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Comparison of transcriptional and translational changes caused by long-term menadione exposure in *Aspergillus nidulans*

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ABSTRACT

Under long-term oxidative stress caused by menadione sodium bisulfite, genome-wide transcriptional and proteome-wide translational changes were compared in *Aspergillus nidulans* vegetative cells. The comparison of proteomic and DNA microarray expression data demonstrated that global gene expression changes recorded with either flip-flop or dendrimer cDNA labeling techniques supported proteome changes moderately with 40% and 34% coincidence coefficients, respectively. Enzyme levels in the glycolytic pathway were alternating, which was a direct consequence of fluctuating gene expression patterns. Surprisingly, enzymes in the vitamin B2 and B6 biosynthetic pathways were repressed concomitantly with the repression of some protein folding chaperones and nuclear transport elements. Under long-term oxidative stress, the peroxide-detoxifying peroxiredoxins and cytochrome c peroxidase were replaced by thioredoxin reductase, a nitroreductase and a flavohemoglobin, and protein degradation became predominant to eliminate damaged proteins.

1. Introduction

In aerobic organisms reactive oxygen species (ROS) are generated continuously as side products of respiration (Li et al., 2009). ROS include hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals ($HO^{\cdot}$). In addition to their important signaling functions in diverse cellular processes (Lara-Ótíz et al., 2003; Cano-Dominguez et al., 2008), ROS are also cytotoxic in prokaryotic and eukaryotic organisms. Not surprisingly, significant efforts are made by the O_2-exposed cells to eliminate harmful ROS through a wide array of both enzymatic and non-enzymatic processes (Pócsi et al., 2004; Li et al., 2009). Higher concentrations of ROS that may originate from exogenous sources or due to intracellular enzyme activities may cause aging and even initiate apoptotic cell death (Perrone et al., 2008; Scheckhuber et al., 2009). ROS generated at low concentrations can trigger an adaptive stress response that makes the cells resistant to lethal concentrations of these toxic oxygen derivatives (Collinson and Dawes, 1992; Jamieson, 1992; Li et al., 2008a).

Gene expression and proteome surveys have identified numerous genes and gene products induced or repressed in response to oxidants in yeasts and filamentous fungi (Godon et al., 1998; Gasch et al., 2000; Chen et al., 2003, 2008; Kim et al., 2006, 2007a). Applications of ROS generating agents, employed at sublethal doses in *Aspergillus nidulans* (Pócsi et al., 2005) and *Saccharomyces cerevisiae* (Gasch et al., 2000; Thorpe et al., 2004), revealed significant differences in gene expression depending on featured chemical, the concentrations of the applied agents and the produced ROS.

Abbreviations: GSH, glutathione; GSSG, glutathione disulfide; ROS, reactive oxygen species; MSB, menadione sodium bisulfite.

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Pócsi et al. (2005) carried out genome-level gene expression data analysis on the oxidative stress response of *A. nidulans*, and they found that 2499 of the 3533 unique PCR-amplified gene probes printed on an EST-based DNA microarray were affected by at least one of the oxidative stress generating agents diamide, H_2O_2 and menadione sodium bisulfite (MSB).

Under the experimental conditions used by Pócsi et al. (2005), diamide, which is a thiol oxidizing compound, caused a quick change in the glutathione/glutathione disulfide (GSH/GSSG) redox status of the cells without influencing intracellular ROS concentrations. On the other hand, the increased peroxide and superoxide concentrations observable under H_2O_2 and MSB exposures could not be separated from GSH/GSSG redox imbalances at any stressor concentration tested. The disturbance of the GSH/GSSG redox balance under H_2O_2 and MSB-treatments was explained by the relatively weak catalase production of *A. nidulans*, which burdened the GSH-dependent enzymatic and non-enzymatic ROS elimination pathways (Pócsi et al., 2005).

The physiological effects of MSB are not limited to the cyclic generation of $O_2^{\cdot-}$ because these anions destroy 4Fe-4S proteins, which leads to the formation of deleterious $OH^{\cdot}$ radicals, and the detoxification of MSB catalyzed by glutathione S-transferase also affects directly the GSH pool of the cells (Toledano et al., 2003; Pócsi et al., 2004). In addition, menadione can chemically modify (arylate) cell components and enhance membrane fluidity (Shertzer et al., 1992). MSB is therefore likely to initiate mixed oxidative/non-oxidative stress when employed at high (above 0.2 mmol l^{-1}) concentrations and for short periods of time in fungal cultures (Pócsi et al., 2005). It is remarkable that a shift from a mixed-type stress response towards a pure oxidative stress response was observed under long-term (6–9 h) exposures of *A. nidulans* cultures to MSB, when the intracellular accumulation of ROS and the decrease in the GSH/GSSG ratio were equally significant, and numerous genes subjected to superoxide, peroxide or GSH/GSSG-dependent transcriptional regulation were responding to oxidative stress (Pócsi et al., 2005).

After completing the analysis of the data obtained from genome-wide gene expression experiments, we addressed the question of whether the large-scale and significant transcriptional changes caused by MSB-treatments would also result in a proteome significantly different from that of unstressed cultures. Kim et al. (2008) reviewed proteomic data collected in the *Aspergillii* up to the year of 2008 and reported only a combined total of 28 cell surface, 102 secreted and 139 intracellular proteins that have been identified in 10 different studies. Taking into consideration the practical significance of these industrially and medically important fungi and the fact that most of them are fully sequenced and their genome annotations have reached an advanced level (Wortman et al., 2009) these numbers are quite modest. Because no proteome study has been carried out yet in oxidative stress-exposed *Aspergillii*, we would also have liked to augment our proteome-level knowledge on the oxidative stress defense system of these Euascomycetes (Miskei et al., 2009).

To compile data for all the requirements, in this study we mapped the intracellular soluble proteome of *A. nidulans* vegetative cells exposed to high-dose (0.8 mmol l^{-1}) MSB for long time periods (6 h). Translational changes triggered by oxidative stress were compared to genome-wide transcriptional changes recorded using EST-DNA-microarrays and flip-flop and dendrimer cDNA population labeling techniques under the same experimental conditions (Pócsi et al., 2005).

2. Materials and methods

2.1. Strain, culture conditions

Aspergillus nidulans FGSC 26 (*biA1*, *veA1*) was used throughout this study and was a gift of S. Rosén (University of Lund, Sweden). Vegetative mycelium was cultivated in minimal nitrate medium and was exposed to 0.8 mmol l^{-1} MSB for 6 h as described before by Pócsi et al. (2005). MSB-treated mycelia were washed with ice-cold phosphate-buffered saline (0.9% w/v NaCl in 0.1 mol l^{-1} phosphate buffer, pH 7.4) and distilled water, and were stored frozen at −20 °C in lysis buffer (20 mmol l^{-1} Tris–HCl, pH 7.6, 10 mmol l^{-1} NaCl, 0.5 mmol l^{-1} deoxycholate) for proteomics studies. In DNA microarray experiments, mycelial sample preparation and storage were performed as previously (Pócsi et al., 2005).

2.2. Proteomics studies

Intracellular soluble protein sample preparation was carried out according to Nandakumar and Marten (2002) with some modifications. Frozen mycelia were disrupted with X-press (AB Biox, Germany), and the endogenous proteases were inactivated by 40 μl ml^{-1} Protease Inhibitor Cocktail (Sigma–Aldrich). The cell debris suspension was centrifuged (6000 g, 4 °C, 10 min), and the supernatant was treated stepwise by 7 μl ml^{-1} RNase/DNase/Mg mix (0.25 mg ml^{-1} RNase, 0.5 mg ml^{-1} DNase, 50 mmol l^{-1} $MgCl_2$; 0 °C; 5 min) and an equal volume of 20% TCA (0 °C; 30 min). Precipitated proteins were separated by centrifugation (6000 g, 4 °C, 20 min), and the pellets were washed twice with ice-cold acetone and were air-dried at room temperature.

In two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), protein samples (protein contents were set to 300 μg, determined by the Non-Interfering Protein Assay Kit of Calbiochem) were applied onto 17 cm immobilized pH gradient (IPG) strips (pH 5–8, Bio-Rad) by passive rehydration for 12 h in a solution containing 7 mol l^{-1} urea, 2 mol l^{-1} thiourea, 2% (w/v) CHAPS, 50 mmol l^{-1} DTT and 0.5% ampholyte (Bio-Lyte 3/10 Ampholyte). Isoelectric focusing (IEF) was performed in a Protean IEF Cell (Bio-Rad) applying the following voltage settings: 0–250 V for 20 min, 250–10,000 V for 2.5 h and, in the final phase, 10,000 V for 8 h. Thereafter, the IPG strips were consecutively incubated in solutions A and B for 20 min each time to reduce and alkylate the proteins. Solution A contained 50 mmol l^{-1} Tris/HCl, pH 8.8, 6 mol l^{-1} urea, 30% (v/v) glycerol, 5% (w/v) SDS and 2% (w/v) DTT, when in solution B DTT was replaced by 6% (w/v) iodoacetamide. The second dimension of 2D-PAGE was performed on 10–14% gradient SDS polyacrylamide gels using the Protean II xi Multi-Cell (Bio-Rad). Gels were stained with Ruthenium II Tris (Rabilloud et al., 2001; Lamanda et al., 2004) and Coomassie Brilliant Blue.

Images of the 2D-PAGE gels were generated using a VersaDoc 4000 imaging system (Bio-Rad), and the analysis of the 2D-images was performed with the PDQuest software (Bio-Rad). Protein samples coming from three independent experiments of each growth condition were analyzed in separate 2D-PAGE runs, and the significances of the differences in the densitometric data gained in MSB-treated and control samples for individual proteins were estimated by the Student's *t*-test.

Protein spots with significantly higher optical densities than their counterparts in either the stress-exposed or the control cultures were cut from 2D-PAGE gels, diced, and then were rinsed with 25 mmol l^{-1} NH_4HCO_3 {prepared in 50% (v/v) acetonitrile/water} to remove SDS and Coomassie Brilliant Blue. The proteins in the spots were digested with side-chain-protected porcine trypsin (Promega, Madison, WI, USA; 25 mmol l^{-1} NH_4HCO_3, 37 °C, 4 h), and the mass spectrometric analysis of the tryptic digests was performed by on-line LC/MSMS using a 3D ion trap (LCQ Fleet, Thermo Fisher Scientific GmbH, Bremen, Germany) connected with a nanoHPLC system (MicroPro, Eldex, USA) and an autosampler (Endurance, Sunchrom, Germany). Peptide fractionations were performed using a 3 μm Atlantis™ dC_{18} column (75 μm × 100 mm; Waters, Milford, MA, USA), equilibrated in 10% (v/v) aqueous solution of acetonitrile, which contained 0.1% formic acid. After sample injection, the

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concentration of acetonitrile was increased to 50% over 40 min (0.1% formic acid, 300 nl min^{-1} flow rate). The mass spectrometer was operated in triple play mode: survey scans were followed by a 6-Da-zoom scan and CID analysis on the most abundant ion in the survey. Singly charged ions were excluded from the precursor selection; and dynamic exclusion was enabled. The MS/MS data were processed with Mascot Distiller (version 2.1.1.0.) with peak picking parameters recommended for ion trap data.

The generated peak-lists were submitted for database searches with Mascot (in-house server v2.2.04.) against the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/) non-redundant database (release 09-26-2007; 55,19,594 sequences). Search parameters were set to 0.6 Da mass accuracy for the precursor ion and 1.0 Da for the fragment ions. Only tryptic cleavages were considered and one missed cleavage was permitted. Carbamidomethylation of Cys-residues was considered as fixed modification, while methionine oxidation, protein N-acetylation and pyroglutamic acid formation from N-terminal Gln residues were regarded as variable modifications. The cut-off score, determined by Mascot using a 0.05 significance threshold ($p \leqslant 0.05$), was 54. To find the exact ORF ID codes and the functions for the proteins, the sequences identified from the tryptic digests were analyzed with the blastp search program of Altschul et al. (1997) in the *Aspergillus* Genome Database (www.broad.mit.edu/). Whenever "hypothetical proteins" with no predicted function were identified, homology search was also carried out *via* translated ORF query *versus* proteins in NCBI BLAST. Homology data were filtered according to the 1E-40 expectation value (*E*) cutoff criteria.

Unless otherwise indicated, proteins with at least four identified peptides and with significant homologies equal to or above the cut-off score 54, and/or with an at least 20% protein sequence coverage (Raman et al., 2005) are presented in this work. In the high and low molecular mass ranges, some proteins with at least two identified peptides and lower sequence coverage were also accepted. On the basis of *Aspergillus* Genome Database (www.broad.mit.edu/), theoretical isoelectric point (pI) and molecular mass (kDa) were calculated for each protein with the Compute pI/kDa tool (Bjellqvist et al., 1993, 1994; Gasteiger et al., 2005; http://ca.expasy.org/tools/pi_tool.html). Biochemical pathway information was extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG; version 51.0; release July 1, 2009; http://www.genome.jp/; Kanehisa and Goto, 2000). The functional clustering of the proteins was carried out using the AmiGO Gene Ontology Database (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi; release August 27, 2009; Carbon et al., 2009). FUN genes were analyzed for putative domains in the Conserved Domain Database of the NCBI (Marchler-Bauer et al., 2005; http://www.ncbi.nlm.nih.gov/sites/entrez?db=cdd).

2.3. Genomics studie

Double printed EST-based DNA chips (3533 unique PCR-amplified probes printed in 2 × 4073 spots; Pócsi et al., 2005) were used to monitor changes in cDNA populations prepared from mRNA pools isolated from MSB-exposed and untreated control cultures. The full description of gene probes including PCR primers, Oklahoma State University contig IDs (OSU contig IDs; PipeOnline [http://bioinfo.okstate.edu/pipeonline/]) and Broad Institute (Cambridge, MA, USA) ORF IDs (Broad Institute *Aspergillus nidulans* Database, http://www.broad.mit.edu/annotation/fungi/aspergillus/) are given at NCBI Gene Expression Omnibus (NCBI GEO; http://www.ncbi.nlm.nih.gov/geo/) on Platforms GPL1752 and GPL1756. Fluorescence labeling of the cDNA populations was carried out following the "flip-flop" protocol of Hedge et al. (2000), where Cy5-dUTP and Cy3-dUTP are incorporated into the cDNAs during the reverse-transcription of the mRNA pools extracted from stress-exposed and control cultures, respectively ("flip"), or *vice versa* ("flop"). After hybridizing the cDNA pools onto the microarrays (Hegde et al., 2000; Pócsi et al., 2005), gene expression levels characterized by fluorescence intensities were read with a GenePix 4000B microarray scanner (Axon Instruments), and the intensity ratios were calculated with GenePix Pro 3.0 software (Pócsi et al., 2005).

Defected spots with false readings were filtered out manually, and data points with background mean +1 SD higher than the spot intensity means for both dyes were also disregarded (Pócsi et al., 2005). Following that, the background-corrected ratios and $\log_2$ ratios (M) of spot intensities were calculated, and the M values were subjected to LOESS-type block-by-block normalization (Leung and Cavalieri, 2003) using SAS for Windows, version 8 (SAS Institute Inc., Cary, NC, USA) software. In further data processing, normalized $\log_2$ ratios (M') were analyzed. Only gene probes with M' values above or below the [+1; −1] M' thresholds value ('twofold rule'; Schena et al., 1996) were considered to respond to MSB-triggered oxidative stress. All DNA microarray data were deposited in NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/index.cgi) on Platform GPL1752 in Folder GSE4713 (flip-flop database).

3. Results and discussion

3.1. mRNA and protein abundances in oxidative stress-exposed *A. nidulans*

Comparing protein concentrations and mRNA expression levels is at an advanced level in yeast research but lagging in filamentous fungi including the *Aspergillii* (Kim et al., 2008). Yeast-based models are of primary importance when the reasons for the poor correlations between mRNA and protein levels typically found in eukaryotic cells are studied and discussed (Gygi et al., 1999; de Nobel et al., 2001; Greenbaum et al., 2003; Beyer et al., 2004; Brockmann et al., 2007; Schmidt et al., 2007; Tuller et al., 2007; de Groot et al., 2007). The correlation depends on both the cellular localization and the physiological function of the proteins (Greenbaum et al., 2003; Beyer et al., 2004; Schmidt et al., 2007; de Godoy et al., 2008; Rossignol et al., 2009), and is influenced by many complex factors including translational activity (Brockmann et al., 2007), protein half-lives (Beyer et al., 2004; Belle et al., 2006) as well as natural and manufactured systematic noise (Greenbaum et al., 2003). It is important to note that data gained by ORF (EST) based DNA microarrays may be distorted to some extent by cross-hybridizations (Iwahashi et al., 2007), which may also influence the conformity of the proteome and transcriptome data.

Similar to general and specific stress responses, which are well-described at the level of transcription, post-transcriptional general and specific stress responses also exist in yeast (Brockmann et al., 2007). Many stress-responsive genes are subjected to the post-transcriptional regulation mechanism "translation on demand" (Beyer et al., 2004; Brockmann et al., 2007), which is crucially important when adapting to an environmental stress that requires a quick cellular response (Brockmann et al., 2007). As a consequence, changes in the expression of mRNA populations do not necessarily correlate with the levels of the translated proteins and *vice versa* (Beyer et al., 2004; Kolkman et al., 2006; Tuller et al., 2007). In general, transcription factors and signaling genes are regulated mainly post-transcriptionally (Brockmann et al., 2007) while many elements of the biosynthetic pathways are controlled transcriptionally (Bro et al., 2003; Washburn et al., 2003; Rossignol et al., 2009).

Because the applicability of yeast-based models in the description of *Aspergillus* stress response systems was limited (Miskei et al., 2009) our primary goal was to gain information on the correlation between protein and mRNA abundances in oxidative stress-exposed *A. nidulans* cells.

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Proteome analysis of MSB-exposed (6 h) *A. nidulans* cultures revealed 82 stress-related intracellular proteins undergoing significant changes (Fig. 1; Supplementary 1). Out of those, 17 proteins were detected in more than one spot and five of them were identified in repressed and induced forms as well (Supplementary 1; Table 1). A survey of the literature and the Aspergillus Stress Database (http://193.6.155.82/AspergillusStress/; Miskei et al., 2009) revealed that merely 19 of the proteins had been related to any kind of stress response thus far (e.g. oxidative, heat, osmotic stress, unfolded protein response; Supplementary 2). DNA microarray databases gained with flip-flop (this study) and dendrimer (Pócsi et al., 2005) cDNA labeling techniques provided us with transcription data for the genes encoding 42 of the 82 identified stress-response proteins (Supplementary 3). We found both flip-flop and dendrimer microarray data for the great majority of these genes (38 of the 42), and the DNA chips used in these studies contained more than one different PCR-amplified probes for 17 stress-related genes (Supplementary 3).

When the correlation between transcriptome and proteome datasets was examined, coincidence between protein levels and gene expressions was found with 6 h MSB-treated proteome (P) and 6 h treated transcriptome samples. Coincidence levels with flip-flop-labeled (F) and dendrimer-labeled (D6) transcriptome datasets were 40% and 34% (Fig. 2, Panels P–F and P-D6, II + RR), respectively. These coincidence coefficients were in good accordance with previous observations correlating mRNAs with protein abundance (Tian et al., 2004; Nie et al., 2006; Brockmann et al., 2007). Poor coincidence coefficients (14–17%) were found comparing 6 h MSB-treated proteome and 0.5–3 h dendrimer data (Fig. 2, Panels P-D0.5, P-D1 and P-D3, II + RR). This may be a consequence of the relatively slowly accumulating oxidative stress in MSB-exposed *A. nidulans* cells (Pócsi et al., 2005). The conformity between proteome and transcriptome data was 29% when proteome was compared to pooled dendrimer data (Fig. 2, Panel P-D0.5–6, II + RR), and the lowest percentage (3%) of opposite proteome and transcriptome changes was recorded with 3 and 6 h transcriptomes (Fig. 2, Panels P-D3 and P-D6, IR + RI). The percentage of proteome changes not reflected in the variations of the transcriptome was higher in the dendrimer-based DNA microarray hybridizations (54–79%; Fig. 2, Panels P-D0.5, P-D1, P-D3 and P-D6, IO + RO) than in the flip-flop-based DNA microarray hybridization (33%, Fig. 2, Panel P–F, IO + RO).

An alternating protein expression pattern was observed for the glycolytic pathway enzymes AcuG (repressed), FbaA (induced), GpdA (repressed), PgkA (induced), EnoA (repressed), PkiA (induced) after 6 h MSB-treatments (Fig. 3). As shown before by Pócsi et al. (2005), the expressions of the glycolytic pathway genes *acuG*, *fbaA*, *gpdA* and *pkiA* were fluctuating (periodically repressed and induced) as a function of the MSB-exposure time (Supplementary 3, Fig. 4A). Theoretically, an alternating protein expression pattern may arise in a metabolic pathway when transcriptional and translational changes are synchronous for the individual genes and gene products but the frequencies of these fluctuations are markedly different (Pócsi et al., 2005). A similar phenomenon has already been observed under lithium treatments of budding yeast cells, when every second gene, namely *PGM2* (*pgmB* ortholog), *FBP1* (*acuG* ortholog), *TDH1* (*gpdA* ortholog) and *GPM2*, *PYK2* (*pkiA* ortholog), was up-regulated in the glycolytic pathway (Bro et al., 2003). Anaerobiosis also affected gene expressions and protein productions in quite different ways in the glycolytic pathway of *S. cerevisiae* because most of the gene expressions remained unchanged but the quantities of a significant number of gene products increased considerably (de Groot et al., 2007).

Opinions on the regulation of glycolytic proteins are dissenting. These proteins may be under transcriptional regulation because genes in the functional categories "metabolism," "energy," and "protein synthesis" exhibit the strongest correlation between mRNA and protein levels in yeasts (Beyer et al., 2004), and a moderate correlation between glycolytic pathway mRNA and protein levels has been recorded by Schmidt et al. (2007). On the other hand, post-transcriptional modulations may also play an important role in the regulation of the glycolytic pathway enzymes (de Groot et al., 2007), and the specific activities of the metabolic enzymes may also influence the observed protein levels (Schmidt et al., 2007). Based on our study, fluctuating mRNA and alternating protein expression levels suggest a remarkably flexible regulation for the glycolytic pathway enzymes in stress-exposed *A. nidulans*.

It is worth noting that gene expression fluctuations are not limited to glycolytic pathway genes as demonstrated by DNA microarray experiments (Table 1; Supplementary 3; Pócsi et al., 2005), Northern blot hybridizations (Pócsi et al., 2005) and real-time reverse-transcription polymerase chain reaction assays (Supplementary 4), and such fluctuating gene expression patterns may also explain, at least in part, the observed asynchrony of the transcriptome and proteome data (Fig. 2).

Nevertheless, for some genes and their protein products transcriptional and translational changes were in good accordance (Supplementary 3), and the gene expressions were either consistently induced (e.g. genes encoding a putative glutathione S-transferase and a FUN protein ortholog to *A. fumigatus* AFUA_2G09530) or repressed (e.g. *hsp70* and *ungA*; Table 1; Fig. 4B). Stress-response genes with minimal variations in their mRNA expression levels after induction like Gst (Fig. 4B) were regulated mainly at transcriptional level and the protein concentrations tended to be less "noisy" in yeast (Brockmann et al., 2007). As a consequence, mRNA levels correlated well with protein concentrations in these cases (Greenbaum et al., 2003; Schmidt et al., 2007).

3.2. Stress-responsive proteins in MSB-treated A. nidulans

Analyzing the proteins described with the GO term 'response to stress', the induced TrxR thioredoxin reductase, a flavohemoprotein (ANID_07169.1) and a nitroreductase (ANID_02343.1) replaced the repressed putative ortholog of budding yeast's mitochondrial Ccp1 cytochrome C peroxidase and the repressed peroxiredoxins in the center of the oxidative stress defense system of MSB-exposed *A. nidulans* (Table 1; Fig. 3; Supplementary 2). The appearance of a flavohemoprotein among the induced proteins may be indicative of developing nitrosative stress in MSB-treated *A. nidulans* mycelia similar to budding yeast cells (Table 1; Liu et al., 2000; Te Biesebeke et al., 2009). In *S. cerevisiae*, both MSB and H_2O_2 treatments have been shown to generate nitrosative stress (Almeida et al., 2007; Osorio et al., 2007).

GSH is the centerpiece of the antioxidative defense system in almost all eukaryotic cells, including fungi. GSH is present in high concentrations in living cells, and is the major reservoir of reduced non-protein sulfur (Pócsi et al., 2004). In MSB-exposed *A. nidulans* mycelium, the GSH concentration drops and a number of GSH-biosynthetic and GSH-regenerating enzymes are induced to maintain a physiologically relevant GSH/GSSG balance (Pócsi et al., 2005). The induction of isoflavone reductase is an indicator of the limited availability of GSH in maize (Petrucco et al., 1996) and, not surprisingly, its ortholog (ANID_08815.1) was also induced in MSB-treated *A. nidulans* (Table 1), when the GSH/GSSG ratio is significantly decreased (Pócsi et al., 2005). Induced glutathione-S-transferases (Gst3 and a putative Gst) were also connected to the oxidative stress response (Table 1) because these enzymes are required to protect eukaryotic cells from peroxide-induced cell death (Pócsi et al., 2005) and the deleterious effects of menadione itself (Emri et al., 1999).

Under long-term, chronic oxidative stress, glucose and ammonia uptake are reduced in fungi (Emri et al., 1997; Osorio et al.,

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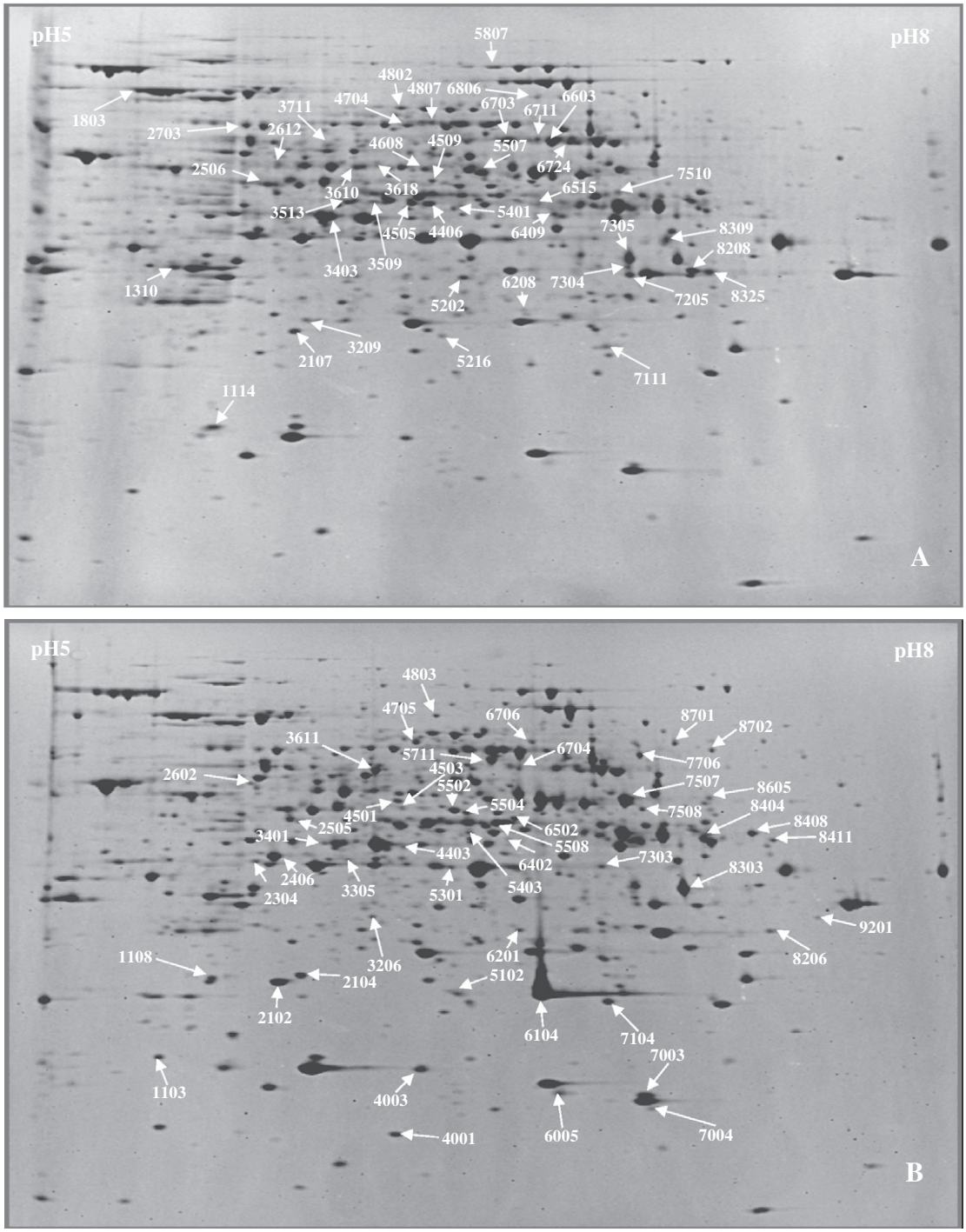

Fig. 1. 2D-PAGE separation and identification of intracellular soluble stress-responsive proteins in MSB-exposed *A. nidulans* vegetative cultures. Parts A and B represent unstressed control and MSB-treated *A. nidulans* FGSC 26 cultures, respectively. Spots with significantly induced (Part B) or repressed (Part A) proteins are localized with arrows and marked with spot ID (also listed in Supplementary 1). Only one of three independent runs is shown.

2004; Li et al., 2008a) and, therefore, cells have to cope with glucose and nitrogen shortages as well, in addition to the neutralization of ROS and the maintenance of the GSH/GSSG and $NADP^+$/NADPH redox balances (Zadzinski et al., 1998; Pócsi et al., 2005; Li et al., 2008b). The appropriate stress-responsive regulation of the carbon and nitrogen metabolic pathways is of cardinal importance in the oxidative stress defense of stress-exposed cells.

In good agreement with this, various enzymes of carbon metabolism characterized with the main GO terms "hexose metabolism" (incorporating glycolysis, gluconeogenesis, pentose phosphate shunt), "TCA cycle", "alcohol metabolism" as well as "carboxylic acid metabolism", "mannitol metabolism" and "amino acid metabolism" were found to be stress-responsive (Table 1). In the glycolytic pathway, the main ATP-producer PkiA pyruvate kinase and PgkA 3-phosphoglycerate kinase were induced together with FbaA fructose 1,6-bisphosphate aldolase (Table 1, Fig. 3). It is important to note that Pgk1p 3-phosphoglycerate kinase and Fba1p fructose 1,6-bisphosphate aldolase were also up-regulated in menadione-

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Table 1
Oxidative stress-responsive proteins in MSB-exposed *Aspergillus nidulans*.

Functions[a]	*A. nidulans* locus ID[b]	Proteomics[c]	Genomics[c]					
			Flip-flop 6 h	Dendrimer 0.5 h	Dendrimer 1 h	Dendrimer 3 h	Dendrimer 6 h	Dendrimer 0.5–6 h
Response to stress								
1-Cys peroxiredoxin, putative	ANID_10223.1	R						
Peroxiredoxin PrxA	ANID_08692.1	R						
cytochrome c peroxidase Ccp1	ANID_10220.1	R						
Flavohemoprotein	ANID_07169.1	I	I	R	R		A	A
Nitroreductase	ANID_02343.1	I						
Thioredoxin reductase TrxR	ANID_03581.1	I						
Glutathione S-transferase GstB	ANID_06024.1	I	I	I	0	0	I	I
Glutathione S-transferase Gst3	ANID_10273.1	I	0					
Hexose metabolism								
UDP-glucose-4-epimerase GalGb	ANID_04727.1	R						
Phosphoglucomutase PgmB	ANID_02867.1	R	R		0			0
Fructose-1,6-bisphosphatase AcuG	ANID_05604.1	R	I	I	0	I	0	I
Fructose 1,6-bisphosphate aldolase FbaA	ANID_02875.1	I	0	0	0	0	0	0
Glyceraldehyde-3-phosphate dehydrogenase GpdA	ANID_08041.1	R	I	0	0	0	0	0
3-Phosphoglycerate kinase PgkA	ANID_01246.1	I						
Enolase EnoA (AcuN)	ANID_05746.1	R						
Pyruvate kinase PkiA	ANID_05210.1	I	I	0	R	I	I	A
Glucose-6-phosphate 1-dehydrogenase GsdA	ANID_02981.1	I	I	0	I		0	I
Ribose 5-phosphate isomerase	ANID_05907.1	I	I	0	R	0	I	A
Transketolase	ANID_09180.1	R						
Transaldolase PppA	ANID_00240.1	I	R	0	R	0	R	R
Tricarboxylic acid cycle								
Aconitase AcoA	ANID_05525.1	R	0	0	0	0	0	0
Hypothetical protein similar to isocitrate dehydrogenase subunit 2 IdpA	ANID_01003.1	I	0	0	0	0	0	0
Mitochondrial malate dehydrogenase MdhA	ANID_06717.1	R						
Malate dehydrogenase, MdhC	ANID_06499.1	R	0	0	0	0	0	0
Alcohol metabolism								
Aldehyde dehydrogenase AldA	ANID_00554.1	R	0	I	R	0	0	A
Zinc-containing alcohol dehydrogenase	ANID_02351.1	I						
Alcohol dehydrogenase	ANID_08406.1	I						
Carboxylic acid metabolism								
Pyruvate decarboxylase PdcA	ANID_04888.1	R	A	0	R	0	R	R
NAD-dependent formate dehydrogenase AciA	ANID_06525.1	R	0	0	0		R	R
Mannitol metabolism								
Mannitol 2-dehydrogenase	ANID_07590.1	I	I	R	0	0	0	R
Cellular amino acid metabolism								
Argininosuccinate synthetase	ANID_01883.1	R						
Fumarylacetoacetate hydrolase FahA	ANID_01896.1	R						
Alanine transaminase	ANID_01923.1	A						
L-ornithine aminotransferase OtaA	ANID_01810.1	I	R					
Ornithine carbamoyltransferase ArgB	ANID_04409.1	R	I	0	0		0	0
Dihydroxy-acid dehydratase	ANID_06346.1	I						
Cystathionine beta-synthase MecA	ANID_05820.1	I						
3-Phosphoserine aminotransferase	ANID_10298.1	R						
NADP-specific glutamate dehydrogenase GdhA	ANID_04376.1	A						
Glutamine synthetase GlnA	ANID_04159.1	R	I	R	0		0	R
Choline oxidase (CodA), putative	ANID_01429.1	I						
Phosphatidyl synthase [*Aspergillus fumigatus* Af293] NCBI	ANID_05564.1	I	I	0	I	I	0	I
Glucose–methanol–choline oxidoreductase	ANID_08547.1	R						
Cellular lipid metabolism								
Myo-inositol-1-phosphate synthase	ANID_07625.1	I	I	R	0	0	0	A
Acetyl-CoA acetyltransferase, putative	ANID_01409.1	R						
Riboflavin biosynthesis								
6,7-Dimethyl-8-ribityl-lumazine synthase RiboG	ANID_10718.1	R	R	R	0	0	R	R
GTP cyclohydrolase II	ANID_10981.1	R	R					
Cytoskeleton organization								
Hypothetical protein similar to fimbrin FimA	ANID_05803.1	I	0	0	0	0	0	0
Chitin biosynthesis								
UDP-N-acetylglucosamine pyrophosphorylase UngA	ANID_09094.1	R	0	R	0	0	R	R
Nucleotide salvage								
Adenine phosphoribosyltransferase 1	ANID_09083.1	R						
Generation of precursor metabolites and energy								
Ubiquinol–cytochrome c reductase iron–sulfur subunit	ANID_02306.1	I						
Inorganic pyrophosphatase IppA	ANID_02968.1	R						

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Table 1 (*continued*)

Functions[a]	*A. nidulans* locus ID[b]	Proteomics[c]	Genomics[c]					
			Flip-flop 6 h	Dendrimer 0.5 h	Dendrimer 1 h	Dendrimer 3 h	Dendrimer 6 h	Dendrimer 0.5–6 h
Signal transduction								
G-protein complex beta subunit CpcB	ANID_04163.1	A	0	R	I	0	0	A
Translation								
Hypothetical protein similar to elongation factor EF-Tu	ANID_01084.1	I						
Elongation factor 2	ANID_06330.1	R						
Translation elongation factor eEF-1B gamma subunit ElfA	ANID_09304.1	I						
Histidyl–tRNA synthetase	ANID_00046.1	I	I		0	0	R	R
Aspartyl–tRNA synthetase Dps1	ANID_04550.1	R	R	0	I		R	A
Protoplast secreted protein 2 [*Aspergillus terreus* NIH2624]NCBI	ANID_00297.1	I						
RNA binding protein [*Aspergillus fumigatus* Af293] NCBI	ANID_05480.1	I	I	I	0	I	I	I
Protein folding, intracellular transport								
Peptidyl–prolyl cis–trans isomerase D Cpr6	ANID_04583.1	R	0		0			0
Hsp70	ANID_05129.1	R	R	R	0	0	R	R
GTP-binding nuclear protein	ANID_05482.1	R	I	0	0	0	0	0
Protein catabolism								
Hypothetical protein similar to proteasome regulatory subunit 8	ANID_05121.1	I						
Proteasome component Pre6	ANID_08054.1	I						
Unknown biological process								
Oxidoreductase	ANID_00179.1	A						
Oxidoreductase, hypothetical	ANID_00895.1	A	R	0	0	I	R	A
Zinc-binding oxidoreductase	ANID_10098.1	I						
NADH:flavin oxidoreductase/NADH oxidase	ANID_05228.1	I						
NADH-dependent flavin oxidoreductase	ANID_06753.1	I						
Zinc-binding oxidoreductase ToxD	ANID_11094.1	I						
NAD binding Rossmann fold oxidoreductase	ANID_02208.1	I						
Isoflavone reductase family protein [*Aspergillus fumigatus* Af293] NCBI	ANID_08815.1	I	I	0	0	0	I	I
Beta-lactamase family protein	ANID_05422.1	R	R	0	0	0	0	0
Conserved hypothetical protein with homology to methyltransferase [*Ajellomyces dermatitidis* ER-3] NCBI	ANID_02561.1	I						
NAD dependent epimerase/dehydratase	ANID_05989.1	R						
FUN proteins								
FUN; tetratricopeptide repeat domain-containing protein	ANID_03987.1	I						
FUN; UPF0160 domain-containing protein MYG1	ANID_04178.1	R						
FUN; DUF833 domain-containing protein	ANID_06058.1	I	0	0	I	I	I	I
FUN; DUF636 domain-containing protein	ANID_07594.1	I	0		0			0
FUN	ANID_10219.1	I	0	0	0	0	0	0
FUN	ANID_10260.1	I	0	R	0		0	R

[a] Putative or verified physiological functions of the stress-response proteins identified in the proteomics studies. Physiological functions were extracted from the *Aspergillus* Comparative Database (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html), the Central *Aspergillus* Data REpository CADRE (Mabey et al., 2004; http://www.cadre-genomes.org.uk/), the *Aspergillus* Genome Database (http://www.aspergillusgenome.org/), the Gene Ontology Database (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi) and the *Saccharomyces* Genome Database (SGD, http://www.yeastgenome.org/).

[b] *A. nidulans* locus ID from the *Aspergillus* Comparative Database (http://www.broadinstitute.org/annotation/genome/aspergillus_group/MultiHome.html).

[c] Letters I, R, 0 and A stand for "significantly induced", "significantly repressed", "no significant induction or repression" and "ambivalent change", respectively. For further explanation of the A "ambivalent change" category in either the genomics or the proteomics studies, see the caption to Fig. 2. A summary of the changes in the gene expression levels can be read in Supplementary 3.

exposed *S. cerevisiae* cells (Kim et al., 2007a). As demonstrated by Pócsi et al. (2005), the expression of some genes encoding glycolytic enzymes was responsive to GSH/GSSG redox imbalance, e.g. FbaA was repressed considerably, and this might result in the intracellular accumulation of fructose-1,6-bisphosphate, a mitochondrion-protectant metabolite (Pócsi et al., 2005). The proteome data challenged this hypothesis because FbaA was clearly induced in MSB-exposed cultures (Table 1).

In the Aspergillus Stress Database (Miskei et al., 2009), GsdA glucose-6-phosphate 1-dehydrogenase, AcuG fructose-1,6-bisphosphatase and GalGb UDP-glucose-4-epimerase from hexose metabolic enzymes are indicated as stress-related proteins (Supplementary 2). Moreover, some data published earlier on GpdA glyceraldehyde-3-phosphate dehydrogenase, EnoA enolase and their yeast orthologs underlined the importance of these enzymes in versatile stress responses. For example, fungal glyceraldehyde-3-phosphate dehydrogenases were reported to participate in osmoadaptation (Kim et al., 2007b), in citric acid stress (Lawrence et al., 2004), as well as in the response to concanamycin (Melin et al., 2002) or amphotericin B (Yu et al., 2007) treatments, and enolases are also well-known participants in various stress responses (Hu et al., 2003; Reverter-Branchat et al., 2004; Entelis et al., 2006; Kwon et al., 2009; Pandey et al., 2009). GAPDH, the budding yeast ortholog of GpdA, was a target of extensive proteolysis under extended (200 min) H_2O_2 treatment, underwent S-nitrosylation and entered to the nucleus where it induced apoptosis (Almeida et al., 2007).

A satisfactory NADPH production is of pivotal importance in the maintenance of the GSH, glutaredoxin and thioredoxin-dependent elements of the antioxidant defense system (Juhnke et al., 1996). In compliance with the NADPH requirement of the stress-exposed cells, the main NADPH-producer enzymes GsdA and isocitrate dehydrogenase were induced.

As far as the nitrogen metabolism is considered, two key enzymes of "cellular amino acid metabolism" were also identified; GdhA NADP-specific glutamate dehydrogenase was found in three

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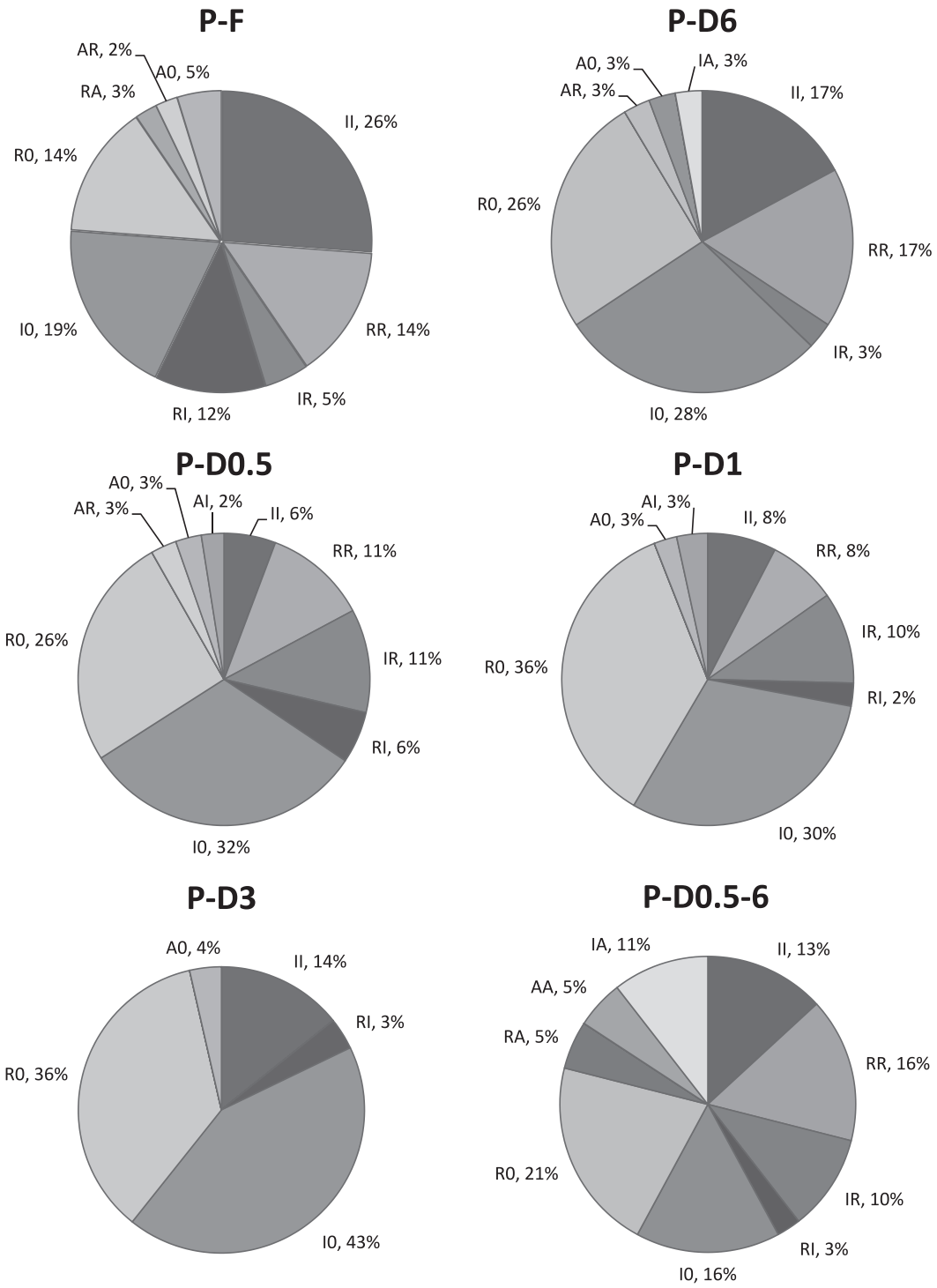

Fig. 2. Comparison of protein and mRNA levels in MSB-exposed *A. nidulans*. The comparison was accomplished on stress-response proteins (P; Table 1) and gene expression databases obtained with dendrimer (D) and flip-flop (F) labeling DNA microarray technology (Supplementary 3; Pócsi et al., 2005). D0.5, D1, D3 and D6 stand for microarray data recorded under 0.5, 1, 3 and 6 h exposures to MSB (Supplementary 3; Pócsi et al., 2005). D0.5–6 symbolizes a unified dataset for the 0.5–6 h dendrimer DNA microarray experiments. Marks I, R, 0 and A stand for "significantly induced", "significantly repressed", "no significant induction or repression" and "ambivalent change", respectively. The "ambivalent change" group included genes with ambiguous or even opposite transcriptional changes recorded on different PCR-amplified gene probes at the same MSB-exposure time or with opposite transcriptional changes recorded on the same gene probe at different MSB-exposure times. In proteomic experiments, the "ambivalent change" group included stress-related proteins with opposite changes in their quantities recorded in separate protein spots.

spots, and the enzyme was induced in two of them under MSB-stress meanwhile GlnA glutamine synthetase was repressed (Table 1; Fig. 3; Supplementary 2). The post-transcriptional regulation of budding yeast's GDH1 (ortholog of GdhA) was observed by several authors (Dang et al., 1996; DeLuna et al., 2001; Griffin et al., 2002; Riego et al., 2002; Kolkman et al., 2006), and the appearance of multiple GdhA spots (both induced and repressed) on the 2D-PAGE gels is in good agreement with these observations. Importantly, the transcription of GdhA was repressed by glucose, induced by nitrogen limitation (Kolkman et al., 2006) and up-regulated under hyp-

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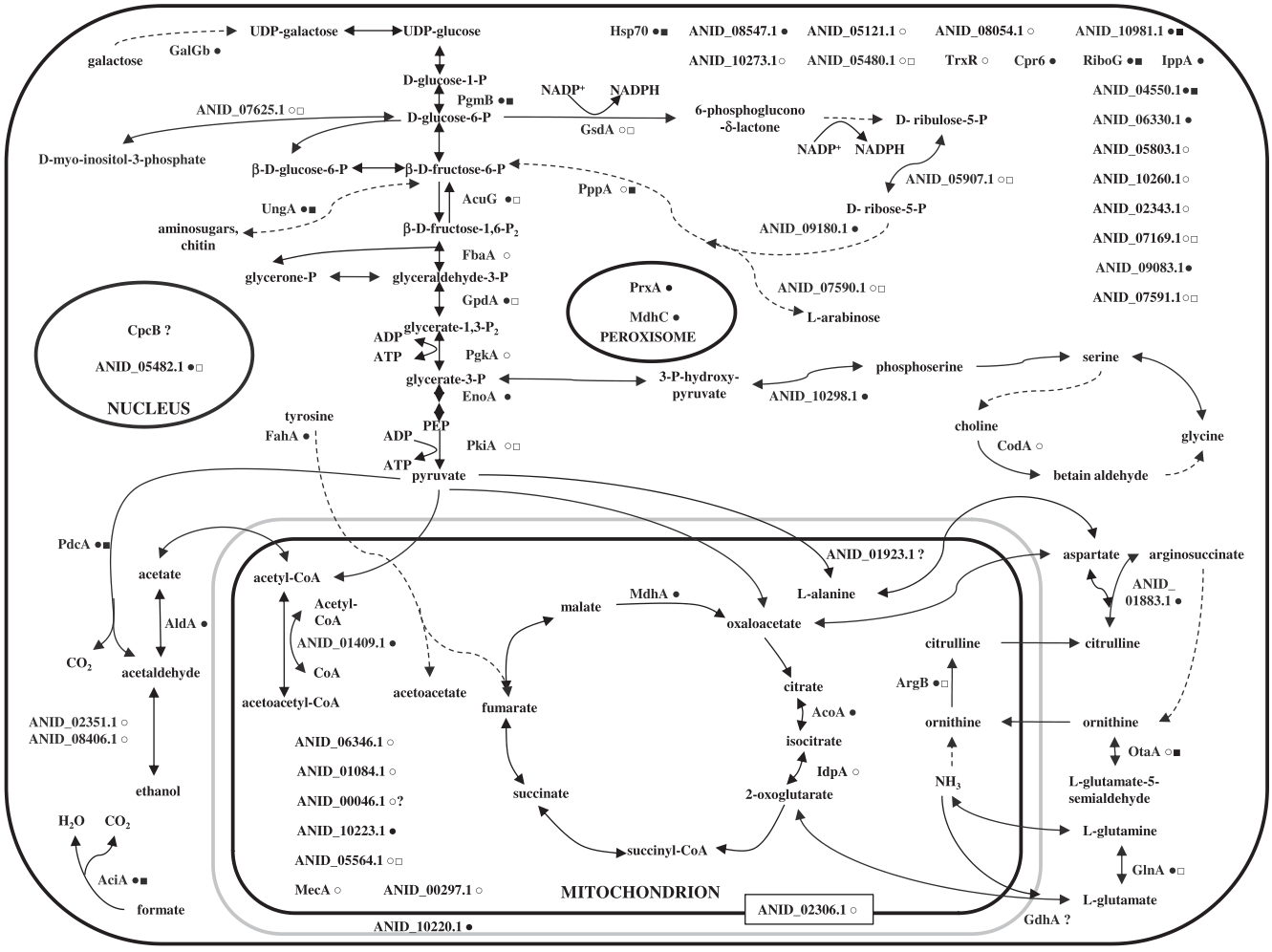

Fig. 3. Metabolic function and schematic cellular localization of MSB-stress-responsive *A. nidulans* proteins. Proteins with putative functions are summarized in Table 1 and are labeled here with their locus IDs. Symbols ○, ●, □ and ■ indicate increased protein, decreased protein, increased mRNA and decreased mRNA levels, respectively (Table 1; Supplementary 3; Pócsi et al., 2005). Question marks refer to 'ambivalent' changes in protein levels (Table 1; Fig. 2). Dashed lines indicate multi-step metabolic pathways. Please note the remarkably alternating protein induction and repression pattern observable in the glycolytic metabolic pathway between the AcuG and PkiA enzymes.

oxic conditions (Shimizu et al., 2009). Changes in the *S. cerevisiae* GLN1 glutamine synthetase (ortholog of GlnA) transcript and protein levels showed poor correlations in large-scale studies (Griffin et al., 2002; Washburn et al., 2003), and opposite transcriptional changes were also observed by us for GlnA in flip-flop (induction) and dendrimer (repression) DNA microarray experiments while the protein level was significantly decreased (Table 1).

The sulfur containing amino acid biosynthetic pathways were represented solely by MecA cystathionine β-synthase among the stress-induced proteins (Table 1; Supplementary 2). MecA catalyzes the homocysteine/cystathionine conversion and, hence, plays an important role in the biosynthesis of cysteine, one of the three amino acids building up GSH (Pócsi et al., 2004). Cysteine can also be synthesized in an alternative pathway, which includes cysteine synthase (*cysB*, ANID_08057.1) and *cysB* was up-regulated under the MSB-treatments (Pócsi et al., 2005). Therefore, both cysteine biosynthetic pathways may operate in oxidative stress-exposed *A. nidulans* hyphae. It is important to note that the cystathionine pathway as well as GSH production were highly induced under cadmium stress in yeast (Vido et al., 2001; Mendoza-Cózatl et al., 2005; Baudouin-Cornu and Labarre, 2006) and in *Blastocladiella emersonii* (Georg and Gomes, 2007). In the latter species, only the cystathionine pathway operates.

Two enzymes in the urea cycle, ArgB ornithine carbamoyltransferase and arginosuccinate synthetase, were repressed in the ornithine–citrulline-L-arginosuccinate bioconversion pathway, however, OtaA L-ornithine aminotransferase was induced, and this may result in the accumulation of ornithine and a subsequent increase in the glutamate biosynthesis. Because the TCA cycle was repressed at malate dehydrogenase and AcoA aconitase (Table 1; Fig. 3), the glutamate requirement of the GSH biosynthesis may be met by the OtaA pathway.

Acetyl-CoA C-acetyltransferase (ANID_01409.1), which is classified under the GO term "fatty acid metabolism" but can be linked to various metabolic pathways including the synthesis and degradation of keton bodies, valine, leucine, isoleucine, the degradation of lysine, the metabolisms of pyruvate and tryptophan, was repressed. Myo-inositol-1-phosphate synthase in the biosynthesis of inositol phospholipids (Reynolds, 2009) and CodA, a putative choline oxidase in the biosynthesis of the osmoprotectant glycine betaine (Park and Gander, 1998; Burg and Ferrais, 2008) were induced together with a phosphatidyl synthase (ANID_05564.1).

Unexpected data were obtained on biosyntheses of vitamins because two enzymes, RiboG 6,7-dimethyl-8-ribityl-lumazine synthase and GTP cyclohydrolase II, both in the riboflavin (vitamin B2) biosynthetic pathway, were strongly repressed together with 3-phosphoserine aminotransferase, which is linked to the syntheses of glycine, serine and threonine but also plays a role in the biosynthesis of pyridoxine (vitamin B6). Riboflavin protects cells from oxidative injuries (Sugiyama, 1991; Perumal et al., 2005), and MSB-

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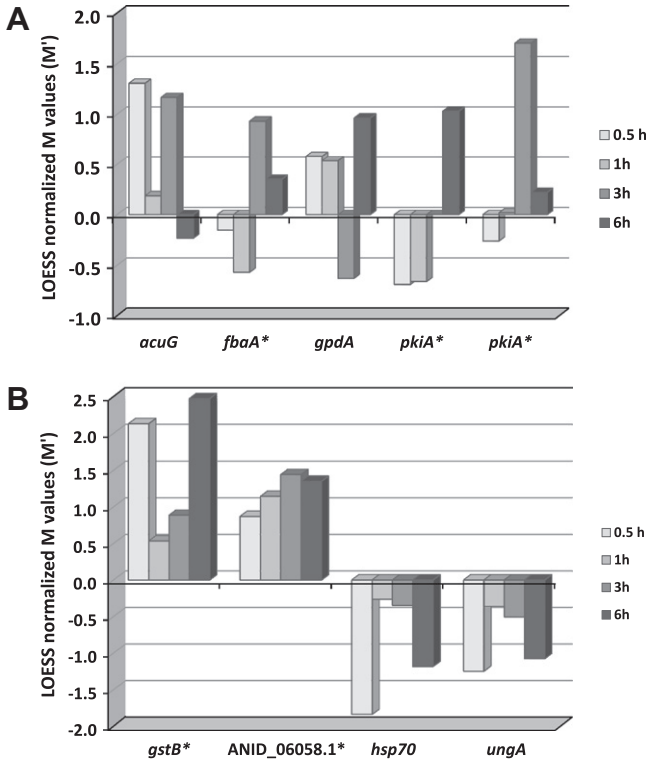

Fig. 4. Typical gene expression patterns recorded under 0.5–6.0 h MSB-treatments on EST-DNA-microarrays using dendrimer cDNA population labeling technique (Pócsi et al., 2005). Gene expression data were imported from NCBI GEO Platform GPL1752, Folder GSE2058. In Part A, the fluctuating transcriptional profiles of the corresponding genes of several glycolytic pathway proteins are shown. The EST–DNA-microarray contained several independent probes for the gene of PkiA (Supplementary 3), and transcriptional changes recorded with two probes are presented here. In Part B, some induced and repressed proteins with consistently induced and repressed gene expressions are shown. Induced proteins are marked with asterisks while repressed proteins are shown without any mark. In both Parts A and B, M stands for the $\log_2$ ratios of the differentially labeled cDNA populations, and the *M* values were normalized using the LOESS method (*M'*; Pócsi et al., 2005).

elicited oxidative stress positively affected the production of riboflavin in *Ashbya gossypii* (Kavitha and Chandra, 2009). Although the photosensitization-coupled cell toxicity of riboflavin is well-documented (Lloyd et al., 1990) further studies are needed to elucidate the physiological significance of the repression of riboflavin biosynthesis in oxidative stress-exposed fungal cells. The possible repression of the vitamin B6 biosynthetic pathway under MSB-treatments is also interesting because Chumnantana et al. (2005) reported on the GSH-pool-stabilizing and cell-vitality-preserving effects of pyridoxine in *S. pombe* exposed to menadione.

MSB-treatment also affected important proteins responsible for the regulation of "transcription, translation", "proper protein folding and transport processes" and "protein catabolism" (Table 1, Supplementary 2). Proper protein folding and nuclear transport seemed to be reduced under oxidative stress since Hsp70 heat shock protein, peptidyl–prolyl cis–trans isomerase D and GSP1/Ran GTP-binding nuclear protein were repressed. On the contrary, two strongly induced proteasome components, Prn8 and Pre6, were identified, which is indicative of an increased degradation of damaged, loss-of-function and improperly-folded proteins. In *S. cerevisiae*, five regulatory subunits of the proteasome were up-regulated under oxidative stress (Haugen et al., 2004). Eight proteins with different functions in translation also responded to MSB-treatments. Interestingly, eEF-2 was found in more than one spot, but all of its isoforms were repressed under oxidative stress while the eEF-Tu and eEF-1Bγ subunits of the elongation factor 1 showed induction.

Among the elements of the signal transduction pathways, only CpcB 'cross-pathway control' protein, which is a transcriptional activator G-protein complex β-subunit, was identified in several spots but the quantities of its isoforms changed oppositely (Table 1, Supplementary 2). The CpcB ortholog proteins in the yeasts *S. cerevisiae* (Cpc2p/Asc1p) and *S. pombe* (Cpc2) are involved in the regulation of differentiation processes (Hoffmann et al., 2000) like sexual differentiation (Jeong et al., 2004) and cell-cell/cell-surface interactions (Valerius et al., 2007). In *A. nidulans*, the induction of the c-Jun homolog CpcA by amino acid limitation resulted in an impaired sexual fruiting body formation, and the RACK1 homolog CpcB repressed the "cross-pathway control" regulatory network in the presence of sufficient amounts of amino acids (Hoffmann et al., 2000). Concanamycin A treatment repressed the transcription of *cpcB* (Melin et al., 2002), and so did the exposure to MSB (Pócsi et al., 2005). MSB-stress-elicited induction of *cpcA* has also been reported by Pócsi et al. (2005).

Several stress-response proteins could not be connected to any known biological function (FUN proteins) but their common characteristic was featuring oxidoreductase domains (Table 1). The structural and functional information available for five other stress-related proteins was even more scarce; three of them possessed conserved domains with unknown function labeled as DUF636 (ANID_07594.1), DUF833 (ANID_06058.1) and UPF0160 (ANID_04178.1) in the Conserved Domain Database of NCBI, meanwhile no domain structure was recognized at all for further gene products (ANID_10219.1 and ANID_10260.1; Table 1). The functional analysis of FUN proteins is now in progress in our laboratory including the generation and phenotypic characterization of gene deletion mutants.

4. Uncited references

Emri et al. (2006), Molnár et al. (2006), Pfaffl (2001) and Pusztahelyi et al. (2006). Q1

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fgb.2010.08.006.

References

Almeida, B., Buttner, S., Ohlmeier, S., Silva, A., Mesquita, A., Sampaio-Marques, B., Osório, N.S., Kollau, A., Mayer, B., Leão, C., Laranjinha, L., Rodrigues, F., Madeo, F., Ludovico, P., 2007. NO-mediated apoptosis in yeast. J. Cell Sci. 120, 3279–3288.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Baudouin-Cornu, P., Labarre, J., 2006. Regulation of the cadmium stress response through SCF-like ubiquitin ligases: comparison between *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and mammalian cells. Biochimie 88, 1673–1685.

Belle, A., Tanay, A., Bitincka, L., Shamir, R., O'Shea, E.K., 2006. Quantification of protein half-lives in the budding yeast proteome. PNAS 103, 3513004–3513009.

Beyer, A., Hollunder, J., Nasheuer, H.-P., Wilhelm, T., 2004. Post-transcriptional expression regulation in the yeast *Saccharomyces cerevisiae* on a genomic scale. Mol. Cell. Proteom. 3, 1083–1092.

Bjellqvist, B., Hughes, G.J., Pasquali, Ch., Paquet, N., Ravier, F., Sanchez, J.-Ch., Frutiger, S., Hochstrasser, D.F., 1993. The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. Electrophoresis 14, 1023–1031.

Bjellqvist, B., Basse, B., Olsen, E., Celis, J.E., 1994. Reference points for comparisons of two-dimensional maps of proteins from different human cell types defined in a pH scale where isoelectric points correlate with polypeptide compositions. Electrophoresis 15, 529–539.

Bro, C., Regenberg, B., Lagniel, G., Labarre, J., Montero-Lomelí, M., Nielsen, J., 2003. Transcriptional, proteomic, and metabolic responses to lithium in galactose-grown yeast cells. J. Biol. Chem. 278, 32141–32149.

Brockmann, R., Beyer, A., Heinisch, J.J., Wilhelm, T., 2007. Post-transcriptional expression regulation: what determines translation rates? PLoS Comput. Biol. 3 (Article No e57).

Burg, M.B., Ferrais, J.D., 2008. Intracellular organic osmolytes. Function and regulation. J. Biol. Chem. 12, 7308–7312.

Cano-Dominguez, N., Alvarez-Delfin, K., Hansberg, W., Aguirre, J., 2008. NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in *Neurospora crassa*. Eukaryot. Cell 7, 1352–1361.

Carbon, S., Ireland, A., Mungall, C.J., Shu, S., Marshall, B., Lewis, S., 2009. Web presence working group. AmiGO: online access to ontology and annotation data. Bioinformatics 25, 288–289.

Chen, D., Toone, W.M., Mata, J., Lyne, R., Burns, G., Kivinen, K., Brazma, A., Jones, N., Bähler, J., 2003. Global transcriptional responses of fission yeast to environmental stress. Mol. Biol. Cell 14, 214–229.

Chen, D., Wilkinson, C.R., Watt, S., Penkett, C.J., Toone, W.M., Jones, N., Bähler, J., 2008. Multiple pathways differentially regulate global oxidative stress responses in fission yeast. Mol. Biol. Cell 19, 308–317.

Chumnantana, R., Yokochi, N., Yagi, T., 2005. Vitamin B6 compounds prevent the death of yeast cells due to menadione, a reactive oxygen generator. Biochim. Biophys. Acta 1722, 84–91.

Collinson, L.P., Dawes, I.W., 1992. Inducibility of the response of yeast cells to peroxide stress. J. Gen. Microbiol. 138, 329–335.

Dang, V.D., Bohn, C., Bolotin-Fukuhara, M., Daignan-Fornier, B., 1996. The CCAAT box-binding factor stimulates ammonium assimilation in *Saccharomyces cerevisiae*, defining a new cross-pathway regulation between nitrogen and carbon metabolisms. J. Bacteriol. 178, 1842–1849.

De Godoy, L.M., Olsen, J.V., Cox, J., Nielsen, M.L., Hubner, N.C., Fröhlich, F., Walther, T.C., Mann, M., 2008. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. Nature 455, 1251–1254.

De Groot, M.J.L., Daran-Lapujade, P., van Breukelen, B., Knijnenburg, T.A., de Hulster, E.A.F., Reinders, M.J.T., Pronk, J.T., Heck, A.J.R., Slijper, M., 2007. Quantitative proteomics and transcriptomics of anaerobic and aerobic yeast cultures reveal post-transcriptional regulation of key cellular processes. Microbiology 153, 3864–3878.

De Nobel, H., Lawrie, L., Brul, S., Klis, F., Davis, M., Alloush, H., Coote, P., 2001. Parallel and comparative analysis of the proteome and transcriptome of sorbic acid-stressed *Saccharomyces cerevisiae*. Yeast 18, 1413–1428.

DeLuna, A., Avendano, A., Riego, L., Gonzalez, A., 2001. NADP-glutamate dehydrogenase isoenzymes of *Saccharomyces cerevisiae*. Purification, kinetic properties, and physiological roles. J. Biol. Chem. 276, 43775–43783.

Emri, T., Pócsi, I., Szentirmai, A., 1997. Glutathione metabolism and protection against oxidative stress caused by peroxides in *Penicillium chrysogenum*. Free Radical Biol. Med. 23, 809–814.

Emri, T., Pócsi, I., Szentirmai, A., 1999. Analysis of the oxidative stress response of *Penicillium chrysogenum* to menadione. Free Radical Res. 30, 125–132.

Emri, T., Molnár, Zs., Veres, T., Pusztahelyi, T., Dudás, G., Pócsi, I., 2006. Glucose mediated repression of autolysis and conidiogenesis in *Emericella nidulans*. Mycol. Res. 110, 1172–1178.

Entelis, N., Brandina, I., Kamenski, P., Krasheninnikov, I.A., Martin, R.P., Tarassov, I., 2006. A glycolytic enzyme, enolase, is recruited as a cofactor of tRNA targeting toward mitochondria in *Saccharomyces cerevisiae*. Genes Dev. 20, 1609–1620.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., Brown, P.O., 2000. Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell 11, 4241–4257.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M.R., Appel, R.D., Bairoch, A., 2005. Protein identification and analysis tools on the ExPASy server. In: Walker, J.M. (Ed.), The Proteomics Protocols Handbook. Humana Press, pp. 571–607.

Georg, R.C., Gomes, S.L., 2007. Transcriptome analysis in response to heat shock and cadmium in aquatic fungus *Blastocladiella emersonii*. Eukaryotic Cell 6, 1053–1062.

Godon, C., Lagniel, G., Lee, J., Buhler, J.M., Kieffer, S., Perrot, M., Boucherie, H., Toledano, M.B., Labarre, J., 1998. A H_2O_2 stimulon in *Saccharomyces cerevisiae*. J. Biol. Chem. 273, 22480–22489.

Greenbaum, D., Colangelo, C., Williams, K., Gerstein, M., 2003. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol. 4 (Article No. 117).

Griffin, T.J., Gygi, S.P., Ideker, T., Rist, B., Eng, J., Hood, L., Aebersold, R., 2002. Complementary profiling of gene expression at the transcriptome and proteome levels in *Saccharomyces cerevisiae*. Mol. Cell. Proteom. 1, 323–333.

Gygi, S.P., Rochon, Y., Franza, B.R., Aebersold, R., 1999. Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. 19, 1720–1730.

Haugen, A.C., Kelley, R., Collins, J.B., Tucker, C.J., Deng, C., Afshari, C.A., Brown, J.M., Ideker, T., Van Houten, B., 2004. Integrating phenotypic and expression profiles to map arsenic response networks. Genome Biol. 5 (Article No. R95).

Hegde, P., Qi, R., Abernathy, K., Gay, C., Dharap, S., Gaspard, R., Earle-Hughes, J., Snesrud, E., Lee, N., Quackenbush, J., 2000. A concise guide to cDNA microarray analysis. Biotechniques 29, 548–562.

Hoffmann, B., Wanke, C., LaPaglia, S.K., Braus, G.H., 2000. C-Jun and RACK1 homologues regulate a control point for sexual development in *Aspergillus nidulans*. Mol. Microbiol. 37, 28–41.

Hu, Y., Wang, G., Chen, G.Y., Fu, X., Yao, S.Q., 2003. Proteome analysis of *Saccharomyces cerevisiae* under metal stress by two-dimensional differential gel electrophoresis. Electrophoresis 24, 1458–1470.

Iwahashi, H., Kitagawa, E., Suzuki, Y., Ueda, Y., Ishizawa, Y., Nobumasa, H., Kuboki, Y., Hosoda, H., Iwahashi, Y., 2007. Evaluation of toxicity of the mycotoxin citrinin using yeast ORF DNA microarray and Oligo DNA microarray. BMC Genomics 8 (Article No. 95).

Jamieson, D.J., 1992. *Saccharomyces cerevisiae* has distinct adaptive responses to both hydrogen peroxide and menadione. J. Bacteriol. 174, 6678–6681.

Jeong, H.T., Oowatari, Y., Abe, M., Tanaka, K., Matsuda, H., Kawamukai, M., 2004. Interaction between a negative regulator (Msa2/Nrd1) and a positive regulator (Cpc2) of sexual differentiation in *Schizosaccharomyces pombe*. Biosci. Biotechnol. Biochem. 68, 1621–1626.

Juhnke, H., Krems, B., Kotter, P., Entian, K.D., 1996. Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress. Mol. Gen. Genet. 25, 456–464.

Kanehisa, M., Goto, S., 2000. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.

Kavitha, S., Chandra, T.S., 2009. Effect of vitamin E and MD supplementation on riboflavin production and stress parameters in *Ashbya gossypii*. Proc. Chem. 44, 934–938.

Kim, I.S., Yun, H.S., Iwahashi, H., Jin, I.N., 2006. Genome-wide expression analyses of adaptive response against MD-induced oxidative stress in *Saccharomyces cerevisiae* KNU5377. Proc. Biochem. 41, 2305–2313.

Kim, I.S., Yun, H.S., Jin, I.N., 2007a. Comparative proteomic analyses of the yeast *Saccharomyces cerevisiae* KNU5377 strain against MD-induced oxidative stress. J. Microbiol. Biotechnol. 17, 207–217.

Kim, Y., Nandakumar, M.P., Marten, M.R., 2007b. Proteome map of *Aspergillus nidulans* during osmoadaptation. Fungal Genet. Biol. 44, 886–895.

Kim, Y., Nandakumar, M.P., Marten, M.R., 2008. The state of proteome profiling in the fungal genus *Aspergillus*. Brief Funct. Genom. Proteom. 7, 87–94.

Kolkman, A., Daran-Lapujade, P., Fullaondo, A., Olsthoorn, M.M.A., Pronk, J.T., Slijper, M., Heck, A.J.R., 2006. Proteome analysis of yeast response to various nutrient limitations. Mol. Sys. Biol. 2, E1–E16.

Kwon, S.J., Cho, S.Y., Lee, K.M., Yu, J., Son, M., Kim, K.H., 2009. Proteomic analyses of fungal host factors differentially expressed by *Fusarium graminearum* infected with *Fusarium graminearum virus*-DK21. Virus Res. 144, 96–106.

Lamanda, A., Zahn, A., Roder, D., Langen, H., 2004. Improved ruthenium II tris (bathophenantroline disulfonate) staining and destaining protocol for a better signal-to-background ratio and improved baseline resolution. Proteomics 4, 599–608.

Lara-Otíz, T., Riveros-Rosas, H., Aguirre, J., 2003. Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol. Microbiol. 50, 1241–1255.

Lawrence, C.L., Botting, C.H., Antrobus, R., Coote, P.J., 2004. Evidence of a new role for the high-osmolarity glycerol mitogen-activated protein kinase pathway in yeast: regulating adaptation to citric acid stress. Mol. Cell Biol. 24, 3307–3323.

Leung, Y.F., Cavalieri, D., 2003. Fundamentals of cDNA microarray data analysis. Trends Genet. 19, 649–659.

Li, Q., Abrashev, R., Harvey, L.M., McNeil, B., 2008a. Oxidative stress-associated impairment of glucose and ammonia metabolism in the filamentous fungus, *Aspergillus niger* B1-D. Mycol. Res. 112, 1049–1055.

Li, Q., McNeil, B., Harvey, L.M., 2008b. Adaptive response to oxidative stress in the filamentous fungus *Aspergillus niger* B1-D. Free Radical Biol. Med. 44, 394–402.

Li, Q., Harvey, L.M., McNeil, B., 2009. Oxidative stress in industrial fungi. Crit. Rev. Biotechnol. 29, 199–213.

Liu, L., Zeng, M., Hausladen, A., Heitman, J., Stamler, J.S., 2000. Protection from nitrosative stress by yeast flavohemoglobin. Proc. Natl. Acad. Sci. USA 97, 4672–4676.

Lloyd, R.E., Rinkenberger, J.L., Hug, B.A., Tuveson, R.W., 1990. Growing *Escherichia coli* mutants deficient in riboflavin biosynthesis with non-limiting riboflavin results in sensitization to inactivation by broad spectrum near-ultraviolet light (320–340 nm). Photochem. Photobiol. 52, 897–901.

Mabey, J.E., Anderson, M.J., Giles, P.F., Miller, C.J., Attwood, T.K., Paton, N.W., Bornberg-Bauer, E., Robson, G.D., Oliver, S.G., Denning, D.W., 2004. CADRE: the central *Aspergillus* data repository. Nucleic Acids Res. 32, D401–D405.

Marchler-Bauer, A., Anderson, J.B., Cherukuri, P.F., DeWeese-Scott, C., Geer, L.Y., Gwadz, M., He, S., Hurwitz, D.I., Jackson, J.D., Ke, Z., Lanczycki, C.J., Liebert, C.A., Liu, C., Lu, F., Marchler, G.H., Mullokandov, M., Shoemaker, B.A., Simonyan, V., Song, J.S., Thiessen, P.A., Yamashita, R.A., Yin, J.J., Bryant, S.H., 2005. CDD: a conserved domain database for protein classification. Nucleic Acids Res. 33, D192–196.

Melin, P., Schnurer, J., Wagner, E.G.H., 2002. Proteome analysis of *Aspergillus nidulans* reveals proteins associated with the response to the antibiotic concanamycin A, produced by *Streptomyces* species. Mol. Genet. Genom. 267, 695–702.

Mendoza-Cózatl, D., Loza-Tavera, H., Hernández-Navarro, A., Moreno-Sánchez, R., 2005. Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. FEMS Microbiol. Rev. 29, 653–671.

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Miskei, M., Karányi, Zs., Pócsi, I., 2009. Annotation of stress-response proteins in the aspergilli. Fungal Genet. Biol. 46, S105–120.

Molnár, Zs., Emri, T., Zavaczki, E., Pusztahelyi, T., Pócsi, I., 2006. Effects of mutations in the GanB/RgsA G protein mediated signaling on the autolysis of *Aspergillus nidulans*. J. Basic Microbiol. 46, 495–603.

Nandakumar, M.P., Marten, M.R., 2002. Comparison of lysis methods and preparation protocols for one- and two-dimensional electrophoresis of *Aspergillus oryzae* intracellular proteins. Electrophoresis 23, 2216–2222.

Nie, L., Wu, G., Zhang, W., 2006. Correlation between mRNA and protein abundance in *Desulfovibrio vulgaris*: a multiple regression to identify of variations. Biochem. Biophys. Res. Commun. 339, 603–610.

Osorio, H., Moradas-Ferreira, P., Gunther Sillero, M.A., Sillero, A., 2004. In *Saccharomyces cerevisiae*, the effect of H_2O_2 on ATP, but not on glyceraldehyde-3-phosphate dehydrogenase, depends on the glucose concentration. Arch. Microbiol. 181, 231–236.

Osorio, N.S., Carvalho, A., Almeida, A.J., Padilla-Lopez, S., Leao, C., Laranjinha, J., Ludovico, P., Pearce, D.A., Rodrigues, F., 2007. Nitric oxide signaling is disrupted in the yeast model for Batten disease. Mol. Biol. Cell 18, 2755–2767.

Pandey, A.K., Jain, P., Podila, G.K., Tudzynski, B., Davis, M.R., 2009. Cold induced *Botrytis cinerea* enolase (BcEnol-1) functions as a transcriptional regulator and is controlled by cAMP. Mol. Genet. Genom. 281, 135–146.

Park, Y.I., Gander, J.E., 1998. Choline derivatives involved in osmotolerance of *Penicillium fellutanum*. Appl. Environ. Microbiol. 64, 273–278.

Perrone, G.G., Tan, S.X., Dawes, I.W., 2008. Reactive oxygen species and yeast apoptosis. Biochim. Biophys. Acta-Mol. Cell Res. 1783, 1354–1368.

Perumal, S.S., Shanthi, P., Sachdanandam, P., 2005. Augmented efficacy of tamoxifen in rat breast tumorigenesis when gavaged along with riboflavin, niacin, and CoQ_{10}: effect on lipid peroxidation and antioxidants in mitochondria. Chem. Biol. Interact. 152, 49–58.

Petrucco, S., Bolchi, A., Foroni, C., Percudani, R., Rossi, G.L., Ottonello, S., 1996. A maize gene encoding an NADPH binding enzyme highly homologous to isoflavone reductases is activated in response to sulfur starvation. Plant Cell 8, 69–80.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29 (Article No. e45).

Pócsi, I., Prade, R.A., Penninckx, M.J., 2004. Glutathione, altruistic metabolite in fungi. Adv. Microb. Physiol. 49, 1–76.

Pócsi, I., Miskei, M., Karányi, Z., Emri, T., Ayoubi, P., Pusztahelyi, T., Balla, G., Prade, R.A., 2005. Comparison of gene expression signatures of diamide, H_2O_2 and MD exposed *Aspergillus nidulans* cultures – linking genome-wide transcriptional changes to cellular physiology. BMC Genom. 6 (Article No. 182).

Pusztahelyi, T., Molnár, Z., Emri, T., Klement, É., Miskei, M., Kerékgyártó, J., Balla, J., Pócsi, I., 2006. Comparative studies of differential expression of chitinolytic enzymes encoded by *chiA*, *chiB*, *chic* and *nagA* genes in *Aspergillus nidulans*. Folia Microbiol. 51, 547–554.

Rabilloud, T., Strub, J.M., Luche, S., van Dorsselaer, A., Lunardi, J., 2001. A comparison between Sypro Ruby and ruthenium II tris (bathophenanthroline disulfonate) as fluorescent stains for protein detection in gels. Proteomics 1, 699–704.

Raman, B., Nandakumar, M.P., Muthuvijayan, V., Marten, M.R., 2005. Proteome analysis to assess physiological changes in *Escherichia coli* grown under glucose-limited fed-batch conditions. Biotechnol. Bioeng. 92, 384–392.

Reverter-Branchat, G., Cabiscol, E., Tamarit, J., Ros, J., 2004. Oxidative damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*: common targets and prevention by calorie restriction. J. Biol. Chem. 279, 31983–31989.

Reynolds, T.B., 2009. Strategies for acquiring the phospholipid metabolite inositol in pathogenic bacteria, fungi and protozoa: making it and taking it. Microbiology 155, 1386–1396.

Riego, L., Avendaño, A., DeLuna, A., Rodríguez, E., González, A., 2002. GDH1 expression is regulated by GLN3, GCN4, and HAP4 under respiratory growth. Biochem. Biophys. Res. Commun. 293, 79–85.

Rossignol, T., Kobi, D., Jacquet-Gutfreund, L., Blondin, B., 2009. The proteome of a wine yeast strain during fermentation, correlation with the transcriptome. J. Appl. Microbiol. 107, 47–55.

Scheckhuber, C.Q., Mitterbauer, R., Osiewacz, H.D., 2009. Molecular basis of and interference into degenerative processes in fungi: potential relevance for improving biotechnological performance of microorganisms. Appl. Microbiol. Biotechnol. 85, 27–35.

Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P.O., Davis, R.W., 1996. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. Proc. Natl. Acad. Sci. USA 93, 10614–10619.

Schmidt, M.W., Houseman, A., Lvanov, A.R., Wolf, D.A., 2007. Comparative proteomic and transcriptomic profiling of fission yeast *Schizosaccharomyces pombe*. Mol. Sys. Biol. 3, 1–12.

Shertzer, H.G., Lastbom, L., Sainsbury, M., Moldeus, P., 1992. Menadione-mediated membrane fluidity alterations and oxidative damage in rat hepatocytes. Biochem-Pharmacol. 43, 2135–2141.

Shimizu, M., Fujii, T., Masuo, S., Fujita, K., Takaya, N., 2009. Proteomic analysis of *Aspergillus nidulans* cultured under hypoxic conditions. Proteomics 9, 7–19.

Sugiyama, M., 1991. Effects of vitamins on chromium(VI)-induced damage. Environ. Health Perspect. 92, 63–70.

te Biesebeke, R., Levasseur, A., Boussier, A., Record, E., Van Den Hondel, C.A.M.J.J., Punt, P.J., 2009. Phylogeny of fungal hemoglobins and expression analysis of the *Aspergillus oryzae* flavohemoglobin gene *fhbA* during hyphal growth. Fungal Biol. 114, 135–143.

Thorpe, G.W., Fong, C.S., Alic, N., Higgins, C.J., Dawes, I.W., 2004. Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes. Proc. Natl. Acad. Sci. USA 101, 6564–6569.

Tian, Q., Stepaniants, S.B., Mao, M., Weng, L., Feetham, M.C., Doyle, M.J., Yi, E.C., Dai, H., Thorsson, V., Eng, J., Goodlett, D., Berger, J.P., Gunter, B., Linseley, P.S., Stoughton, R.B., Aebersold, R., Collins, S.J., Hanlon, W.A., Hood, L.E., 2004. Integrated genomic and proteomic analyses of gene expression in mammalian cells. Mol. Cell. Proteom. 3, 960–969.

Toledano, M.B., Delaunay, A., Biteau, B., Spector, D., Azevedo, D., 2003. Oxidative stress responses in yeast. In: Hohman, S., Mager, W.H. (Eds.), Yeast Stress Responses. Springer-Verlag, Berlin, pp. 305–387.

Tuller, T., Kupiec, M., Ruppin, E., 2007. Determinants of protein abundance and translation efficiency in *S. cerevisiae*. PloS Comput. Biol. 3, 2510–2519 (Article No. e248).

Valerius, O., Kleinschmidt, M., Rachfall, N., Schulze, F., López Marín, S., Hoppert, M., Streckfuss-Bömeke, K., Fischer, C., Braus, G.H., 2007. The *Saccharomyces* homolog of mammalian RACK1, Cpc2/Asc1p, is required for FLO11-dependent adhesive growth and dimorphism. Mol. Cell. Proteom. 6, 1968–1979.

Vido, K., Spector, D., Lagniel, G., Lopez, S., Toledano, M.B., Labarre, J., 2001. A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. J. Biol. Chem. 276, 8469–8474.

Washburn, M.P., Koller, A., Oshiro, G., Ulaszek, R.R., Plouffe, D., Deciu, C., Winzeler, E., Yates III, J.R., 2003. Protein pathway and complex clustering of correlated mRNA and protein expression analyses in *Saccharomyces cerevisiae*. PNAS 100, 3107–3112.

Wortman, J.R., Gilsenan, J.M., Joardar, V., Deegan, J., Clutterbuck, J., Andersen, M.R., Archer, D., Bencina, M., Braus, G., Coutinho, P., von Döhren, H., Doonan, J., Driessen, A.J., Durek, P., Espeso, E., Fekete, E., Flipphi, M., Estrada, C.G., Geysens, S., Goldman, G., de Groot, P.W., Hansen, K., Harris, S.D., Heinekamp, T., Helmstaedt, K., Henrissat, B., Hofmann, G., Homan, T., Horio, T., Horiuchi, H., James, S., Jones, M., Karaffa, L., Karányi, Z., Kato, M., Keller, N., Kelly, D.E., Kiel, J.A., Kim, J.M., van der Klei, I.J., Klis, F.M., Kovalchuk, A., Krasevec, N., Kubicek, C.P., Liu, B., Maccabe, A., Meyer, V., Mirabito, P., Miskei, M., Mos, M., Mullins, J., Nelson, D.R., Nielsen, J., Oakley, B.R., Osmani, S.A., Pakula, T., Paszewski, A., Paulsen, I., Pilsyk, S., Pócsi, I., Punt, P.J., Ram, A.F., Ren, Q., Robellet, X., Robson, G., Seiboth, B., van Solingen, P., Specht, T., Sun, J., Taheri-Talesh, N., Takeshita, N., Ussery, D., vanKuyk, P.A., Visser, H., van de Vondervoort, P.J., de Vries, R.P., Walton, J., Xiang, X., Xiong, Y., Zeng, A.P., Brandt, B.W., Cornell, M.J., van den Hondel, C.A., Visser, J., Oliver, S.G., Turner, G., 2009. The 2008 update of the *Aspergillus nidulans* genome annotation: a community effort. Fungal Genet. Biol. 46, S2–S13.

Yu, L., Zhang, W., Wang, L., Yang, J., Liu, T., Peng, J., Leng, W., Chen, L., Li, R., Jin, Q., 2007. Transcriptional profiles of the response to ketoconazole and amphotericin B in *Trichophyton rubrum*. Antimicrob. Agents Chemother. 51, 144–153.

Zadzinski, R., Fortuniak, A., Bilinski, T., Grey, M., Bartosz, G., 1998. Menadione toxicity in *Saccharomyces cerevisiae* cells: activation by conjugation with glutathione. Biochem. Mol. Biol. Int. 44, 747–759.

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