Germination of conidia of *Aspergillus niger* is accompanied by major changes in RNA profiles

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Abstract: The transcriptome of conidia of *Aspergillus niger* was analysed during the first 8 h of germination. Dormant conidia started to grow isotropically two h after inoculation in liquid medium. Isotropic growth changed to polarised growth after 6 h, which coincided with one round of mitosis. Dormant conidia contained transcripts from 4 626 genes. The number of genes with transcripts decreased to 3 557 after 2 h of germination, after which an increase was observed with 4 780 expressed genes 8 h after inoculation. The RNA composition of dormant conidia was substantially different than all the subsequent stages of germination. The correlation coefficient between the RNA profiles of 0 h and 8 h was 0.46. They were between 0.76–0.93 when profiles of 2, 4 and 6 h were compared with that of 8 h. Dormant conidia were characterised by high levels of transcripts of genes involved in the formation of protecting components such as trehalose, mannitol, protective proteins (*e.g.* heat shock proteins and catalase). Transcripts belonging to the Functional Gene Categories (FunCat) protein synthesis, cell cycle and DNA processing and respiration were over-represented in the up-regulated genes at 2 h, whereas metabolism and cell cycle and DNA processing were over-represented in the up-regulated genes at 4 h. At 6 h and 8 h no functional gene classes were over- or under-represented in the differentially expressed genes. Taken together, it is concluded that the transcriptome of conidia changes dramatically during the first two h and that initiation of protein synthesis and respiration are important during early stages of germination.

Key words: Aspergillus niger, conidia, germination, transcriptome.

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INTRODUCTION

Conidia are the main vehicles of distribution for fungi (Navarro-Bordonaba & Adams 1996) and are characterised by a dormant state and transported via different media such as water and air. Air-dispersed conidia possess moderate resistance towards low water activity conditions, high and low temperature, UV radiation and other stressors like reactive oxygen species. The dormancy of these cells is broken upon exposure to water, air, and / or inorganic salts, amino acids and fermentable sugars (Osherov & May 2001, Thanh et al. 2005). The environmental conditions are signaled by receptor(s) via Ras/MAPK and cAMP/PKA signal-transduction pathways (Osherov & May 2000, Liebmann et al. 2004, Reyes et al. 2006, Zhao et al. 2006). Upon activation of germination, the disaccharide trehalose and the polyol mannitol are degraded (Witteveen & Visser 1995, Thevelein 1996, d’Enfert et al. 1999, Fillinger et al. 2001, Ruijter et al. 2003, Dijksterhuis et al. 2007). As a consequence, glycerol is formed, which is indicative for an active glycolysis (d’Enfert 1997).

The first morphological change in spore germination is isotropic growth. During this process, also called swelling, the diameter of the spore increases two fold or more. It involves water uptake and a decrease in the micro-viscosity of the cytoplasm (van Leeuwen et al. 2010). Moreover, molecules are directed to the cell cortex to enable addition of new plasma membrane and cell wall (Bartnicki-Garcia & Lippman 1977, Momany 2002). Isotropic growth is concomitant with metabolic activities such as respiration, and DNA, RNA, and protein synthesis (Mirkes 1974, Osherov & May 2001). Isotropic growth is followed by polarised growth that results in the formation of a germ tube. During this phase, the morphogenetic machinery is redirected to the site of polarisation. This machinery includes the cytoskeleton, the vesicle trafficking system, landmark proteins, signaling pathways and endocytic partners like Rho GTPase modules, polarsome and Arp2/3 complexes (d’Enfert 1997, Momany 2002, Harris & Momany 2004, Harris 2006). Moreover, the lipid composition of the plasma membrane changes by the appearance of sterol-rich domains (Van Leeuwen et al. 2008). At later stages of development the growth speed of the germ tube increases and the functional organisation of the hyphal tip area acquires its full potential as judged by zones of endocytosis and exocytosis and the presence of the Spitzenkörper (Taheri-Talesh et al. 2008, Köhli et al. 2008). By branching and inter-hyphal fusions (Glass et al. 2004) a fungal mycelium is established.

Genera of the order Eurotiales (*e.g.* *Penicillium*, *Aspergillus* and *Paecilomyces*) produce numerous single-celled conidia that are abundant in air samples (McCartney & West 2007). These genera are associated with food spoilage and are able to form a wide panel of mycotoxins (Frisvad et al. 2007). In addition, they can act as opportunistic pathogens (Burrell 1991). *Aspergillus niger* is a world-wide food spoiler and can also infect harvested crops (Snowdon 1990). Moreover, it is an important cell factory (Meyer et al. 2011). The impact of *A. niger*, the availability of its genome sequence and whole genome microarrays (Pel et al. 2007) makes...
this an attractive fungal model system. So far, only the asexual stage of *A. niger* has been identified. Formation of conidia involves a complex developmental pathway (Krijgsheld et al. 2013). In this study, the transcriptome of conidia of *A. niger* was studied during dormancy and germination. Most changes in the transcriptome occurred early in germination (i.e. before isotropic growth). The data show that the transcriptome of dormant conidia is distinct from that of conidia during all stages of germination.

**MATERIALS AND METHODS**

Organism and growth conditions

The *A. niger* strain N402 (Bos et al. 1988) and its derivative RB#9.5 were used in this study. The latter strain expresses a gene encoding a fusion of sGFP and the histone protein H2B under regulation of the *mpdA* promoter. For spore isolation, strains were grown for 12 days at 25 °C on complete medium (CM) containing per liter: 1.5 % agar, 6.0 g NaNO₃, 1.5 g KH₂PO₄, 0.5 g KCl, 0.5 g MgSO₄, 4.5 g D-glucose, 0.5 % casamino acids, 1 % yeast extract and 200 μl trace elements (containing per liter: 10 g EDTA, 4.4 g ZnSO₄·7H₂O, 1.0 g MnCl₂·4H₂O, 0.32 g CoCl₂·6H₂O, 0.32 g CuSO₄·5H₂O, 0.22 g (NH₄)₆Mo₇O₂₄·4H₂O, 1.5 g CaCl₂·2H₂O, and 1.0 g FeSO₄·7H₂O). Conidia were harvested in ice-cold ACES-buffer (10 mM ACES, 0.02 % Tween-80, pH 6.8). To this, the colony surface was gently rubbed with a sterile T-spatula and the conidal suspension was filtered through sterile glass wool. Conidia were washed in ice-cold ACES-buffer, resuspended in CM and kept on ice until further processing on the same day.

Microscopy

Samples of liquid cultures were placed on poly-l-lysine (Sigma) coated cover slips (Van Leeuwen et al. 2008). After removal of the medium, the cover slips with the immobilised cells were placed upside-down onto an object glass overlaid with a thin (< 0.5 mm) layer of 2 % water agar. Any remaining liquid was removed with filter paper. Images were captured with a Zeiss Axioskop 2 plus microscope (Zeiss, Oberkochen, Germany) equipped with a HBO 100 W mercury lamp and a AxioCam MRc (Zeiss, Germany) camera using standard FITC (λ = 450–490 nm, FT510, LP520) filters. A minimal number of 93 cells for each time point was counted for the enumeration of the number of nuclei in dormant and germinating conidia.

RNA extraction

For isolation of RNA, 3 x 10⁹ conidia were inoculated in 300 ml CM. Three cultures were shaken at 125 rpm at 24 °C for each RNA isolation. At each time point, 15 ml culture medium was sampled from the biological triplicates. Samples were pooled and centrifuged (3000 x g, 10 min, 4 °C). The supernatant was mixed thoroughly with 1000 μl isopropanol. RNA was precipitated at 12.000 x g (10 min, 4 °C) and the pellet was air-dried for 5 min. The pellet was then resuspended in 100 μl RNase-free water. This was followed by addition of 700 μl of RLT buffer (without β-mercaptoethanol) and 500 μl 96 % ethanol. RNA was further purified using the RNeasy® Mini kit (Qiagen) according to the RNA Cleanup protocol. The concentration of RNA was measured with the Nanodrop ND-1000 spectrophotometer (NanoDrop Tech., Wilmington, USA). The quality was assayed with an Agilent 2100 Bioanalyzer™, using an RNA Nano LabChip® (Agilent Technology, Palo Alto, CA, USA).

**cDNA labeling, microarray hybridisation and data analysis**

cDNA labeling, microarray hybridisation, and scanning were performed at ServiceXS (Leiden, The Netherlands) according to Affymetrix protocols. In brief, 2 µg of total RNA was used to generate Biotin-labeled antisense cRNA with the Affymetrix Eukaryotic One-Cycle Target Labeling and Control reagents. Quality of the cRNA was assayed using the Agilent 2100 Bioanalyzer™. Subsequently, labeled cRNA of biological triplicates for each time point was used for the hybridisation of Affymetrix *A. niger* Genome Genechips (Pel et al. 2007). After an automated process of washing and staining, absolute values of expression were calculated from the scanned array using the Affymetrix Command Console v. 1. software. Arrays were globally scaled to a target value (TGT) of 100 using the average signal from all gene features using Microarray Suite v. 5.0. (MAS5.0) in Refiner Array Affymetrix IVT Arrays 5.2 of Genedata Expressionist (Basel, Switzerland). Arrays were then normalised on the median in Genedata Analyst (Basel Switzerland). The array data has been deposited in NCBI’s Gene Expression Omnibus (Edgar et al. 2002) and is accessible through GEO Series accession number GSE36439 (www.ncbi.nlm.nih.gov/geo/). MAS5.0 detection calls were calculated using the median in Genedata Analyst (Basel Switzerland). The array data has been deposited in NCBI’s Gene Expression Omnibus (Edgar et al. 2002) and is accessible through GEO Series accession number GSE36439 (www.ncbi.nlm.nih.gov/geo/). MAS5.0 detection calls were used to calculate the number of absent / present calls on each probe set. Filtering was performed by setting the value of all probesets that satisfied the criteria of a Benjamini Hochberg False Discovery rate of p=0.05 (Benjamini & Hochberg 1995) was applied. The Functional Catalogue (FunCat; Munich Information Center for Protein Sequence) was used for functional classification of genes (Ruepp et al. 2004).
RESULTS

Conidial germination

Conidial germination of A. niger has a maximal rate between 30 – 34 °C, with more than 90 % germination after 6 h (Abdel-Rahim & Arbab 1985). In this study A. niger was grown at 25 °C enabling us to separate the different stages of germination in time (Fig. 1A). Isotrophic growth was observed between 2 and 6 h after inoculation and germ tubes were formed between 6 and 8 h. An A. niger reporter strain (RB#9.5) expressing a fusion of the H2B histone protein and the sGFP protein under control of the mpdA promoter was used to monitor nuclear division (Fig. 1B). Dormant conidia were predominately bi-nucleate (85 %), the remainder being uni-nucleate. Nuclear division was shown to occur between 6 and 8 h of germination. After 8 h, 42 % and 34 % of the germinating conidia contained 3 or 4 nuclei, respectively, 10 % and 14 % of the conidia still had 1 or 2 nuclei, respectively.

Transcriptional profiling

Most methods for RNA isolation from fungal tissue are based on extraction with phenol or phenol based reagents like TRizol® (Invitrogen, Breda, The Netherlands). Using this method we were unable to extract RNA from dormant conidia and from conidia during early stages of germination. Therefore, a novel RNA extraction method for conidia of A. niger was developed (see Materials and Methods) resulting in high quality intact RNA (see online Supplemental Fig. 1). This method was used to isolate RNA from dormant (0 h) and germinating (2, 4, 6, and 8 h) conidia. RNA from three independent biological replicates were used for hybridisation of A. niger Affymetrix microarray chips representing 14,259 open reading frames (Pel et al. 2007, Jacobs et al. 2009). MAS5.0 detection calls were used to determine the number of expressed genes. Dormant conidia contained transcripts from 4626 genes (Fig. 2A, 3A). The number of expressed genes decreased to 3,557 after 2 h of germination. This was followed by a gradual increase to 4,780 genes 8 h after inoculation. Correlation of expression showed that the RNA profile of dormant conidia was
most different when compared to the other samples (Fig. 2B). The correlation coefficient of the profiles at 0 h and 8 h was 0.46. Correlation increased from 0.76 to 0.93 when the profiles of 2 h, 4 h, and 6 h were compared to that of 8 h. A principal component analysis (PCA) showed similar results (Fig. 2C). According to the PCA, the 0 h sample was substantially different from all other time points in that it contributes for the majority of the first principal component while the variation in the other time points was predominantly confined to the second principal component.

Comparison of gene expression during conidial germination

In dormant conidia, 1986 genes were expressed that were subsequently absent at the 2 h time point (Fig. 3A). These numbers were markedly lower (i.e. between 179 and 290) when the other stages were compared. A similar trend was obtained when the numbers of down-regulated genes with a fold change ≥ 2 were compared (Fig. 3B). In fact, the differences are even stronger. A number of 1959 genes were down-regulated between 0 h and 2 h, whereas between 3 and 45 genes were down-regulated when the other stages were compared. The number of up-regulated genes was also highest between 0 h and 2 h (i.e. 1161 genes) when compared to the other stages (i.e. between 16 and 383 genes). This difference was less when the number of present calls was taken into account (Fig. 3A).

A Fisher exact test showed that transcripts belonging to the functional gene classes protein synthesis and protein fate are over-represented in the RNA profile of dormant spores. Transcripts belonging to the functional category protein synthesis and its subcategories translation and initiation were over-represented in the up-regulated genes, while amino acid degradation was under-represented. Taken together, these data indicate that initiation of translation and respiration are key processes for initial stages of germination.

During later stages of germination (between 2 and 8 h of germination) the changes in expression of functional gene classes were smaller. In fact, no functional gene classes are over- or under-represented in the differentially expressed genes at 6 h and 8 h. The categories metabolism and cell cycle and DNA processing were over-represented in the up-regulated genes at 4 h. The latter suggests that the conidium prepared itself for mitosis which occurs a few hours later.

Specific transcriptional changes associated to conidial germination

Regulation

So far, asexual development has not been studied in A. niger. However, its genomic sequence predicts that mechanisms of asexual development are similar, if not identical, to that in A. nidulans (Pel et al. 2007, Krijgsheld et al. 2013). The expression of genes predicted to be involved in regulation of asexual development is given in Table 2. Levels of the master regulator of asexual development brlA (An01g10540) were very low in dormant conidia and absent in germinating conidia. Transcription factor genes that are operating more downstream from brlA including medA (An02g02150), abaA (An01g03750) and possibly hymA (An02g08420, Karos & Fisher 1999) and dopA (An02g08420, Pascon & Miller 2000) were present at higher levels than brlA and did show clear higher expression after 2 h (medA), a general trend of down regulation (abaA) or a general trend in upregulation (hymA and dopA). In contrast, stuA (An05g00480) was clearly down-regulated when germination was initiated. Genes that are predicted to directly activate genes involved in conidium formation and stress resistance (i.e. wetA (An01g08890), af11 (An14g06250, An02g07070, An12g10230) and sakA (An08g05850)) had high transcript levels in dormant conidia and invariably showed strong down-regulation.
FadA (An08g06130), SfaD (An18g02090) and FlbA (An02g03160) are members of one the signaling pathways that regulates the transition from vegetative growth to conidiation. Their genes are clearly expressed in germinating spores, but transcripts were also shown to be present in dormant spores. Gene fadA, which encodes an α-subunit of heterotrimeric G-proteins, was up-regulated after 2 hours of germination. The Gβ-subunit encoded by gene sfaD also showed a clear tendency in up-regulation during germination. Interestingly, flbA, which represses this signaling pathway showed a trend to down-regulation. Surprisingly, an α-subunit of the heterotrimeric G-proteins namely GanB (An08g05820), which is involved in conidial germination in A. nidulans, is lowly expressed in germinating conidia of A. niger. It signals via adenylate cyclase (An11g01520) and via the protein kinase PkaC (An02g04270) together with its regulator PkaR (An16g03740, Lafon et al. 2005)). The latter genes do exhibit characteristic expression patterns in A. niger conidia, including a very high accumulation of transcripts in dormant conidia, a strong drop at 2 hours of germination (but not to zero) and clear tendencies of up-regulation during further germination. This expression pattern is very similar to that of a gene which has a strong similarity to the Gpr1 receptor in yeast (An07g08810), which has a function as a nutrient sensing G-protein coupled receptor (Kraakman et al. 1999).

| Table 1. Over- (E) and under- (S) representation of functional gene classes in the pool of genes that are up- and down-regulated between t = 0 h and t = 2 h and between t = 2 h and t = 4 h after inoculation of conidia of A. niger. |
|---------------------------------|-----------------|-----------------|
| **01 METABOLISM**               |                 |                 |
| 01.01.10 amino acid degradation (catabolism) | S               |                 |
| 01.02.01 nitrogen and sulfur utilisation |                 |                 |
| 01.03 nucleotide metabolism      |                 |                 |
| 01.03.01 purine nucleotide metabolism | E               |                 |
| 01.03.04 pyrimidine nucleotide metabolism | E               |                 |
| 01.05.01 C-compound and carbohydrate utilisation |                 | E               |
| 01.05.07 C-compound, carbohydrate transport |                 | S               |
| 01.20.05 biosynthesis of acetic acid derivatives |                 |                 |
| 01.20.05.01 biosynthesis of acetoacetate, acetone, hydroxybutyril acid | S               |                 |
| 01.20.35 biosynthesis of secondary products derived from L-phe and L-tyr | S               |                 |
| 01.20.37 biosynthesis of peptide derived compounds | S               |                 |
| **02 ENERGY**                   |                 |                 |
| 02.11.05 accessory proteins of electron transport and energy conservation | E               |                 |
| 02.13 respiration               |                 |                 |
| 02.13.03 aerobic respiration     |                 |                 |
| **03 CELL CYCLE AND DNA PROCESSING** | E               |                 |
| 03.01.03 DNA synthesis and replication |                 |                 |
| 03.03.01 mitotic cell cycle and cell cycle control |                 |                 |
| **04 TRANSCRIPTION**            |                 |                 |
| 04.01.01 rRNA synthesis         |                 |                 |
| 04.01.04 rRNA processing        |                 |                 |
| 04.05.05 mRNA processing (splicing, 5’-, 3’-end processing) | S               |                 |
| 04.05.01 mRNA synthesis         | S               | S               |
| **05 PROTEIN SYNTHESIS**        |                 |                 |
| 05.04 translation               |                 |                 |
| 05.04.01 initiation             |                 |                 |
| **06 PROTEIN FATE (folding, modification, destination)** |                 |                 |
| 06.07.05 modification by ubiquitination, deubiquitination | S               |                 |
| 06.13.01 cytoplasmic and nuclear degradation | S               |                 |
| **11 CELL RESCUE, DEFENSE AND VIRULENCE** |                 |                 |
| **29 TRANSPOSABLE ELEMENTS, VIRAL AND PLASMID PROTEINS** | S               | S               |
| **40 SUBCELLULAR LOCALISATION** |                 |                 |
| **99 UNCLASSIFIED PROTEINS**    | S               | S               |
Gene fluG (An14g03390) encodes a protein that is involved in the production of an extracellular factor that leads to an up-regulation of the transcription factor gene brlA. Several fb-genes play a role in this upstream regulation of brlA and three An genes (An02g05420, An12g08230, An01g04830) are highly similar to these factors. These genes were only lowly expressed (one of the two fbC analogues is up-regulated during germination), while fluG exhibits clear tendencies up-regulation of > 2-fold compared to the previous time point. As noted above, brlA is not expressed during germination. Up-regulation of fluG may fulfill another role in growth of A. niger.

Different studies have stressed the importance of a RasA signaling pathway during germination of A. nidulans conidia (Som & Kolaparthi 1994, Osherov & May 2000, Fillinger et al. 2001, Harispe et al. 2008). A gene similar to a RasA GTP-binding protein (An01g02320, rasA) is strongly up-regulated 2 h after inoculation, while rasB (An05g00370) is not expressed at all during germination.

Three A. niger genes (An01g10790, An04g02110, An12g10240) are highly homologous to (late) conidiation factors of N. crassa (Roberts & Yanofsky 1989). These transcripts show high accumulation in dormant spores of A. niger, but were detected at much lower levels at all stages of germination.

Compatible solutes

Trehalose and mannitol are needed to protect proteins and membranes against heat, drought and other stressors. These compatible solutes accumulate in dormant conidia and are degraded during germination (d’Enfert et al. 1999, Ruijter et al. 2004, Van Leeuwen et al. 2013). Conidia of A. oryzae and A. nidulans contain 0.7–1.4 pg trehalose per spore which is comparable to 2–4 % of the spore wet weight (d’Enfert & Fontaine 1997, Sakamoto et al. 2009). Trehalose biosynthesis occurs by the action of trehalose-6-phosphate synthase (TPS). It links UDP-glucose to glucose-6-

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Table 2. Expression of regulatory genes in germinating conidia of A. niger. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 7000 expression units. If the outline of the boxes is dashed, the value of gene expression is significantly differentially expressed (> 2-fold) compared to the previous time point. SS = strong similarity; S = similarity; WS = weak similarity. Anid = Aspergillus nidulans; Hsap = Homo sapiens; Mgri = Magnaporthe grisea; Ncras = Neurospora crassa; Pans = Podospora anserina; Scer = Schizosaccharomyces cerevisiae.

| Name                     | Description                                      | Dormant | 2 h | 4 h | 6 h | 8 h |
|--------------------------|--------------------------------------------------|---------|-----|-----|-----|-----|
| An01g10540               | SS to developmental regulatory protein BrlA - Anid| 17      | 12  | 12  | 12  | 12  |
| An02g02150               | SS to Medusa (MedA) - Anid [truncated ORF]       | 20      | 94  | 70  | 54  | 63  |
| An01g03750               | SS to protein AbA - Anid                          | 86      | 76  | 61  | 51  | 27  |
| An05g00480               | SS to transcription factor involved in differentiation StuA - Anid | 115 | 12 | 12 | 23 | 60 |
| An02g08420               | SS to hypha-like metulae protein HymA - Anid      | 33      | 27  | 60  | 92  | 96  |
| An01g09800               | SS to regulatory protein WetA - Anid              | 548     | 26  | 31  | 26  | 26  |
| An14g06250               | WS to transcription factor Atf1+ - Spom            | 183     | 23  | 17  | 19  | 26  |
| An02g07070               | SS to transcription factor Atf1 - Spom             | 1024    | 42  | 69  | 69  | 69  |
| An12g10230               | S to ATF/CREB-family transcription factor Atf21 - Spom | 262 | 12 | 13 | 13 | 14 |
| An06g05850               | SS to osmosensitivity MAP kinase OSM1 (SacA) - Mgris | 1191 | 220 | 293 | 327 | 416 |
| An09g06130               | SS to GTP-binding regulatory protein alpha chain FadA - Anid | 300 | 700 | 588 | 601 | 677 |
| An08g05820               | SS to G protein alpha subunit Mod-D - Pans (ganB)| 66      | 16  | 22  | 29  | 28  |
| An18g02090               | SS to G-protein beta subunit StfA - Anid          | 65      | 95  | 100 | 122 | 144 |
| An02g03160               | SS to developmental regulator FlbA - Anid         | 178     | 141 | 103 | 102 | 62  |
| An19g06110               | SS to related a-agglutinin core protein Aga1 - Ncra | 81      | 56  | 47  | 54  | 54  |
| An11g01520               | SS to adenylate cyclase Mac1 - Mgr                | 532     | 70  | 78  | 100 | 131 |
| An02g04270               | cAMP-dependent protein kinase catalytic subunit PkaC - Anid | 263 | 17 | 74 | 81 | 100 |
| An16g03740               | cAMP-dependent protein kinase regulatory subunit PkaR - Anid | 800 | 20 | 87 | 128 | 262 |
| An07g08810               | SS to G protein-coupled receptor Gpr1 - Scer      | 1800    | 977 | 140 | 171 | 132 |
| An02g01560               | SS to G protein-coupled receptor Edg-4 - Hsap (gprD)| 14 | 18 | 12 | 17 | 16 |
| An01g06290               | SS to hypothetical protein related to VeA - Ncra  | 186     | 55  | 72  | 66  | 58  |
| An14g03390               | SS to FluG - Anid                                  | 137     | 58  | 65  | 170 | 300 |
| An02g05420               | SS to putative zinc finger protein (FlbC) - Anid   | 25      | 46  | 49  | 43  | 45  |
| An12g08230               | SS to zinc finger protein FlbC - Anid              | 22      | 27  | 17  | 22  | 12  |
| An01g04830               | SS to myb-like DNA binding protein FbdB - Anid     | 12      | 31  | 12  | 26  | 14  |
| An01g02320               | SS to GTP-binding protein A-ras - Anid             | 38      | 152 | 161 | 288 | 359 |
| An05g00370               | SS to Ras-2 protein - Neurospora crassa           | 15      | 12  | 12  | 12  | 12  |
| An02g08420               | SS to developm. reg. of asex. and sex. reproduction DopA - Anid | 32 | 20 | 49 | 66 | 107 |
| An01g10790               | SS to hypothetical conidiation-specific protein Con-10 - Ncras | 2061 | 30 | 57 | 100 | 99 |
| An04g02110               | S to Con-8 - Ncras                                  | 7110    | 28  | 49  | 21  | 17  |
| An12g10240               | SS to conidiation-specific protein pCon-10a - Ncras| 2876    | 12  | 12  | 12  | 12  |
phosphate resulting in trehalose-6-phosphate (d’Enfert et al. 1999, Avonce et al. 2006). In the next step, the phosphate is removed by trehalose-6-phosphate phosphatase (TPP), which results in the formation of trehalose. Transcripts of tpsA (An08g10510), tpsC (An14g02180), tpsB (An13g00400) and tppC (An07g08720) were found in dormant conidia (Table 3). Their levels dropped strongly 2 h after inoculation. Expression of tpsA, tpsC and tppB increased gradually after 2 h, while tppC was not up-regulated. Other predicted tps and tpp genes (i.e. An02g07770 and An11g10990) also showed a gradual increase during germination.

The transcript level of the gene encoding neutral trehalase (An01g09290) was high in conidia. This is the major enzyme needed for trehalase degradation during germination (d’Enfert et al. 1999). Transcript levels dropped dramatically during early germination and showed a clear increase during isotropic growth (d’Enfert & Fontaine 1997). The gene encoding acid trehalase (An01g09290) is involved in extracellular trehalase degradation during vegetative growth (d’Enfert & Fontaine 1997). Transcript levels of this gene are low in dormant conidia but clearly increase during germination. Taken together, transcripts of most trehalose-synthesising and degrading enzymes are relatively abundant in dormant conidia. After a strong decrease of the levels at 2 h, their expression gradually increases.

Manitol is present in higher amounts than trehalose in A. niger conidia and makes up 10–15 % of the dry weight (Witteveen & Visser 1995, Ruijter et al. 2003). Manitol dehydrogenase (MDT) converts manitol into fructose and vice versa. Fructose enters glycolysis if it is converted via fructose-6-phosphate and fructose-1,6-diphosphate into glyceraldehyde-3-phosphate. Fructose-6-phosphate can be reduced to mannitol-1-phosphate by mannitol-1-phosphate dehydrogenase (MPD) or can enter glycerol metabolism. Transcripts of mtdA (An15g005450, R.P. de Vries, personal communication) and mpdA (An02g05830) were abundant in dormant spores (Table 3). Like the genes involved in synthesis and degradation of trehalose, levels of mtdA and mpdA initially strongly dropped, after which they gradually increased during germination.

### Table 3. Expression of genes involved in metabolism of compatible solutes

| Name                  | Description                                                                 | Dormant | 2 h  | 4 h  | 6 h  | 8 h  |
|-----------------------|-----------------------------------------------------------------------------|---------|------|------|------|------|
| An08g10510            | trehalose-6-phosphate synthase subunit 1 TpsA - Anig                       | 871     | 44   | 100  | 175  | 270  |
| An14g02180            | SS to trehalose-6-phosphate synthase TpsB - Anig                           | 456     | 6    | 59   | 69   | 134  |
| An07g08710            | α,α-trehalose-phosphate synthase 2 TpsB - Anig                             | 121     | 45   | 100  | 87   | 99   |
| An02g07770            | SS to trehalose synthase Tsase - Gnfola frondosa                           | 139     | 22   | 106  | 173  | 494  |
| An13g00400            | SS to reg. sub. treh-6-P synthase/phosphatase complex Tps3 - Scer          | 392     | 34   | 19   | 21   | 45   |
| An07g08720            | SS to 123K chain α,α-trehalose-phosphate synthase Tal1 - Scer              | 286     | 19   | 31   | 19   | 27   |
| An11g10990            | SS to TPP of patent WO200116357-A2 - Scer                                  | 96      | 104  | 123  | 136  | 198  |
| An01g09290            | SS to neutral trehalase (TreB) - Anid                                       | 1203    | 17   | 60   | 87   | 178  |
| An01g01540            | SS to α,α-trehalase TreA - Anid                                            | 22      | 31   | 42   | 66   | 329  |
| An02g05830            | SS to manniitol-1-phosphate 5-dehydrogenase MtdID - Smut                   | 140     | 16   | 27   | 30   | 153  |
| An15g05450            | SS to NADPH-dependent carbonyl reductase S1 - Candida magnoliace           | 425     | 84   | 894  | 606  | 862  |
| An03g02430            | SS to manniitol dehydrogenase Mtd - Pseudomonas fluorescens                | 645     | 36   | 77   | 119  | 200  |
| An02g07610            | SS to manniitol transporter Mat1 - Apium graveolens                        | 467     | 12   | 12   | 12   | 12   |

Conidiation, heat shock proteins and other protective factors

A number of abundant transcripts in dormant conidia are predicted to encode protective proteins (Table 4). The levels of these transcripts have dropped sharply at 2 h. Gene An02g07350 encodes a protein that is homologous to group 3 LEA proteins that protect seeds against drought stress (Chakrabortee et al. 2007, Tompa & Kovacs 2010). The putative protective proteins also include dehydrin-like proteins as described in A. fumigatus (An13g01110 and An14g05070, Wong Sak Hoi et al. 2011) and heat shock proteins. For instance, the protein encoded by An06p01610 is homologous to heat-shock protein Hsp9 of Schizosaccharomyces pombe. This protein is also very similar to Hsp12 of S. cerevisiae that has been designated as LEA-like and which has been shown to stabilise the plasma membrane (Sales et al. 2000). Gene An01g13350 encodes a homologue of the heat shock protein Hsp104, which together with trehalose provides acquired heat resistance when expressed in yeast cells (Elliott et al. 1996). A number of 10 other genes predicted to encode heat shock proteins (e.g. An15g05410, An07g09990 and An18g00600) also show high accumulation in dormant spores, but some are even further up-regulated at later stages (e.g. An16g09260, An11g00550, and An08g05300). Interestingly a transcript (An01g00160) that is predicted to be a regulator of the unfolded protein response (Hac1p in yeast) is also highly present in dormant conidia.

Catalase, superoxide dismutase, glutathione, and thioredoxin also protect conidia by opposing oxidative stress that occurs during air transport or after rewetting of dried spores. Transcripts of genes similar to catalase encoding genes (i.e. An01g01830, An12g10720, An09g03130, and An08g08920) were highly present in dormant conidia, but to a much lesser extent in germinating spores. Gene An07g03770, which is predicted to encode a superoxide dismutase, had high mRNA levels in dormant cells. After an initial sharp drop, mRNA levels of this gene increased again 4 h after inoculation. Transcripts of genes involved in the synthesis of glutathione (i.e. An02g06560, An01g15190 and An09g06270) were highly represented in dormant conidia, but were somewhat down-regulated in germinating spores. In contrast, genes predicted to encode thioredoxin showed similar levels of transcripts in dormant and germinating conidia. Taken together, mRNA of genes encoding catalase, superoxide dismutase and genes involved in the synthesis of glutathione and thioredoxin are abundant in dormant conidia and show a strong drop after start of germination. These data suggest that stress resistance of conidia may drop strongly very early during germination.

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Cell wall modulation

Conidia of *A. niger* possess a relatively thick, layered cell wall, of which the pigmented outer cell wall is shed during germination (Tiedt 1982). The spores contain complex melanin pigments (see Krijgsheld et al. 2012). Transcripts of three genes (An03g03750, An09g05730, An14g05350) of the melanin synthesis pathway (Jorgenson et al. 2010) were present in dormant conidia but disappeared during germination. Transcripts of five out of seven genes that code for proteins with similarity to hydrophobins are highly accumulated in dormant conidia and drop strongly upon activation of germination.

Transcripts of genes encoding cell wall degrading and synthesising enzymes were present at every stage of germination (Table 5). A transcript similar to a GPI-anchored chitinase ChiA (An09g06400, Yamazaki et al. 2008) was most highly expressed 6–8 h after inoculation. This enzyme is associated with polarised growth in *A. nidulans*, which makes sense while germ tubes were formed 6–8 h after inoculation. Gene An04g1430 which also has strong similarity to ChiA was highly expressed 8 h after inoculation but transcripts were also abundant in dormant conidia. This suggests an active role during other processes, for instance during spore formation. Chitin synthases are the counterparts of chitinases and reported to be present in the fungal cell (Horiuchi 2009). Transcripts of An07g05570 (*chs1*, *A. nidulans*), An09g04010 (*chsC*, *A. fumigatus*) and An12g10380 (*chsC*, *A. fumigatus*) accumulated 2 h after inoculation, whereas transcripts of An02g02340 and 02360 were present at all stages. The latter genes were similar to csmA, a class V chitin synthase with a myosin motor domain, which has been associated with hyphal tip growth (Takeshita et al. 2005).

Transcripts of two genes that encode glucanases that degrade glucan in the cell wall were observed during germination; one gene (An08g10740) is up-regulated during germination while the other (An12g09130) showed its highest accumulation in dormant conidia. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 6500 expression units. For further explanation see Table 2. Mmar = *Methylobacter marinus*; Nmen = *Neisseria meningitidis*; Pchry = *Penicillium chrysogenum*; Zmay = *Zea mays*.

| Name | Description | Dormant | 2 h | 4 h | 6 h | 8 h |
|------|-------------|---------|-----|-----|-----|-----|
| An02g07350 | WS to group 3 Lea protein MgI3 - Zmay | 4559 | 20 | 122 | 72 | 74 |
| An13g01110 | S to hypothetical protein An14g05070 (dehydrin) - Ang | 550 | 15 | 32 | 19 | 18 |
| An14g05070 | WS to heterokaryon incompatibility protein Het-C (dehydrin) - Ncra | 785 | 12 | 106 | 19 | 13 |
| An06g16160 | SS to the heat shock protein Hsp10 - Spom | 4577 | 80 | 248 | 525 | 460 |
| An01g13350 | SS to heat shock protein Hsp104 - Socr | 365 | 13 | 28 | 40 | 61 |
| An15g05410 | SS to heat shock protein Hsp30- Anid | 1275 | 31 | 27 | 32 | 30 |
| An19g06650 | SS to heat shock protein Hsp30 - Anid | 610 | 37 | 93 | 118 | 97 |
| An07g09990 | SS to heat shock protein Hsp70 - Ajellomyces capsulata | 4139 | 1247 | 2733 | 2140 | 1895 |
| An03g04000 | S to the heat shock protein Hsp42 - Socr | 61 | 23 | 28 | 21 | 19 |
| An11g08220 | S to heat shock protein Hsp70 patent WO20034465-A2 - Nmen | 476 | 12 | 12 | 12 | 12 |
| An19g06060 | SS to heat shock protein Hsp30 - Ncras [truncated ORF] | 513 | 12 | 12 | 38 | 12 |
| An11g00550 | SS to chaperonin Hsp10 - Socr | 303 | 537 | 704 | 647 | 685 |
| An09g05300 | SS to heat shock protein Hsp70 Pss1+ - Spom | 251 | 513 | 803 | 692 | 558 |
| An12g04940 | SS to mitochondrial heat shock protein Hsp60 - Socr | 522 | 1011 | 1211 | 1158 | 1036 |
| An16g09260 | SS to DnaK-type molecular chaperone Ssb2 - yeast Socr | 3585 | 6678 | 6741 | 6299 | 6103 |
| An08g03480 | SS to the mitochondrial heat shock protein Hsp78p - Socr | 618 | 57 | 201 | 217 | 361 |
| An01g00160 | S to regulator of unfolded protein response (UPR) Hac1p - Socr | 668 | 219 | 244 | 441 | 619 |
| An08g08920 | SS to catalase C CatC - Anid | 78 | 24 | 56 | 29 | 29 |
| An09g03130 | SS to catalase CatB - Anid | 3106 | 24 | 34 | 29 | 37 |
| An12g10720 | SS to catalase Methanosaarcina Barkeri | 1978 | 12 | 12 | 12 | 12 |
| An01g01830 | SS to catalase/peroxidase CpeB - Streptomyces reticuli | 555 | 27 | 49 | 49 | 61 |
| An07g3770 | SS to Cu,Zn superoxide dismutase SodC - Afum | 3091 | 12 | 529 | 856 | 1380 |
| An02g05650 | SS to glutathione S-transferase iso1 - Rhodococcus sp. | 379 | 12 | 49 | 75 | 185 |
| An01g15190 | SS to glutathione-dependent formaldehyde dehydrogenase Fdh - Mmar | 568 | 12 | 12 | 12 | 12 |
| An09g06270 | SS put.glutath.-depend. formal. dehydrogen. SPBC1198.01 - Spom | 6489 | 26 | 396 | 239 | 323 |
| An16g06100 | S to glutathione S-transferase Gat1 - Ascaris suum | 64 | 12 | 28 | 18 | 12 |
| An13g02540 | SS to glutathione S-transferase Gt1 - Socr | 150 | 14 | 16 | 39 | 176 |
| An02g08110 | SS to glutathione peroxidase Hyr1 - Socr | 251 | 50 | 132 | 193 | 337 |
| An19g02500 | SS to thioredoxin - Anid | 1002 | 241 | 164 | 142 | 208 |
| An01g08570 | SS to thioredoxin reductase TrxB - Pchry | 77 | 138 | 148 | 135 | 128 |
| An11g07230 | S to mitochondrial thioredoxin of patent W08832863-A2 - Rattus sp. | 194 | 22 | 20 | 12 | 15 |
| An03g02980 | SS to thioredoxin - Anid | 65 | 154 | 206 | 191 | 242 |
conidia and 8 h old germlings. A gene similar to a glucan synthase of Paracoccidioides brasiliensis (Sorais et al. 2010, An06g01550) was highly up-regulated 2 h after inoculation and levels remained high up to 8 h. Another gene that is predicted to encode a glucan synthase (An09g04010, similar to mok1 of S. pombe, Katayama et al. 1999) had similar expression levels throughout germination. Other genes shown in Table 5 are related to cell wall processing. Their encoding proteins make cross-links between 1,6- and 1,3 glucans and between glucans and chitin (see Fontaine et al. 1997, Rodriguez-Peña et al. 2000, Cabib 2009). These genes had accumulated transcripts in dormant conidia (An08g03580), after 2 h or 8 h. During all stages of germination, specific activities of enzymes can be seen, but most are highly expressed after 2 h.

**DISCUSSION**

In this study the transcriptome of dormant and germinating conidia of the fungus *A. niger* was studied. In fact, this is the first report describing a whole genome expression analysis of dormant and germinating conidia within the class Eurotiomycetes. This class contains, among others, the genera *Aspergillus* and *Candida*. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 7100 expression units. For further explanation see Table 2. Afum = *Aspergillus fumigatus*; Cabi = *Candida albicans*; Ccin = *Coprinopsis cinerea*, Cmin = *Coniothyrium minitans*; Pbra = *Paracoccidioides brasiliensis*, Tree = *Trichoderma reesei.*

| Name            | Description                                      | Dormant | 2 h   | 4 h   | 6 h   | 8 h   |
|-----------------|--------------------------------------------------|---------|-------|-------|-------|-------|
| An14g03350      | SS to yellow-green 1 Agy1 - Afum                 | 111     | 24    | 25    | 15    | 12    |
| An09g05730      | SS to polyketide synthase Ab1 - Afum             | 46      | 12    | 12    | 17    | 21    |
| An03g03750      | SS to brown 2 Ab2 - Afum                         | 59      | 14    | 14    | 16    | 19    |
| An03g02360      | S to the spore-wall fungal hydrophobin DewA - Anid| 1286    | 12    | 15    | 12    | 12    |
| An03g02400      | SS to the spore-wall fungal hydrophobin DewA - Anid| 3110    | 60    | 60    | 36    | 34    |
| An04g08500      | SS to rodletless protein RodA - Anid              | 252     | 19    | 16    | 17    | 18    |
| An12g05020      | S to hydrophobin Hfb1 - Tree                      | 12      | 12    | 12    | 12    | 64    |
| An07g03340      | SS to hydrophobin Hyp1 - Afum                    | 812     | 63    | 48    | 35    |
| An08g09880      | WS to hydrophobin Coh1 - Coin                    | 159     | 17    | 17    | 12    | 12    |
| An09g06400      | SS to chitinate ChaA - Anid                      | 32      | 17    | 17    | 17    | 12    |
| An04g01430      | WS to the chitinate ChaA - Anid                   | 987     | 26    | 123   | 310   | 1341  |
| An06g01000      | SS to protein related to chitinase 3 precursor - Ncra| 68      | 162   | 768   | 588   | 634   |
| An07g05570      | SS chitin synthase Chs1 - Anid                   | 20      | 17    | 17    | 12    | 12    |
| An09g04010      | SS to chitin synthase ChsC - Afum                 | 145     | 134   | 134   | 128   | 160   |
| An12g10380      | SS to chitin synthase C ChsC - Afum               | 100     | 134   | 134   | 128   | 160   |
| An02g02340      | SS to the chitin synthase with a myosin motor-like domain CsmA - Anid| 228     | 188   | 232   | 227   | 281   |
| An02g02360      | SS to CsmA - Anid [truncated ORF]                | 76      | 174   | 163   | 175   | 213   |
| An09g02290      | SS to chitin synthase ChsE - Anid                 | 29      | 32    | 14    | 46    | 107   |
| An08g10740      | SS to ZmGnsN3 glucanase of patent WO200073470-A2 - Zmay| 177     | 226   | 473   | 597   | 617   |
| An12g09130      | S to glucanase ZmGnsN3 of patent WO200073470-A2 - Zmay| 333     | 24    | 135   | 205   | 454   |
| An06g01550      | SS to glucan synthase Fks - Pbra                  | 181     | 2417  | 1597  | 1490  | 1737  |
| An17g02120      | SS to 1,3-beta-glucan synthase Gs-1 - Ncra       | 68      | 132   | 111   | 120   | 119   |
| An09g03070      | SS to alpha-glucan synthase Mok1 - Spom           | 432     | 481   | 333   | 357   |
| An04g09890      | SS to cell wall alpha-glucan synthase Ags1 - Spom| 14      | 45    | 17    | 19    |
| An11g07660      | S to exo-1,3-beta-glucanase Xog - Cabi            | 71      | 52    | 77    | 117   |
| An03g05290      | S to glucan 1,3-beta-glucosidase Bgt2 - Scer      | 23      | 215   | 134   | 151   |
| An07g04650      | S to exo-beta-1,3-glucanase Bgt2 - Scer          | 233     | 12    | 12    | 12    | 29    |
| An19g00990      | SS to the exo-1,3-glucanase Cmg1 - Cmin           | 36      | 213   | 628   | 1297  |
| An16g08800      | SS to endoglucanase Egf - Anig                   | 34      | 30    | 30    | 51    | 127   |
| An03g06220      | SS to beta (1-3) glucanosyltransferase Gel3 - Afum| 12      | 12    | 12    | 27    | 98    |
| An16g02850      | SS to cell wall glucosidase Ch1 - Scer            | 69      | 52    | 52    | 112   | 142   |
| An01g11010      | SS to the cell wall protein Ch1 - Scer            | 54      | 78    | 78    | 54    | 156   |
| An07g01160      | SS to cell wall protein Utr2 - Scer               | 12      | 102   | 83    | 109   |
| An07g07530      | SS to cell wall protein Utr2 - Scer               | 80      | 692   | 660   | 793   |
| An08g03580      | SS to 1,3-beta-glucosyltransferase Bgt1 - Afum    | 7135    | 104   | 75    | 56    |
| An10g00400      | SS to beta(1,3)glucanosyltransferase Gel1 - Afum  | 36      | 455   | 480   | 751   |
| An16g07040      | S to beta-1,3-glycosyltransferase Bgt1 - Afum [truncated ORF] | 14      | 481   | 1148  | 2037  |

Table 5. Expression of genes involved in the production of enzymes involved in cell wall synthesis or processing. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 7100 expression units. For further explanation see Table 2. Afum = *Aspergillus fumigatus*; Cabi = *Candida albicans*; Ccin = *Coprinopsis cinerea*, Cmin = *Coniothyrium minitans*; Pbra = *Paracoccidioides brasiliensis*, Tree = *Trichoderma reesei.*
Penicillium. The data show that the RNA profile of dormant conidia is substantially different when compared to all other stages of germination, each of which is characterised by a typical morphology. A transcriptome reorganisation was shown to take place before the stage of isotropic growth, after which RNA profiles changed gradually. These changes are illustrated by the correlation coefficients of the profiles of dormant conidia and germinating conidia 8 h after inoculation (0.46) and those of conidia 2, 4 and 6 h after inoculation with that of 8 h after inoculation (0.76 to 0.93).

Dormant conidia

About half of the 14 253 genes are expressed in a vegetative mycelium of A. niger (Levin et al. 2007), while approximately 40 % of the genes are active in the aerial structures (i.e. aerial hyphae, conidiophores, conidiospores) (Bleichrodt et al. 2013). Transcripts of 33 % of the genes were detected in dormant conidia, which is in good agreement with the finding that 42 % and 27 % of the genes had transcripts in dormant conidia of Fusarium graminearum (Seong et al. 2008) and Aspergillus fumigatus (Lamarre et al. 2008). The lower complexity of the RNA in conidia when compared to the vegetative mycelium and the aerial structures is explained by the fact that these spores represent a single cell type. In contrast, vegetative mycelium and aerial structures consist of different types of hyphae and cells (Krijgsheld et al. 2013). For instance, the vegetative mycelium consists of hyphae that differ in age, in morphology and in the environmental conditions they are exposed to. In fact, even expression profiles of neighboring hyphae are highly different (Vinck et al. 2005, 2011, De Bekker et al. 2011). It would be interesting to assess to which extent dormant spores are also heterogenic with respect to their RNA profiles.

Lamarre et al. (2008) showed that the RNA profile of dormant conidia of A. fumigatus only changes marginally during a storage period of one year. It is thought that the mRNAs in dormant conidia function as a pool of pre-packed mRNAs primed for translation (Osherov et al. 2002, Lamarre et al. 2008). This would enable the conidium to respond quickly and specific after the onset of germination. Indeed, a Fisher exact test showed that transcripts belonging to the functional gene classes protein synthesis and its subcategories translation and initiation were over-represented in the up-regulated genes. Moreover, the categories energy (including the sub-category respiration), cell cycle & DNA processing as well as transcription (with its sub-categories rRNA synthesis and rRNA processing) were over-represented. On the other hand, genes involved in mRNA processing were under-represented in the up-regulated genes. Taken together, the composition of the RNA profiles of the dormant conidium and conidia 2 h after inoculation indicate that protein synthesis is key during early germination. A similar phenomenon has been described for A. fumigatus (Lamarre et al. 2008). The importance of protein synthesis in early stages of germination is also indicated by the fact that the protein synthesis inhibitor cycloheximide prevents isotropic growth, while inhibitors of the cytoskeleton and DNA- and RNA synthesis had no effect (Osherov & May 2000).

In this study, the distinct morphological changes that occur during germination are not correlated with the highest change in the transcriptome. This is of interest as Kasuga et al. (2005) concluded that transcriptional and morphogenetic change during conidial germination are highly coupled in case of N. crassa. Two h after inoculation, transcripts of only 3 557 genes were present in the conidia of A. niger. This number increased to 4 780 8 h after inoculation. Differential expression of genes was relatively low. Between 16 and 383 genes were ≥ 2-fold up-regulated between 2 and 4 h, 4 and 6 h, and 6 and 8 h. On the other hand only 3–45 genes were ≥ 2-fold down-regulated during these stages. The minor changes in gene expression is also illustrated by the fact that during 2 and 8 h post-inoculation only the categories metabolism and cell cycle and DNA processing were over-represented in the up-regulated genes between 2 and 4 h. The latter suggests that the conidium prepared itself for mitosis which indeed occurred a few hours later. Taken together, germination of A. niger is typified by one large transcriptional transition (i.e. during the first two h after inoculation). Germination in the protonozoa Dictyostelium discoideum is also characterised by such a transcriptional transition (Xu et al. 2004).

Further studies on conidial germination should provide mechanisms underlying the transition of the RNA profile early during spore germination. Perturbation of early germination with natamycine showed that transcriptome reorganisation occurs to a similar scale despite the presence of the antifungal (Van Leeuwen et al. 2013). This suggests that transcriptome reorganisation is a relatively endogenous process that plays an important role in the transition from a stabilised fungal conidium towards a vegetative growing cell.

Germinating conidia

Lamarre et al. (2008) studied changes in expression profiles during early germination of A. fumigatus conidia by means of macro-arrays that covered approximately 3 000 genes. Differential expression of near 800 genes (80 % being up-regulated) was observed after 30 min of germination at 37 °C. In our study, a whole genome expression analysis was performed during early germination. The first two h after inoculation (i.e. before the stage of isotropic growth) is characterised by disappearance of transcripts. As mentioned above, transcripts of 1986 genes were no longer detected. On the other hand, 917 genes became active. Transcripts belonging to the functional gene classes protein synthesis and its subcategories translation and initiation were over-represented in the up-regulated genes. Moreover, the categories energy (including the sub-category respiration), cell cycle & DNA processing as well as transcription (with its sub-categories rRNA synthesis and rRNA processing) were over-represented. On the other hand, genes involved in mRNA processing were under-represented in the up-regulated genes. Taken together, the composition of the RNA profiles of the dormant conidium and conidia 2 h after inoculation indicate that protein synthesis is key during early germination. A similar phenomenon has been described for A. fumigatus (Lamarre et al. 2008). The importance of protein synthesis in early stages of germination is also indicated by the fact that the protein synthesis inhibitor cycloheximide prevents isotropic growth, while inhibitors of the cytoskeleton and DNA- and RNA synthesis had no effect (Osherov & May 2000).

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SUPPLEMENTARY INFORMATION

A

| Time (h) | µg/10^8 conidia | 260/280 | 260/230 | 260/270 |
|----------|------------------|---------|---------|---------|
| 0 h      | 7.9 ± 1.4        | 2.1     | 2.3     | 1.24    |
| 2 h      | 19.7 ± 12.0      | 2.2     | 2.5     | 1.26    |
| 4 h      | 31.1 ± 12.0      | 2.2     | 2.5     | 1.25    |
| 6 h      | 58.6 ± 2.4       | 2.1     | 2.4     | 1.23    |
| 8 h      | 52.9 ± 7.8       | 2.1     | 2.4     | 1.22    |

B

Fig. S1. Quality control of RNA extracted from dormant and germinating conidia. (A) Spectrophotometric data showing concentration and quality relevant ratios. (B) Two examples of Agilent 2100 Bioanalyzer™ electropherograms.