor presence (1), absence (0) or ambivalence (A)

A noticeable feature in the proteome of both strains was that for a number of cell wall acting enzymes with high transcription levels during carbon starvation, protein was detected only at a low level or not at all. These include the GH81 endo-β-1,3-glucanase EngA, the GH18 chitinase CfcA as well as the GH76 putative α-1,6-mannanase An07g07700.

A number of differences were found between the extracellular proteins of the wild-type and *ΔflbA* (Table 2). The GH28 endo-polygalacturonase PgalI and GH5 cellulase EglB were detected in increased quantities in *ΔflbA* during exponential growth and carbon starvation, as might be expected from their gene expression levels. The

<i>ΔflbA</i>		SP ^b	GPI ^c	CAZy family	Name	Function
Day 3	Day 6					
+++	+++	1	0	GH28	PgaB	endo-polygalacturonase
-	-	1	0	GH3		putative β-glucosidase
.*	-	1	1	CBM18-GH16	CrhB	chitin-glucan transferase
-	-	1	0	GH17	BgtE	putative β-1,3-glucanotransferase, generating β-1,6 linkages
.*	-	1	0	GH36	AgIC	α-galactosidase
-	+++	1	0	GH28	PgaD	endo-polygalacturonase
-	.*	1	0	GH3		putative β-glucosidase
-	-	1	1	GH16		endo-β-1,3-glucanase
+++	++++	1	0	CE8	PmeA	pectin methylesterase
-	-	1	0	GH79		β-glucuronidase
++	++	0	0	GH29		putative α-L-fucosidase
-	-	1	0	GH11	XynB	endo-β-1,4-xylanase
++++	++++	1	0	GH12	EglA	cellulase
+++	+++	1	0	GH20		putative β-N-acetylhexosaminidase
-	.*	1	1	GH18	CtcA	endo-chitinase, group B
-	-	1	0	GH28	RhgB	rhamnogalacturonase
-	-	1	0	GH79		putative β-glucuronidase
+++	+++	1	0	GH5	Man5A	endo-β-1,4-mannanase
-	-	1	0	GH43		putative β-1,4-xylosidase
-	+++	1	0	GH28	PgaA	endo-polygalacturonase
.*	.*	1	0	GH71	AgnD	putative α-1,3-glucanase
-	++	1	0	PL1	PelA	pectin lyase A
+++	+++	1	0	GH28	XghA	endo-xylogalacturonan hydrolase
+++	+++	1	1	CBM18-GH16	CrhC	chitin-glucan transferase
-	-	1	0	GH43	AbnD	putative endo-arabinanase
-	.*	1	0	GH78		putative α-L-rhamnosidase
-	+++	1	1	GH76	DfgE	putative α-1,6-mannanase
-	.*	0	0	GH17	BgtC	putative β-1,3-glucanotransferase generating β-1,6 linkages
-	-	1	0	GH75		chitosanase
-	-	1	0	GH13-GT5	AgsE	α--glucan synthase
-	-	0	0	CBM48		putative glycogen binding module

< 50 ng/ml

> 4000 nI/ml

α-1,3-glucanase AgnB was detected in the wild-type during carbon starvation but not in *ΔflbA*, in contrast to its gene expression levels, that were up-regulated to a similar extent in both strains (day 1 and 3) or up-regulated (day 6) in only *ΔflbA*. The detection of the chitinolytic GH20 β-N-acetyl-glucosaminidase NagA fits with the gene expression profile, the amount of NagA protein in the culture filtrate of *ΔflbA* is reduced compared to that in the wild-type. The GH47 α-1,2-mannosidase An01g12550 as well as GH92 putative α-mannosidases An08g03060, An08g08370 and An14g04240 were detected at lower levels in *ΔflbA* when compared to the wild-type.

Hydrolytic enzyme activities in culture filtrates. The proteolytic and hydrolytic enzyme activities in the culture filtrates of batch fermentations of the 3 strains was studied. Protease activity (Fig. 4A) increased strongly at the onset of carbon starvation (> 0 h) but subsequently remained stable in time. Throughout the fermentation, proteolytic activity remained lowest for the $\Delta flbA$ strain.

Carbohydrate hydrolytic capacity in the culture filtrates was determined by assessing the combined activity of endo- and exo-acting enzymes on the carbohydrates in the fungal cell wall. Incubation of fermentation filtrates with isolated fungal cell walls resulted in the release of galactose and glucose. In the wild-type strain enzyme activities releasing galactose (Fig. 4B) and glucose (Fig. 4C) increased during carbon starvation (0 - 140 h). Compared to the wild-type the culture filtrates of the $\Delta brlA$

Fig. 4. Enzyme activities detected in culture filtrate of batch cultures of strains N402 (squares), $\Delta flbA$ (triangles) and $\Delta brlA$ (dots). Protease activity (A), total hydrolytic activity releasing galactose (B) and glucose (C) from the isolated *A. niger* cell walls and GH exo-activity releasing N-acetyl-glucosamine from GlcNAc- β -pNP (D). Values are given as mean $\pm$ SE of measurements performed for two biological duplicates.

strain had similar glucose releasing activity but lower galactose releasing activity. The culture filtrate of the $\Delta flbA$ strain contained lower activity in both cases. No release of mannose and N-acetyl-glucosamine from cell walls was detected, indicating that mannanase and chitinase activity was absent (data not shown). The activity of exo-acting N-acetyl-hexosaminidase was determined separately using the pNP labeled substrate N-acetyl-glucosamine- β -pNP (Fig. 4D). Hydrolysis increased in time throughout starvation, in the wild-type and both developmental mutants. Thus, although no cell wall acting chitinase activity was detected in culture filtrates, exo-chitinolytic activity was present.

Cell wall carbohydrate composition. Differential expression and activity of CAZymes and cell wall polymer synthases in strains N402, $\Delta flbA$ and $\Delta brlA$ during autolysis and sporulation may result in changes in cell wall composition. Therefore the carbohydrate monomer composition of cell walls was analyzed at seven time points during exponential growth and autolysis. At the end of the exponential growth phase, the total carbohydrates in wild-type strain N402 cell walls were composed of 13 % glucosamine, 8 % galactose, 73 % glucose and 6 % mannose (Fig. 5A), consistent with the previously reported composition of *A. niger* cell walls (Johnston, 1965). The glucosamine may arise from either N-acetyl-glucosamine or glucosamine, present in the cell wall in chitin or chitosan since the used acid hydrolysis removes the acetyl group from carbohydrates. During carbon starvation, the relative galactose and mannose content remained similar while the glucosamine content increased to 24 % and glucose content decreased to 63 % at day 6 of carbon starvation.

During the exponential growth phase of $\Delta flbA$ strain (Fig. 5B), the relative amount of glucosamine detected in cell walls was significantly higher than in wild-type (24 versus 13 %), whereas the relative amount of glucose was lower (61 versus 73 %). Also small variations in galactose content were observed during carbon starvation but otherwise no significant changes were detected in carbohydrate composition during the course of the batch cultivation. Cell walls of strain $\Delta brlA$ (Fig. 5C) from the exponential phase contained less glucose (64 %) compared to wild-type (73 %) and, although with $p = 0.06$ not significant, seemed to contain relatively more glucosamine. During carbon starvation (> 0 h), the monomer composition of the cell walls of this strain was not significantly different from the wild-type.

Discussion

When filamentous fungi encounter carbon starvation, complex responses are initiated to ensure their survival. Turnover of hyphal biomass by autophagy and cell wall degradation may provide the energy and building blocks required to fuel cellular differentiation and sporulation. Here we present an overview of the physiological effects of the mutational inactivation of the developmental regulators FlbA and BrIA that coordinate the expression of carbohydrate-active enzymes, the key effectors of cell wall remodeling during autolysis and sporulation. Under carefully controlled, reproducible growth conditions, changes in gene transcription, protein secretion, enzyme activities, and their combined effects on the fungal cell wall were investigated.

During growth on solid media, the $\Delta brlA$ strain shows an a-conidial phenotype as recently described (Krijgsheld *et al.*, 2013b); the mutant strain differentiates to form aerial hyphae and conidial stalks, but does not produce mature conidiophores. De-

Fig. 5. Carbohydrate composition of the cell walls of strains N402 (A), $\Delta flbA$ (B) and $\Delta brlA$ (C). Values are given as mean $\pm$ SE in % of total moles of carbohydrate as detected in isolated cell walls of two independent growth experiments.

letion of *flbA* results in inhibition of sporulation and the formation of long aerial hyphae, as described by (Krijgsheld *et al.*, 2013b). In addition, we showed here that these hyphae collapse in the center of the colony, resulting in a phenotype similar to that of an *A. nidulans* strain carrying a deletion of *flbA* (Wieser *et al.*, 1994).

During the exponential growth phase in the submerged fermentation, three important differences were identified for $\Delta flbA$ compared to the wild-type and $\Delta brlA$ strains. First, the growth rate and the amount of accumulated biomass was lower, in contrast to the lack of differences in biomass levels reported after 7 days of growth as sandwiched colonies on solid media containing xylose (Krijgsheld *et al.*, 2013b). Secondly, the transcriptome data suggest that production of glycogen is reduced in $\Delta flbA$, in view of reduced transcription of the glycogen synthase and glycogen branching enzyme encoding genes. In addition, glycogen synthase may be inactivated at the protein level by phosphorylation; the putative regulatory subunit of protein phosphatase 1 - that dephosphorylates glycogen synthase - is down-regulated, and deletion of *flbA* may result in continuous activation of protein kinase Pka (similar as reported for *A. nidulans*, (Shimizu & Keller, 2001)) which phosphorylates glycogen synthase. Such a reduction in glycogen synthase gene transcription and enzyme activity may be the result of stress experienced during the exponential growth, as described for *Neurospora crassa* (Cupertino *et al.*, 2012). As a result of reduced glycogen synthesis, the $\Delta flbA$ strain may have accumulated less carbon- and energy reserves to withstand future stress conditions such as carbon starvation. Thirdly, the up-regulated transcription of the CWI pathway target *agsA* and the increased amount of chitin in the cell wall, which may be a result of CWI pathway activation (Ram *et al.*, 2004), both indicate that the CWI pathway is activated during exponential growth, thus reflecting a requirement for cell wall reinforcement. Indeed the width of the cell wall of strain $\Delta flbA$ is thinner compared to that of the wild-type (Krijgsheld *et al.*, 2013b). The changes in enzymes (putatively) responsible for cell wall synthesis and modification identified in this study provide a molecular explanation for this finding.

During carbon starvation in the submerged fermentation, *flbA* deletion resulted in a reduction of transcription of the β -N-acetyl-glucosaminidase encoding gene *nagA*, the endo- β -1,3-glucanase encoding gene *engA* as well as genes An16g02910 and An08g08370 that encode GH92 α -mannosidases. Expression levels were increased for β -1,6-glucanase encoding gene An03g00500, the gene encoding α -1,3-

glucanase AgnB, and a number of genes encoding putative plant cell wall acting enzymes. The $\Delta flbA$ strain reportedly has a hyper-autolytic phenotype (Wieser *et al.*, 1994). In this study this is reflected in a rapid decrease in its biomass during the first day of carbon starvation (Fig. 3A). However, the transcriptome and proteome data of strain $\Delta flbA$ show that induction of autolytic genes and enzymes was rather limited. Also the enzyme activities detected in the culture filtrates by this strain were lower or equal to those of the wild-type, also giving no indication for hyper-induction of autolytic enzymes. Extracellular GlcNAc-pNP degrading activity was reduced in $\Delta flbA$, corresponding with a reduction in *nagA* transcription and NagA protein concentration. It is possible that the enzymes responsible for cell wall degradation are associated with the cell wall and not present in the culture filtrate. However, the amount of biomass and the cell wall composition of strain $\Delta flbA$ remained unchanged after 1 day of carbon starvation, signifying changes to the wall may be limited. The rapid decrease in biomass during the first day of carbon starvation combined with the lower enzyme activity and lack of cell wall monomer changes indicate that other processes than cell wall degradation may play an important role in the observed autolytic phenotype of the $\Delta flbA$ strain.

The proteomics analysis indicates an increase in the number of total proteins and CAZymes during growth of the $\Delta flbA$ strain. The *flbA* gene thus is an interesting target for industrial strain improvement. This is in agreement with the previously reported enriched secretome for strain $\Delta flbA$ grown on solid media (Krijgsheld *et al.*, 2013b).

Surprisingly, for a number of genes that had a strongly up-regulated transcription during carbon starvation, such as those encoding chitinase CfcA, β -1,3-glucanase EngA and putative α -1,6-mannanase An07g07700, the corresponding proteins were not detected by the proteome analysis. These enzymes may escape detection by being located in or on the fungal cell wall, or may be unstable under the cultivation conditions. The absence of CfcA, the main chitinase expressed under carbon starvation conditions, and possibly that of other enzymes, may result in incomplete degradation of cell walls during carbon starvation (Nitsche *et al.*, 2012). Indeed, we did not detect endo-chitinase or mannanase activity in culture filtrates of any of the strains under the used cultivation conditions. Furthermore, the composition of the cell wall showed a relative increase of the (N-acetyl-)glucosamine content during carbon starvation, indicating a relative increase in the chitin and/or chitosan content.

Together these findings support the conclusion that chitinase, and possibly other activities such as mannanase activity, are required for complete cell wall degradation. Cultivation at pH 3 may lead to inactivation, degradation or increased cell wall association of these enzymes. Preliminary experiments with the wild-type strain showed that when pH control was released upon the onset of carbon starvation, the chitinase CfcA, β -1,3-glucanase EngA and putative α -1,6-mannanase An07g07700 were found in high concentrations in the culture filtrates. During these cultivations the pH increased to 5.8 and microscopy showed increased fragmentation compared to the pH 3 controlled cultivations. In Chapter 3 we show that deletion of the gene encoding chitinase CfcA indeed results in reduced hydrolysis of cell wall chitin during carbon starvation at elevated pH.

During submerged cultivation of the $\Delta brlA$ strain, the growth rate, the autolytic degradation of biomass and the cell wall composition were very similar to the wild-type strain. By comparing the $\Delta brlA$ transcriptome with that of the wild-type and $\Delta flbA$ strains, we identified a small subset of genes encoding CAZymes that is strictly dependent on BrlA for expression. This strongly suggests that their encoded enzymes are important for the formation of mature conidiophores. All these genes were up-regulated in the aerial structures of sporulating *A. niger* colonies growing on agar plates (Chapter 4); this shows that their function is conserved under different conditions of sporulation, and is not limited to sporulation in submerged liquid cultures. In addition to the *brlA* dependent CAZymes, a second set of enzymes was identified that are strongly up-regulated during sporulation, but are also expressed under vegetative conditions. These enzymes thus appear to have a role in sporulation but also during other growth conditions. This paper presents the first overview of the *A. niger* set of CAZyme-encoding genes that are controlled by the conidiation-specific regulatory pathway. This is of interest to understand the molecular processes underpinning cell wall changes during sporulation. Also, as fungal sporulation requires a significant investment of carbon and energy, manipulation of this process to redirect energy and building blocks to other metabolic routes may allow industrial strain improvement. This may be applicable especially under conditions where *brlA* deletion is not desirable, for example when potential effects on secondary metabolite production need to be avoided. The effect of *brlA* inactivation in *A. niger* on secondary metabolite production is still unknown, and although the link between secondary metabolite production and sporulation is often mediated through StuA (Sigl *et al.*, 2011; Twumasi-Boateng *et al.*, 2009), *brlA* inactivation did decrease mycotoxin pro-

duction in *A. fumigatus* (Coyle *et al.*, 2007). Thus, influencing sporulation by modifying expression of key *brlA* target genes provides an interesting alternative.

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Table S1. Normalized gene expression levels of all analysed genes encoding CAZy enzymes

ORF	wild-type				$\Delta flbA$				$\Delta briA$				SP ^b	GP ^c	CAZy family
	Exp ^a	Day 1	Day 2	Day 6	Exp	Day 1	Day 3	Day 6	Exp	Day 1	Day 3	Day 6			
An03g06550	268.3	91.7	17.2	10.2	183.5	98.8	2.6	3.9	154.9	41.8	7.2	1.9	1	0	GH15-CBM20
An11g03340	31.1	2.6	1.4	0.6	13.4	3.3	0.4	0.4	13.0	1.8	0.7	0.2	1	0	GH13
An02g10550	0.7	48.4	119.5	95.0	0.8	67.5	51.0	36.3	1.7	34.9	26.5	12.4	1	0	GH43
An04g06920	161.2	26.7	17.9	8.2	100.6	17.1	3.4	3.8	90.4	29.3	32.4	8.6	1	0	GH31
An01g11010	18.0	87.8	57.3	38.9	10.2	24.5	12.4	7.0	8.0	12.0	6.2	1.5	1	1	GH16
An03g05290	33.7	24.3	18.2	13.1	30.3	19.9	8.6	5.2	3.8	0.9	1.0	0.9	1	1	GH17
An15g02300	0.4	0.6	0.6	0.7	0.4	1.5	0.3	0.3	0.4	0.5	0.4	0.3	1	0	GH54-CBM42
An18g03570	2.6	47.1	67.1	75.2	5.2	21.6	3.8	3.0	5.5	41.5	32.1	12.6	1	0	GH3
An09g00670	45.4	71.7	57.3	66.5	52.7	62.5	29.9	18.5	22.9	34.6	24.3	11.9	1	1	GH72-CBM43
An01g12450	7.1	28.0	23.7	36.2	2.9	21.1	3.6	1.2	3.5	10.6	6.3	2.5	1	0	GH55
An06g00170	0.4	18.6	14.4	10.8	0.4	11.4	4.0	2.6	0.4	8.3	5.2	3.5	1	0	GH27-CBM13
An04g08730	4.9	18.7	18.2	16.2	2.7	19.4	12.5	16.1	2.0	8.4	9.3	7.3	1	0	GH125
An09g02240	0.8	63.6	50.7	49.7	1.5	8.0	10.2	3.7	1.4	38.3	34.9	17.7	1	0	GH20
An03g00500	1.9	1.9	2.9	2.9	4.4	24.4	15.4	19.7	3.9	2.1	2.9	3.4	1	0	GH30
An01g12550	0.8	95.8	85.8	55.5	0.5	101.3	108.5	65.9	0.6	50.3	62.4	42.4	1	0	GH47
An03g05530	3.8	2.8	3.2	2.0	18.6	8.2	4.4	3.6	5.1	1.8	1.4	1.0	1	0	GH12
An16g06800	2.8	1.8	2.6	2.6	27.9	5.5	2.3	2.2	2.7	1.2	1.7	2.0	1	0	GH5-CBM1
An11g01540	0.9	1.9	1.0	0.5	2.1	0.8	0.6	0.7	1.7	0.9	1.1	0.7	1	1	GH16
An01g10930	44.5	38.9	35.9	18.7	22.1	31.5	19.0	14.8	26.9	44.4	39.4	22.0	1	0	GH31
An01g04560	0.9	7.9	19.2	27.0	1.3	22.0	7.3	1.3	1.2	6.7	5.4	4.6	1	0	GH16
An08g03060	0.3	8.5	5.0	5.0	0.3	2.9	2.0	1.1	0.4	2.7	3.0	2.4	1	0	GH92
An02g11150	1.5	13.6	4.7	4.0	0.9	5.5	4.7	2.8	1.2	5.3	3.2	1.7	1	0	GH27
An09g02160	0.3	5.5	3.3	2.8	0.3	20.5	15.3	8.3	0.3	2.3	1.2	1.7	1	0	CE12
An12g08280	1.0	0.6	0.8	0.6	0.7	0.4	0.4	0.4	0.8	0.5	0.4	0.4	1	0	GH32
An14g04240	0.2	4.9	3.1	1.9	0.3	4.3	1.7	0.6	0.2	1.9	2.3	1.5	1	0	GH92
An08g10780	0.4	1.7	0.4	0.5	0.4	0.9	0.5	0.5	0.4	0.4	0.7	1.1	1	0	GH43-CBM35
An03g04190	13.2	8.8	3.7	1.6	21.9	24.9	2.8	1.6	6.5	4.0	2.3	2.4	1	0	EXPN-CBM63
An02g07020	1.3	69.6	75.9	83.9	2.6	79.2	91.0	57.7	2.0	37.9	73.2	73.6	0	0	GH18
An04g06930	6.0	2.8	3.9	1.8	7.4	0.8	0.6	0.4	6.0	3.3	3.1	1.3	1	0	GH13
An01g01540	5.4	7.5	6.7	7.4	4.1	4.2	3.4	3.5	3.3	5.9	5.7	3.4	1	0	GH65
An08g05230	0.5	2.0	12.1	9.3	0.6	5.2	1.1	0.6	0.4	1.2	1.3	0.6	1	0	GH61
An03g00960	0.4	2.1	8.7	7.6	0.4	2.3	6.6	17.9	0.4	5.1	1.9	0.5	1	0	GH62
An15g05370	0.3	0.6	0.7	0.8	6.7	3.6	0.9	0.5	0.3	0.7	0.4	0.3	1	0	GH28
An07g09330	0.3	0.5	2.0	0.7	0.3	2.5	0.4	0.4	0.3	0.7	0.7	0.7	1	0	GH7
An08g08370	0.3	34.1	31.3	40.0	0.3	8.3	3.2	5.1	0.3	9.0	20.5	31.0	1	0	GH92
An03g01050	0.4	1.3	0.8	0.7	0.4	0.6	0.6	0.6	0.3	0.9	1.1	4.6	1	0	GH5
An01g00330	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	1	0	GH51
An11g06540	1.0	0.8	0.5	0.5	0.8	0.7	0.4	0.4	0.5	0.6	0.5	0.5	1	0	GH2
An09g01190	0.8	2.5	1.5	1.1	0.8	19.9	21.4	13.2	0.7	1.4	1.1	2.3	1	0	GH43
An14g01800	0.3	1.0	0.6	0.5	0.3	1.2	0.8	0.5	0.3	0.4	0.3	0.3	1	0	GH27
An14g01790	0.4	1.3	0.8	0.5	0.4	0.9	0.6	0.5	0.3	0.6	0.4	0.3	0	0	CBM35
An01g09960	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.2	1	0	GH3
An18g04100	0.6	3.3	12.6	14.6	0.6	1.1	0.9	0.7	0.5	5.6	3.7	1.4	1	0	GH28
An07g08640	0.4	29.5	25.9	6.0	0.3	52.0	36.3	51.2	0.3	8.3	10.3	7.6	1	A	GH71
An14g02670	0.6	1.3	6.4	3.3	0.8	3.2	0.9	0.8	0.6	0.9	0.9	0.6	1	0	GH61-CBM1
An01g03090	0.9	29.9	51.0	52.7	1.2	19.5	18.9	9.3	1.0	16.6	39.8	40.1	1	0	GH81
An01g14600	0.6	0.6	1.3	1.1	0.6	0.7	0.5	0.4	0.5	1.3	0.8	0.5	1	0	GH11
An07g07700	1.3	27.3	32.6	24.4	1.3	29.1	27.7	20.7	1.1	17.2	16.0	12.7	1	0	GH76
An01g03340	0.7	0.7	0.8	0.8	1.1	1.4	1.4	1.0	0.7	0.7	0.9	1.0	1	0	GH12
An10g00400	9.9	15.7	42.7	42.1	9.8	3.4	2.1	1.3	23.3	15.7	15.8	5.2	1	1	GH72
An04g09650	0.3	0.5	0.3	0.3	0.2	0.5	0.3	0.4	0.2	0.3	0.3	0.2	1	1	GH76
An01g12150	1.6	10.8	2.9	2.1	1.2	8.9	1.2	0.8	1.0	7.4	1.8	1.0	1	0	GH35
An14g03520	2.7	5.9	5.2	5.3	4.9	7.4	9.1	11.2	4.7	4.6	5.7	6.4	1	1	GH76
An02g13580	0.4	0.8	19.4	38.9	0.3	1.1	0.8	0.6	0.2	0.9	0.3	0.2	1	0	GH18-CBM18
An01g01920	0.5	1.8	1.2	1.7	0.4	0.8	0.4	0.4	0.4	0.5	0.4	0.3	1	0	GH20
An02g13180	0.3	3.2	1.8	2.4	0.3	1.8	0.7	0.3	0.3	1.5	0.9	0.4	1	0	GH55
An01g11660	0.3	0.3	0.4	0.4	0.4	3.2	1.3	0.3	0.3	0.3	0.4	0.3	1	0	GH7-CBM1
An08g07350	13.3	7.9	4.9	3.8	16.8	9.1	3.8	3.2	8.3	3.8	2.6	2.4	1	1	GH72
An02g04900	2.5	0.7	0.5	0.5	8.5	1.1	0.6	0.6	10.4	1.3	0.6	0.4	1	0	GH28
An11g00200	0.4	1.1	2.6	1.5	0.5	0.7	0.9	1.6	0.4	6.5	1.6	1.0	1	0	GH3
An07g07530	4.4	3.3	3.6	4.3	21.1	8.1	7.7	7.3	4.8	2.3	6.8	5.9	1	1	CBM18-GH16
An16g07040	48.7	36.1	39.9	30.7	59.9	30.6	11.2	8.8	48.0	24.7	14.2	4.1	1	0	GH17
An09g00260	6.5	0.8	0.4	0.4	2.3	3.0	1.6	0.8	1.4	0.4	0.3	0.5	1	0	GH36
An09g03260	0.6	0.4	1.0	1.3	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.9	1	0	GH28
An11g06080	0.3	3.0	1.5	1.0	0.4	2.0	1.3	0.9	0.5	1.8	3.1	4.2	1	0	GH3
An08g03580	0.6	0.7	1.2	1.7	0.7	0.8	0.6	0.5	0.9	2.9	1.8	2.1	1	0	GH17
An03g00940	0.4	0.4	2.7	5.1	0.4	0.4	0.7	7.0	0.3	1.5	2.0	0.5	1	0	GH10
An13g01260	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.3	0.5	0.7	0.7	1	0	GH92
An14g05340	0.2	0.3	7.0	8.7	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	GH105
An02g00850	1.2	2.2	5.6	9.8	1.3	1.4	1.0	0.9	1.1	1.2	1.1	1.0	1	1	GH16
An03g06310	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.3	0.4	0.3	1	0	CE8
An02g11890	0.3	0.6	0.6	0.8	0.3	0.3	0.3	0.3	0.2	0.4	0.7	0.8	1	0	GH79
An13g02110	0.3	0.3	0.2	0.2	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0	0	GH29
An01g00780	0.5	0.5	0.7	0.6	0.5	0.6	0.6	0.5	0.7	0.5	0.3	0.3	1	0	GH11
An14g02760	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.2	0.3	0.3	1	0	GH12
An03g02960	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.2	0.4	0.3	0.4	0.3	1	0	GH20
An04g09360	0.5	1.1	1.1	1.1	0.5	1.3	1.6	1.6	0.4	1.4	1.6	1.9	1	0	CE12
An09g06400	33.1	9.5	6.8	5.5	3.4	0.9	0.4	0.5	16.3	3.8	1.6	0.6	1	1	GH18
An15g03550	0.4	0.4	0.8	0.8	0.4	2.0	0.7	0.5	0.4	0.6	0.5	0.3	1	0	GH43
An14g04200	0.3	0.4	0.5	0.4	0.3	0.8	0.6	0.7	0.3	0.3	0.3	0.2	1	0	GH28
An14g01620	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.4	0.4	1	0	GH79
An09g05920	0.3	0.4	11.0	37.0	0.3	0.4	0.4	0.4	0.3	0.5	0.5	0.6	1	0	GH18
An05g01320	0.5	0.4	0.4	0.4	1.0	0.9	0.8	0.9	0.7	0.9	0.8	1.0	1	0	GH5
An02g09050	0.6	0.6	2.3	5.5	0.8	0.7	0.5	0.6	0.7	0.8	0.7	0.6	1	1	GH72
An01g10350	1.4	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.7	0.9	1.0	0.9	1	0	GH35
An03g05380	0.8	0.8	0.8	0.8	0.9	3.3	1.5	1.1	1.0	1.3	0.9	0.4	1	0	GH12
An11g03120	0.6	10.5	8.6	5.6	0.5	11.6	12.7	9.3	0.5	11.4	8.2	4.8	1	0	GH43
An08g01900	0.3	0.3													

An08g09610	22.4	21.0	21.8	16.2	7.4	4.6	4.7	1.9	38.4	31.9	24.2	12.4	1	0	GH71
An13g02510	1.4	0.9	0.9	0.8	1.1	0.9	1.1	1.3	2.2	1.1	1.2	1.1	1	0	GH16
An14g04370	2.1	1.6	1.5	1.6	2.3	1.7	1.4	1.5	1.5	1.5	1.5	1.7	1	0	PL1
An12g05010	0.3	0.3	0.5	0.6	0.3	0.5	0.3	0.3	0.3	0.3	0.4	0.3	1	0	CE1
An09g03100	5.4	12.6	12.2	15.3	6.9	4.6	2.4	0.5	3.8	5.8	4.5	3.0	1	1	GH13
An04g09700	0.3	0.3	0.3	0.4	0.3	0.9	0.3	0.3	0.3	0.3	0.3	0.2	1	0	GH28
An07g01160	2.4	0.9	2.0	2.4	13.6	7.2	4.3	9.0	2.9	1.9	5.6	7.2	1	1	CBM18-GH16
An08g11070	0.7	0.5	0.4	0.6	0.6	0.6	0.5	0.5	0.6	0.5	0.5	0.4	1	0	GH32
An16g06120	0.6	0.4	0.4	0.4	0.6	0.4	0.4	0.5	0.5	0.3	0.4	0.3	1	1	GH72-CBM43
An04g03170	0.4	0.6	0.4	0.4	0.3	0.3	0.3	0.3	0.3	1.2	0.4	0.4	1	0	GH1
An04g09690	0.3	0.3	0.3	0.3	0.4	0.5	0.3	0.3	0.3	0.3	0.3	0.3	1	0	CE8
An16g02730	1.7	1.2	1.3	0.8	4.8	1.4	0.6	0.6	2.4	0.9	0.5	0.4	1	0	GH43
An01g06620	1.0	1.9	2.2	2.1	1.2	2.3	2.4	2.6	1.3	3.0	2.9	2.9	1	0	GH78
An16g08090	3.0	1.5	1.0	0.8	6.2	2.9	1.2	1.3	3.4	2.0	1.4	1.7	1	1	GH76
An18g05940	0.3	0.7	0.8	1.1	0.4	0.5	0.4	0.4	0.3	1.1	0.7	0.6	1	0	GH53
An07g04650	1.1	4.5	5.1	4.4	1.1	7.4	9.0	6.8	1.6	7.2	10.3	8.2	0	0	GH17
An02g02540	0.9	1.1	1.1	1.2	1.0	1.3	1.6	1.8	0.9	1.3	1.8	3.6	1	0	CE16
An15g07800	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.2	1	1	GH13
An12g05700	0.3	0.5	0.5	0.4	0.3	0.4	0.3	0.3	0.3	0.7	0.4	0.3	0	0	GH78
An01g04760	2.6	6.6	7.9	8.7	1.9	4.8	6.8	7.6	3.1	7.7	12.4	9.3	0	0	GT41
An03g05260	0.4	0.4	0.4	0.4	0.5	2.8	2.1	1.3	0.4	0.3	0.4	0.3	1	0	GH75
An01g11520	0.3	0.2	0.3	0.3	0.3	0.3	0.4	0.5	0.3	0.3	0.3	0.3	1	0	GH28
An09g03300	0.9	1.1	2.5	2.9	1.4	2.3	0.7	0.7	0.8	0.7	0.8	0.8	1	0	GH31
An01g01340	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0	0	GH88
An05g02100	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH13
An06g03060	9.0	6.4	1.8	1.3	11.5	6.1	2.4	1.5	15.7	5.1	2.1	2.2	1	1	GH76
An07g04930	0.6	0.8	0.7	0.6	0.8	0.6	0.6	0.7	0.7	0.7	0.5	0.6	1	0	GH43
An09g03070	3.7	1.9	3.5	4.0	6.7	1.2	1.1	0.8	4.4	1.9	2.5	3.0	1	0	GH13-GT5
An03g03740	3.6	10.9	4.9	2.5	3.2	16.3	11.2	9.7	2.8	14.5	14.3	5.7	0	0	GH1
An01g06630	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.4	1	0	GH2
An16g03720	1.4	3.0	3.5	5.0	1.2	1.4	2.5	2.3	1.4	3.2	7.5	9.3	0	0	CBM48
An07g00240	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GH78
An02g00610	0.5	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.5	0.5	0.4	0.4	1	0	GH2
An15g01890	3.3	3.6	4.1	4.7	3.5	4.0	4.9	5.1	4.1	4.4	4.4	4.0	0	0	GH3
An03g06740	0.4	0.4	0.5	0.6	0.4	0.4	0.5	0.5	0.3	0.4	0.5	0.6	1	0	GH28
An12g07020	4.0	2.2	1.1	1.0	5.8	2.5	1.4	1.0	7.1	4.1	3.0	2.7	1	0	GT32
An10g00390	0.5	0.6	0.5	0.5	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.6	1	0	AA8-AA3
An04g06990	5.7	5.7	2.1	1.9	1.4	1.8	1.0	0.9	6.4	4.4	1.8	1.2	1	0	GH47
An11g07660	2.5	1.1	1.0	0.8	2.0	1.2	1.1	1.1	2.8	1.3	1.1	1.5	0	0	GH5
An01g09810	0.6	2.0	0.9	0.8	0.7	1.0	1.1	0.9	0.7	0.9	0.8	0.8	0	0	GT4
An11g00680	1.0	0.7	0.7	0.8	0.7	0.7	0.6	0.8	0.7	0.5	0.8	0.9	0	0	GT1
An03g02990	3.8	4.0	3.4	3.6	5.4	3.3	4.3	4.2	7.2	5.8	7.1	7.8	0	0	GT8
An01g09290	4.2	2.7	7.7	8.2	3.3	3.5	4.8	5.0	5.4	4.6	5.3	4.9	0	0	GH37
An07g06430	8.2	2.6	2.7	2.6	6.2	1.6	0.9	1.1	9.4	3.3	3.5	3.2	1	0	GT24
An15g07810	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH13-GT5
An14g07420	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	1	0	CBM18-GH18
An14g07390	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	GH11
An09g00270	13.6	2.4	1.2	1.0	7.1	10.0	6.1	3.7	6.6	3.0	0.9	2.0	0	0	GH36
An15g04550	0.4	0.3	0.3	0.2	0.4	0.3	0.3	0.3	0.7	0.3	0.3	0.2	1	0	GH11
An14g02170	0.4	0.3	0.8	0.6	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.3	1	0	CE5
An12g07500	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.5	0.6	0.7	1	0	GH28
An11g04040	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH28
An02g12450	0.6	0.6	0.5	0.5	0.5	0.4	0.6	0.6	0.5	0.6	0.6	0.8	1	0	GH28
An01g14650	0.7	0.5	0.7	1.6	0.6	0.4	0.5	0.5	0.5	0.4	0.4	0.4	1	0	GH28
An03g02080	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	GH28
An06g02070	0.4	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.2	0.3	1	0	GH28
An11g06320	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	1	0	GH28
An07g01000	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	1	0	GH28
An11g08700	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH28
An15g00320	1.3	1.4	1.0	1.2	2.6	1.7	0.7	0.5	1.2	1.6	1.4	1.7	0	0	GH32
An06g02420	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0	0	GH32
An11g03200	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	1	0	GH32
An01g01000	2.6	14.8	5.4	2.1	1.0	6.9	5.9	3.2	1.6	8.5	4.2	3.7	0	0	GT25
An01g01250	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH2
An01g01320	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.5	0.3	0.3	0.2	1	0	GH27
An01g01870	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	1	0	GH74-CBM1
An01g04880	0.7	2.1	1.4	1.5	0.7	1.2	1.2	1.0	0.9	3.7	4.2	2.3	0	0	GH31
An01g05360	1.0	7.8	9.1	7.8	1.1	4.8	7.2	6.0	2.1	10.9	12.6	18.6	1	0	GH18
An01g06120	6.1	21.7	12.1	12.9	5.3	9.7	9.6	7.7	3.5	21.2	10.8	8.7	0	0	GH13
An01g06500	0.4	2.5	3.6	4.8	0.4	0.7	2.2	3.9	0.4	4.1	7.9	8.7	1	A	GH76
An01g08460	0.8	1.1	0.9	0.7	0.5	0.8	0.9	0.8	0.7	1.8	0.9	0.9	1	0	GT22
An01g09110	3.1	2.7	3.6	3.7	3.2	3.3	5.4	5.9	3.8	4.4	4.7	5.2	1	0	GT1
An01g09510	2.2	1.7	2.1	2.4	2.8	2.2	2.6	2.4	2.7	1.5	1.3	1.3	1	0	GT31
An01g09910	2.0	1.4	1.9	1.6	1.9	1.4	1.7	1.7	1.9	1.1	1.5	1.5	0	0	GT4
An01g11670	0.2	0.2	0.2	0.2	0.2	1.0	2.6	0.5	0.2	0.2	0.2	0.2	1	0	GH5-CBM1
An01g11970	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.7	0.3	0.3	0.2	0	0	GH16
An01g13340	3.1	3.2	2.0	1.9	1.7	1.8	1.8	1.7	1.7	2.1	2.8	3.1	0	0	CE4
An01g13610	1.1	0.7	0.6	0.7	1.1	0.8	0.7	0.8	0.9	0.6	0.6	0.6	0	0	GH13
An01g14670	0.2	0.3	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH28
An02g00140	0.4	0.8	0.6	0.5	0.5	0.7	0.9	0.9	0.5	1.5	1.3	1.4	0	0	GH43
An02g00730	0.3	0.3	0.9	0.7	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	1	0	CE5
An02g01400	0.8	0.8	0.7	0.7	0.9	1.4	1.7	1.9	0.9	0.9	0.8	0.9	1	0	GH43
An02g02340	6.0	15.1	21.0	18.2	8.1	11.5	15.6	14.5	8.4	16.2	21.7	20.7	0	0	Myosin_motor-GT2
An02g02360	2.4	3.9	4.6	3.3	3.7	3.5	3.5	3.0	2.2	0.8	2.0	2.4	0	0	Myosin_motor-GT2
An02g02660	0.6	1.1	0.8	0.7	0.5	0.6	0.6	0.6	0.8	1.6	1.1	1.1	1	0	GH76
An02g02980	2.1	0.9	1.0	1.0	2.7	1.0	1.0	1.1	2.6	1.0	1.2	1.1	1	0	GT59
An02g03070	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.2	0.3	0.3	1	A	GH72-CBM43
An02g03260	0.3	0.3	0.6	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	1	0	GH13-GT5
An02g03630	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0	0	GT1
An02g03980	0.3	0.2	0.8	1.3	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.3	0	0	GH16
An02g05260	5.3	17.3	28.8	25.3	0.7	0.7	1.4	1.1	1.8	5.1	10.7	6.1	1	0	CBM21
An02g05730	0.5	0.5	0.4												

An02g07590	1.0	3.6	1.9	1.5	0.7	0.8	1.1	1.4	0.9	3.1	2.6	2.0	0	0	GH3
An02g07770	1.4	3.4	3.6	4.6	3.1	4.1	7.4	8.6	0.8	2.6	9.7	13.5	0	0	GT4
An02g07960	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0	0	GT1
An02g09270	0.4	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.4	0.3	0.3	0.3	1	0	AA3
An02g09940	6.1	5.6	6.9	7.4	6.2	4.8	7.4	6.1	7.1	5.0	7.3	8.3	1	0	GT15
An02g10310	13.1	12.2	12.9	14.2	2.0	5.8	6.5	5.6	8.0	7.9	6.2	5.6	0	0	GT3
An02g10490	1.8	1.5	1.3	1.5	1.8	1.8	3.0	4.2	2.8	2.2	4.2	4.8	0	0	GH16
An02g11410	2.3	0.7	2.1	3.0	0.6	0.5	0.5	0.4	1.7	0.7	0.6	0.5	0	0	GT4
An02g11560	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.3	0.2	0.3	1	0	AA3
An02g11720	2.1	26.0	20.7	22.0	2.0	20.1	18.9	19.5	2.1	14.7	16.4	16.0	0	0	GH38
An02g12630	4.1	2.1	2.2	2.0	3.6	1.8	1.5	1.6	3.7	2.1	2.1	1.6	0	0	GT57
An02g13240	1.4	3.7	2.2	1.5	1.2	1.5	1.7	2.0	1.3	3.3	3.0	2.2	0	0	GH13
An02g13450	8.5	1.7	0.5	0.4	3.0	0.5	0.3	0.3	8.5	1.9	0.5	0.4	0	0	GT69
An02g13520	1.2	1.6	2.3	2.1	1.2	1.3	1.3	1.3	0.9	1.0	1.5	1.6	1	0	GT31
An02g13530	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	CE4
An02g14160	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.4	0.3	0.2	0	0	GT69
An03g01030	18.6	18.8	6.2	2.9	7.5	10.6	4.1	2.2	12.5	8.0	6.3	8.2	0	0	GH38
An03g01090	0.6	0.4	0.5	0.4	0.6	0.5	0.4	0.4	0.5	0.5	0.8	1.3	1	0	PL1
An03g00740	0.9	0.7	1.0	1.5	0.9	0.8	0.8	0.8	0.8	0.6	0.6	0.7	1	1	GH76
An03g01090	5.5	5.2	4.3	2.7	6.5	1.7	1.6	2.3	13.0	6.0	6.7	7.2	1	0	GT32
An03g02860	0.3	0.3	0.4	1.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GT32
An03g02880	0.3	0.3	0.4	0.8	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.3	1	0	GT25
An03g04410	7.1	4.8	4.3	3.9	5.5	3.5	4.0	4.3	6.9	6.3	5.5	5.6	1	0	GT2
An03g05010	2.8	1.6	2.0	2.7	3.8	2.3	2.2	2.0	4.2	2.3	2.6	2.2	1	0	GT62
An03g05330	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0	0	GH3
An03g05740	3.9	0.9	0.9	0.7	4.1	1.7	0.7	0.8	5.7	2.0	5.1	8.4	0	0	GT2
An03g06220	0.4	0.4	0.5	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.5	1	1	GH72-CBM43
An03g06360	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GT2
An04g01260	3.7	3.7	4.3	4.4	4.5	3.9	4.6	4.4	4.9	4.3	3.6	2.4	0	0	GT62
An04g02700	0.4	0.7	0.4	0.4	0.3	0.4	0.3	0.3	0.4	0.8	0.5	0.4	0	0	GH36
An04g04110	1.4	2.1	2.7	3.3	1.2	2.5	2.7	3.2	1.5	3.0	3.9	4.3	0	0	GT22
An04g04530	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.5	0.4	0.4	0.4	1	0	GH75
An04g04630	1.3	0.7	0.7	0.7	4.2	1.8	1.6	1.8	1.7	1.1	1.1	1.3	1	0	GT69
An04g04670	3.1	3.0	1.9	0.9	8.9	3.6	1.1	0.8	3.6	4.4	1.9	1.6	0	0	GH18
An04g04790	0.7	0.5	0.5	0.6	0.6	0.3	0.2	0.3	0.7	0.4	0.4	0.4	0	0	GT69
An04g05330	1.4	1.4	1.4	1.5	1.7	2.2	2.8	3.1	1.3	1.4	1.6	2.4	0	0	GT76
An04g05940	3.2	2.8	2.8	3.9	3.6	3.5	3.9	3.8	3.0	3.3	3.9	4.5	0	0	GT34
An04g06730	1.7	0.5	0.4	0.4	0.4	0.4	0.4	0.4	1.1	0.4	0.4	0.4	1	0	GT71
An04g06900	1.9	2.0	2.4	2.0	1.9	2.4	2.4	2.4	1.7	0.7	0.9	1.0	1	0	GT31
An04g07110	0.5	0.4	0.4	0.4	0.5	0.3	0.4	0.3	0.3	0.3	0.3	0.2	1	0	CE4
An04g08370	0.7	0.6	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.4	0.5	0.4	0	0	GT1
An04g08550	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	1	0	GH61
An04g08820	6.8	3.0	3.7	3.9	6.2	2.8	2.8	3.0	5.9	2.6	3.3	2.9	0	0	GT57
An04g09070	0.6	0.7	0.6	0.7	0.5	0.5	0.6	0.6	0.6	0.7	0.7	0.8	0	0	GH78
An04g09890	1.6	1.3	0.8	0.6	5.5	1.3	0.7	0.6	1.7	0.7	0.5	0.5	1	0	GH13-GT5
An04g10340	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GT1
An05g01750	8.4	25.9	6.1	4.9	1.5	16.6	10.3	3.4	7.3	14.1	11.0	4.9	1	0	GT32
An05g02310	23.5	12.7	8.4	5.1	27.2	8.6	1.5	1.2	33.5	19.1	19.6	11.6	1	0	GT32
An05g02320	6.5	4.4	4.3	3.0	7.2	3.1	1.6	1.5	6.3	3.7	2.9	2.5	1	0	GT32
An05g02330	2.3	2.4	2.2	1.6	4.2	3.8	2.2	1.8	3.1	3.4	2.3	1.7	0	0	GT69
An05g02410	0.5	5.9	2.6	2.3	0.5	1.8	1.8	1.9	0.3	1.9	3.5	3.3	0	0	GH2
An05g02440	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	1	0	GH28
An06g00290	0.5	0.5	0.5	0.4	0.5	0.5	0.7	0.7	0.4	0.5	0.5	0.6	1	0	GH35
An06g00510	0.7	0.5	0.5	0.4	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.5	1	0	GH71
An06g00810	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0	GH71-CBM24-CBM24-CBM24
An06g01100	1.5	1.0	1.1	1.0	1.3	0.9	1.4	1.4	1.5	1.0	1.3	1.4	1	0	GT33
An06g01140	0.6	0.5	2.3	4.5	0.5	0.4	0.4	0.5	0.5	0.5	0.4	0.4	0	0	GT1
An06g01510	7.6	10.4	11.2	9.3	5.0	10.5	8.7	7.6	5.0	5.5	6.9	6.0	1	0	GH47
An06g01530	0.8	0.6	2.0	3.9	0.8	1.0	0.9	1.2	0.6	0.5	1.0	1.2	1	0	GH17
An06g01550	60.8	68.2	73.2	86.7	61.9	64.4	69.7	62.1	51.3	56.3	60.0	64.7	0	0	GT48
An06g02040	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GH3
An06g02060	0.4	0.3	0.4	0.4	0.3	0.4	0.4	0.5	0.3	0.4	0.4	0.5	1	0	GH5
An06g02460	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	GH3
An07g00350	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	1	0	GH31
An07g01110	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0	0	GT2
An07g01740	0.5	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.3	0	0	GT25
An07g01780	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GT34
An07g03100	3.1	5.9	10.1	5.4	3.0	6.1	5.5	5.0	2.6	10.3	13.7	13.2	0	0	CE1
An07g03380	2.6	1.5	2.1	2.0	4.3	2.2	2.7	3.1	2.9	1.9	1.8	1.8	0	0	GT21
An07g04420	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.6	0	0	GH35
An07g04940	0.9	0.8	0.6	0.8	0.8	0.9	1.1	1.5	0.9	0.6	0.8	1.0	1	0	GT32
An07g05570	2.4	2.3	2.2	2.1	3.2	1.8	1.8	2.1	3.0	3.8	2.4	1.8	0	0	GT2
An07g06610	2.7	8.5	7.6	6.2	2.3	6.9	7.5	6.9	3.3	6.9	6.5	5.5	0	0	GT1
An07g07630	0.7	0.7	0.6	0.6	0.6	0.5	0.6	0.6	0.4	0.4	0.4	0.5	1	0	GH3
An07g08710	6.5	6.7	7.1	4.4	4.9	2.5	2.4	1.0	7.0	7.3	5.0	2.9	0	0	GT20
An07g08720	2.0	8.9	8.7	5.4	1.0	1.7	1.4	0.7	0.7	1.2	1.4	1.1	0	0	GT20
An07g08950	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.2	1	0	GH5
An07g09760	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH3
An07g10350	32.8	26.9	27.1	29.5	32.3	25.7	30.5	32.3	22.0	16.8	22.3	25.8	0	0	GT39
An08g01100	2.1	2.8	2.7	2.1	1.6	1.7	2.0	2.2	2.2	2.9	4.5	4.9	1	0	GH5
An08g01440	1.2	9.1	1.5	0.8	0.6	0.6	0.9	1.1	4.3	12.9	2.6	1.3	1	0	CE3
An08g01710	0.4	5.3	3.9	2.5	0.3	2.1	1.9	1.6	0.5	7.4	4.1	2.0	0	0	GH51
An08g01760	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.3	1	0	GH6
An08g05290	0.3	0.6	0.5	0.4	0.3	0.3	0.3	0.3	0.5	1.2	0.6	0.5	1	0	GT2
An08g05790	3.5	71.0	84.4	79.2	2.5	25.0	42.4	35.8	2.9	59.8	80.0	64.0	0	0	GT35
An08g07020	2.1	1.7	1.6	1.6	1.7	1.3	1.9	1.7	1.7	1.4	1.2	1.4	0	0	GT22
An08g08240	0.6	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.3	1	0	GH3
An08g09030	0.4	0.4	0.6	0.7	0.4	0.4	0.4	0.7	0.3	1.0	5.1	10.6	1	0	GH18
An08g09120	0.5	0.4	0.4	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0	0	GT1
An08g09140	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH78
An08g09990	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0	GT2
An08g10510	4.0	10.0	10.5	10.1	4.0	6.0	7.								

An09g00100	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0	0	GT32
An09g00790	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	1	0	CE5
An09g00880	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	1	GH51
An09g01010	0.6	0.4	0.5	0.5	0.7	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0	0	CE5
An09g01560	1.9	1.1	1.6	2.0	2.9	1.3	1.3	1.5	1.6	1.1	1.2	1.4	1	0	GT31
An09g02290	3.4	8.1	9.0	7.7	2.1	7.2	6.1	5.6	2.5	6.9	5.6	4.7	0	0	GH2
An09g03110	0.5	0.3	0.2	0.3	1.3	0.4	0.3	0.3	0.8	0.3	0.4	0.3	0	0	GH13
An09g04010	6.7	14.7	14.5	15.1	3.9	9.9	9.0	8.3	8.4	13.3	9.6	6.5	0	0	GT2
An09g05880	15.4	9.1	10.1	10.7	9.6	5.5	6.1	5.5	10.7	9.4	7.5	6.1	1	0	GH31
An09g06260	2.6	1.6	2.0	2.9	2.4	1.5	1.2	1.2	2.8	2.4	3.8	4.3	1	0	GH71
An09g06340	2.7	3.3	2.7	2.2	2.7	3.6	5.9	5.8	4.1	5.1	3.5	3.9	0	0	GH18
An10g00290	0.3	0.2	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH78
An10g00870	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	PL1
An11g00110	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	1	0	CE5
An11g00390	0.3	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	1	0	PL4
An11g00920	0.5	0.8	0.8	0.9	0.5	0.6	0.7	0.8	0.5	0.7	1.0	1.0	1	0	CBM18-CE4-CBM18
An11g01160	0.4	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.2	0	0	GH18
An11g01240	2.1	1.2	0.5	0.5	0.5	0.6	0.5	0.4	5.7	3.3	1.0	0.5	0	0	GH76
An11g02090	4.1	2.1	1.6	2.3	3.0	1.2	0.8	0.8	4.4	1.6	0.9	1.1	0	0	GT25
An11g02100	0.3	0.4	0.6	1.4	0.3	0.4	0.6	0.5	0.3	0.6	0.4	0.4	1	0	GH1
An11g03210	0.5	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0	0	GH32
An11g04030	0.3	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	1	0	PL1
An11g05580	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	1	0	AA3
An11g05600	0.9	7.2	1.3	0.6	0.3	0.3	0.4	0.4	11.0	14.1	16.2	6.2	1	0	AA3
An11g05860	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0	GH18
An11g06330	0.6	0.5	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	1	0	GH27
An11g07490	0.2	0.5	0.5	0.3	0.4	0.8	0.5	0.3	0.3	0.3	0.3	0.3	1	0	GT32
An11g09030	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	1	0	GT1
An11g09890	6.4	3.7	3.4	3.4	5.1	2.7	2.5	2.7	6.5	3.3	3.5	3.5	0	0	GT39
An11g10260	2.3	6.6	4.3	2.1	1.4	3.4	4.7	2.1	1.5	8.9	4.4	1.7	1	0	GT8
An11g10610	2.2	1.8	1.5	1.7	3.1	2.1	2.2	2.3	2.7	2.2	1.7	2.4	0	0	GT8
An11g10990	10.3	5.3	6.2	6.7	9.7	6.3	5.9	5.5	10.3	4.6	5.8	6.3	0	0	GT20
An12g00100	7.5	7.4	8.8	6.6	1.4	2.3	6.8	5.1	3.4	3.8	3.7	4.2	1	0	GT2
An12g00340	1.4	1.6	2.0	2.2	1.0	1.2	1.7	1.6	1.4	2.0	1.8	1.7	1	0	GH47
An12g00950	0.3	0.4	0.4	0.4	0.4	0.8	1.0	0.9	0.5	0.6	0.7	0.8	1	0	GH28
An12g01800	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	1	0	GH71-CBM24-CBM24-CBM24
An12g01850	0.9	6.2	3.9	5.0	0.8	3.7	3.4	4.7	1.0	6.8	5.7	6.4	0	0	GH2
An12g01880	0.9	0.9	0.9	0.8	1.0	0.9	0.9	0.8	1.0	0.9	0.8	0.7	1	0	GT50
An12g02220	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.3	0.3	0.3	1	0	CBM1-GH6
An12g02450	2.1	1.2	0.5	0.5	1.0	0.4	0.3	0.3	0.8	0.6	0.4	0.3	1	0	GH13-GT5
An12g02460	4.9	3.3	1.1	1.0	2.8	1.2	0.7	0.7	2.2	1.5	0.5	0.5	1	1	GH13
An12g02540	0.4	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.3	1	0	GH61
An12g02550	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3	0.3	1	0	CE1
An12g03070	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.4	0.2	0.3	0.5	0.8	0	0	GH15
An12g04480	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.2	0.2	0.2	1	0	CE4
An12g04610	0.9	0.7	0.7	0.7	0.8	0.7	0.7	0.6	0.7	0.6	0.6	0.6	1	0	GH61
An12g05330	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0	0	CBM18-GH18
An12g05930	0.6	0.4	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.4	0.4	0.4	0	0	GH76
An12g08720	3.8	2.0	2.5	2.8	3.7	1.9	1.8	1.8	3.5	1.1	1.4	1.3	1	0	GT31
An12g09860	0.6	2.0	0.5	0.4	0.4	0.5	0.4	0.3	0.4	1.2	0.3	0.3	0	0	GT25
An12g10160	1.3	1.4	1.4	1.4	1.4	1.4	1.5	1.6	1.3	1.5	1.3	1.3	1	0	GT31
An12g10380	22.5	5.0	2.7	2.3	30.5	8.1	1.8	1.8	20.2	5.4	2.0	1.7	0	0	GT2
An12g10440	0.7	1.5	0.7	0.6	0.6	0.6	0.8	0.5	1.3	0.8	0.8	0.5	1	0	GT4
An13g00400	0.9	1.2	1.1	1.5	1.2	1.3	1.3	1.3	1.1	2.2	3.5	4.4	1	0	GT20
An13g03710	0.4	0.5	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.5	0.4	0.4	0	0	GH13
An14g00660	5.4	4.6	4.0	4.5	3.4	1.6	0.5	0.5	4.6	2.2	1.3	1.0	0	0	GT2
An14g01130	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	PL4
An14g01330	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.4	1	0	GH79
An14g01770	1.1	1.7	1.8	1.7	1.0	10.3	4.1	2.6	0.7	1.3	1.3	1.2	1	0	GH3
An14g02180	0.5	0.8	1.3	3.5	0.7	1.1	1.6	1.8	0.7	2.1	9.1	21.4	0	0	GT20
An14g02920	0.4	0.8	0.6	0.5	0.3	0.5	0.5	0.6	0.4	1.5	0.9	0.6	0	0	GH105
An14g03910	6.5	5.8	6.3	6.0	9.1	5.4	4.3	5.3	11.0	10.5	10.6	8.8	1	0	GT15
An14g04190	10.9	42.6	23.7	22.1	2.2	7.2	9.5	8.4	4.1	23.2	21.6	13.3	0	0	CBM48-GH13
An14g05490	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH16
An14g05800	0.8	1.1	1.5	1.3	0.8	0.8	1.0	1.2	0.7	1.4	1.3	0.7	1	0	GH67
An14g05820	0.5	0.6	0.7	0.7	0.6	0.5	0.5	0.5	0.5	1.1	0.7	0.4	1	0	GH35
An14g05910	3.7	1.3	1.4	1.5	2.3	1.2	1.7	2.1	4.5	2.3	2.9	2.8	0	0	GT4
An14g06060	1.8	1.8	1.4	1.3	1.8	2.3	1.6	1.8	1.8	2.3	1.7	1.8	1	0	GT71
An14g07140	0.6	0.5	0.4	0.5	0.7	0.5	0.5	0.5	0.5	0.4	0.3	0.4	1	0	GT32
An15g00630	4.5	3.2	3.4	2.8	5.3	2.3	2.2	2.2	2.7	1.4	1.7	1.8	1	0	GT32
An15g00840	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2	1	0	CBM18-GH18
An15g00910	1.3	1.2	1.3	1.3	1.7	1.4	1.6	1.6	1.3	0.8	1.1	1.3	0	0	CBM48
An15g00920	0.5	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.3	0.4	0.4	1	0	GT71
An15g01420	11.3	3.2	4.1	4.4	6.6	1.9	1.8	2.2	6.1	3.3	3.4	3.2	1	0	GH63
An15g02570	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0	0	CBM18-CBM18
An15g03330	0.7	1.4	2.4	4.1	1.1	1.1	1.4	1.1	1.0	2.3	2.6	2.7	0	0	GT34
An15g04260	0.3	0.3	0.3	0.4	0.4	0.4	0.5	1.1	0.3	0.5	0.3	0.3	0	0	GH33
An15g04530	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0	0	GH78
An15g04570	0.5	0.5	0.4	0.4	0.6	0.5	0.5	0.5	0.7	0.5	0.4	0.4	1	0	GH61-CBM1
An15g04760	0.3	0.3	0.9	0.4	0.3	0.3	0.3	0.3	0.3	0.5	0.6	0.6	1	0	GH71
An15g04800	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH3
An15g04810	6.8	4.9	3.3	1.7	11.6	6.4	3.7	2.5	10.5	6.9	3.6	2.2	1	0	GT71
An15g04860	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0	0	GT1
An15g04900	2.0	1.3	1.2	1.3	1.6	1.5	1.6	1.6	1.3	1.0	1.1	1.4	1	0	GH61-CBM1
An15g05350	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.3	1	0	GH16
An15g06230	1.3	1.0	1.7	1.7	2.1	1.5	1.8	2.1	2.2	1.8	2.5	3.1	0	0	GT62
An15g07160	2.7	2.3	3.4	4.2	2.4	2.4	2.2	2.2	1.9	2.7	3.2	4.3	1	0	PL1
An15g07370	0.3	0.3	2.2	7.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	CBM14
An15g07760	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	1	0	GH26
An16g00540	0.3	0.2	0.2	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.2	1	0	GH95
An16g02100	4.3	7.3	7.1	7.5	4.5	5.1	5.1	4.9	3.5	11.5	10.2	12.8	0	0	GH5
An16g02760	0.4	1.4	1.8	1											

An16g02850	1.2	0.8	3.7	2.5	3.1	1.1	1.3	1.5	1.2	0.9	1.1	0.9	1	0	GH16
An16g02910	0.5	16.7	9.1	6.6	0.5	2.6	0.5	0.4	0.4	13.3	5.0	2.5	1	0	GH92
An16g04330	7.2	5.1	6.8	6.9	7.8	6.3	10.3	12.3	9.4	7.7	8.0	9.3	0	0	GT2
An16g05970	1.0	0.7	0.6	0.5	4.8	1.3	0.9	0.9	4.3	1.0	0.9	1.0	1	0	GT1
An16g06590	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0	0	GH53
An16g08490	12.7	10.2	11.5	10.0	9.2	8.6	8.8	9.4	7.6	5.9	6.5	6.4	0	0	GT39
An16g08570	22.1	5.6	6.9	7.5	15.2	4.6	3.9	3.7	16.8	5.4	4.8	6.0	1	0	GT66
An16g09040	0.6	5.8	7.5	10.3	0.5	6.5	8.4	9.5	0.6	5.4	10.0	11.2	0	0	CE9
An16g09090	0.6	0.8	1.7	3.1	0.8	1.1	1.6	2.0	0.7	1.4	3.4	5.3	0	0	GH3
An17g00300	1.3	1.4	1.1	0.9	1.2	1.1	1.0	1.0	1.3	2.0	1.3	0.9	1	0	GH3
An17g00520	0.5	0.5	0.5	0.5	0.5	0.4	0.4	0.4	0.6	0.7	0.7	0.5	0	0	GH3
An18g00730	57.2	20.9	3.6	1.6	3.7	1.4	0.9	1.0	59.0	20.2	2.1	0.8	1	0	GT69
An18g01410	0.6	0.6	1.3	2.5	0.7	0.6	0.6	0.5	0.7	0.5	0.6	0.6	1	0	GH76
An18g01640	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0	0	GT4
An18g02170	9.0	8.9	10.0	9.7	9.4	8.5	7.6	9.0	8.8	6.5	6.6	5.8	0	0	GT15
An18g02360	2.6	0.8	1.2	1.4	2.2	0.8	0.8	0.9	2.5	0.8	1.1	1.2	0	0	GT58
An18g04420	1.4	2.0	1.5	1.2	0.6	0.9	1.0	1.1	0.7	1.1	1.3	1.4	0	0	GT8
An18g04560	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.3	0.4	0.4	1	0	CE4
An18g04800	0.8	0.6	0.6	0.7	1.0	1.1	1.1	1.2	1.8	1.5	1.4	1.4	1	0	GH78
An18g05620	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.4	0.3	0.4	0.8	0.6	0	0	GH31
An18g05910	2.8	3.3	3.8	3.8	2.5	2.7	3.3	3.4	2.2	3.6	3.5	3.5	1	0	GT4
An18g06220	2.0	0.7	0.6	0.8	1.6	0.6	0.6	0.5	2.3	0.6	0.6	0.5	0	0	GH47
An19g00090	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	1	0	GH55
An19g00100	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.3	0.3	1	0	CBM18-CBM18-CBM18-GH18
An19g00270	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1	0	PL1
An07g08940	0.5	0.7	0.7	0.7	0.5	1.3	0.9	0.8	0.5	0.5	0.6	0.7	1	0	CE16
An12g06930	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1	0	GH13
An02g11360	6.7	3.1	7.6	4.5	5.3	3.9	3.5	1.6	8.5	6.6	5.9	4.1	1	0	GH114
An08g04330	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	1	0	GH114
An07g04010	2.6	2.9	2.0	1.4	2.8	1.3	0.7	0.7	2.6	1.9	0.9	0.6	1	0	GT8
An16g09050	1.5	3.7	11.1	12.3	1.0	5.5	4.6	4.6	0.9	3.9	11.2	13.5	0	0	AA3
An18g04440	1.3	1.0	0.9	0.6	1.1	0.6	0.3	0.3	1.7	1.3	0.8	0.6	1	0	GT90
An01g04570	2.7	3.8	4.7	6.6	2.2	1.5	1.8	2.1	2.3	3.7	2.9	2.4	0	0	GT90
An01g02730	0.5	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.4	0.5	0.4	0.3	1	0	CBM50-CBM50
An02g02790	0.4	0.5	0.4	0.3	0.4	0.4	0.4	0.4	0.5	0.8	1.5	0.9	0	0	GT90
An01g12020	0.3	0.2	0.2	0.3	0.5	0.3	0.3	0.3	0.5	0.2	0.2	0.2	0	0	GT2
An04g09400	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.3	0	0	GT2
An09g05170	0.9	10.0	27.9	32.2	2.2	23.9	12.7	8.4	1.0	8.1	6.1	1.8	1	0	AA11
An15g02350	0.9	0.7	51.9	147.0	0.7	0.6	0.5	0.6	0.6	0.6	0.5	0.5	1	0	AA11

Mean gene expression levels given as % of *actA* expression during exponential growth.

^a Exp: exponential growth, Day 1,3 or 6; carbon starvation day 1, 3, 6

^b SP: predicted signal peptide presence (1) or absence (0)

^c GPI: predicted GPI-anchor presence (1), absence (0) or ambivalence (A)