

1 **Transcriptome-based modeling reveals that oxidative stress induces modulation of the**
2 **AtfA-dependent signaling networks in *Aspergillus nidulans***

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37

38 Abbreviations: endoplasmic reticulum, ER; menadione sodium bisulfite, MSB; *t*-
39 butylhydroperoxide, tBOOH

40

41

42 **Abstract**

43 To better understand the molecular functions of the master stress-response regulator AtfA in
44 *Aspergillus nidulans*, transcriptomic analyses of the *atfA* null mutant and the appropriate
45 control strains exposed to menadione sodium bisulfite (MSB), *t*-butylhydroperoxide and
46 diamide induced oxidative stresses were performed. Several elements of oxidative stress
47 response were differentially expressed. Many of them, including the down-regulation of
48 mitotic cell cycle as well as the MSB stress specific up-regulation of FeS cluster assembly
49 and the MSB stress specific down-regulation of nitrate reduction, tricarboxylic acid cycle as
50 well as ER to Golgi vesicle-mediated transport showed AtfA-dependence. To elucidate a
51 potential global regulatory role of AtfA governing expression of a high number of genes with
52 very versatile biological functions, which are stress specific, we devised a model based on the
53 comprehensive transcriptomic data. Our model suggests that an important function of AtfA is
54 to modulate the transduction of stress signals. Although it may regulate directly only a limited
55 number of genes these include elements of the signaling network, *e.g.* members of two-
56 component signal transduction systems. **AtfA acts in a stress-specific manner, which may**
57 **increase further the number and diversity of AtfA-dependent genes.** Our model sheds light on
58 the versatility of the physiological functions of AtfA and its orthologs in fungi.

59

60 Key words: *Aspergillus nidulans*; oxidative stress; interplay between signaling pathways;
61 bZIP-type transcription factors; AtfA

62

63 Oxidative stress is commonly defined as a physiological state when the negative
64 effects of reactive oxygen species (ROS) significantly decrease the fitness of stress-
65 exposed cells. Besides its practical importance, *e.g.* oxidative stress occurs frequently
66 during host-pathogen interactions, decomposition of xenobiotics, biosorption of heavy
67 metals *etc.* [1-4], oxidative stress response is frequently studied in fungal biology to
68 understand how these microbes are able to adapt to their rapidly changing environment
69 and, in more general, to elucidate the molecular mechanisms of stress signalings and
70 regulations [5-7]. Several events have been identified in the course of oxidative stress
71 response so far and many of them, including the activation of glutaredoxin-thioredoxin
72 and DNA repair systems, production of antioxidant enzymes and NADPH or inhibition
73 of cell cycle, are observed commonly in a wide spectrum of species [4, 8-11].

74 AtfA and its orthologues (*e.g.* Atf1 in the fission yeast *Schizosaccharomyces pombe* or
75 Atf2 in mammals) are conserved bZIP oxidative stress response elements regulated by MAPK
76 (mitogen-activated protein kinase) pathways in eukaryotes [12-13]. In *S. pombe*, Atf1 forms a
77 heterodimer with Pcr1 and this heterodimer participates in meiotic recombination,
78 maintenance of heterochromatin structure, regulation of certain genes related to sexual
79 differentiation, beside induction of stress responsive genes under oxidative, heat, reductive,
80 osmotic and starvation stresses [13-17]. AtfA has been characterized as a regulator of conidial
81 stress tolerance in *A. nidulans*, *A. fumigatus* and *A. oryzae* [18-21]. As an example, more than
82 half of the conidia specific genes are regulated in an AtfA-dependent manner in *A. fumigatus*;
83 among them up-regulation of conidial stress-related genes and down-regulation of genes
84 related to germination are notable [20]. AtfA regulates several processes in vegetative hyphae
85 in filamentous fungi. It contributes to stress tolerance and/or alters secondary metabolism in
86 *A. nidulans* [12, 22-24], *Fusarium graminearum* [25], *Fusarium oxysporum* [26], *Botrytis*
87 *cinerea* [27], *Magnaporthe oryzae* [28], *Claviceps purpurea* [29]. AtfA is an important
88 component of a central multiple stress-signaling pathway also regulating development in
89 filamentous fungi as well [24]. AtfB, an orthologue/paralogue of AtfA is an important
90 transcription factor which integrates mycotoxin production and oxidative stress response in
91 *Aspergillus parasiticus* and probably in other aspergilli as well [30]. Most recently, the
92 involvement of the bZIP type transcription factors AtfA-D in the orchestration of stress
93 responses mounted against various types of environmental stress was also demonstrated in *A.*
94 *fumigatus* [31]. AtfA also influence asexual and/or sexual development in *Neurospora crassa*,
95 *A. nidulans* and *F. graminearum* [12, 24-25, 32]. Owing to its importance in the regulation of
96 stress tolerance and secondary metabolism, AtfA significantly contributes to the virulence of

97 plant pathogenic fungi [25, 27-29], and it is also essential for the virulence of the human
98 pathogenic *A. fumigatus* [19-20, 31]. The involvement of AtfA in virulence and/or mycotoxin
99 production in several fungi explains the ceaseless interest in this bZIP-type transcription
100 factor.

101 In a previous study, we investigated the genome-wide transcriptional changes
102 mounted in *A. nidulans*, when it was exposed to six types of stress, including oxidative
103 stress (menadione sodium bisulfite (MSB), low and high concentration of H₂O₂, *t*-
104 butylhydroperoxide (tBOOH), diamide) and high osmolarity stress (NaCl) [23].
105 Transcriptional changes taking place in stress-exposed vegetative tissues of
106 exponentially growing *A. nidulans* were recorded and compared in an oxidative stress
107 sensitive $\Delta atfA$ mutant as well as in the appropriate control strains [23]. The observed
108 stress responses were quite different at the level of the stress responsive genes, which
109 was unexpected since out of the six studied stress conditions five were oxidative
110 stresses [23]. In this study, we carried out a [functional categorization](#) of the stress
111 responsive genes to identify gene groups and biological processes which were under
112 AtfA control in oxidative stress treated vegetative hyphae. To reach these goals, three
113 stress treatments (by MSB, tBOOH and diamide) were chosen and studied in details
114 because the selected oxidative stress conditions were similar to each other in strength,
115 according to the high and comparable numbers of stress responsive genes and the
116 significant and also comparable growth inhibitions observed in stress-exposed cultures
117 [23]. As a result, several AtfA-dependent elements and cellular events of oxidative
118 stress response were identified based on stress-elicited transcriptional changes,
119 including the down-regulation of mitotic cell cycle genes, nitrate reduction,
120 tricarboxylic acid cycle and ER to Golgi vesicle-mediated transport or the up-regulation
121 of FeS cluster assembly genes. To elucidate how AtfA is able to regulate these versatile
122 biological processes we set up a model based on transcriptomic data which suggests that
123 the main function of AtfA is to modulate the signaling network operating under
124 oxidative stress.

125

126 **Materials and methods**

127 *Strains and culture conditions*

128 The *A. nidulans* TNJ 92.4 (*pyrG89*, *AfupyrG*⁺; *pyroA4*; $\Delta atfA::pyroA$; *veA*⁺) and
129 THS30.3 (*pyrG89*, *AfupyrG*⁺; *pyroA*⁺; *veA*⁺) strains as a $\Delta atfA$ gene deletion mutant and

130 the appropriate control strain, respectively [23], were used in this study. The strains
131 were maintained on Barratt's minimal medium [33], and the inoculated agar plates were
132 incubated at 37 °C for 6 d. Conidia harvested from these cultures were used to inoculate
133 submerged liquid cultures. All liquid cultivations were carried out in Erlenmeyer flasks
134 (500 ml) containing 100 ml Barratt's minimal medium, inoculated with 1×10^8 conidia
135 and incubated at 37 °C and at 3.7 Hz shaking frequency for 20 h [23]. Stress exposures
136 were carried out at 16 h using cultures with similar biomass concentrations as described
137 earlier [23]. The applied stressor concentrations (0.12 mM MSB, 0.8 mM tBOOH and
138 1.8 mM diamide) were close to those used by other researchers earlier [12, 18] and
139 reduced (but did not block completely) the growth of both strains with similar intensity
140 [23]. Samples were taken at 0.5 h for RNA isolation and at 4 h for measuring specific
141 enzyme activities, sterol contents and extracellular siderophore contents after stress
142 exposures. Independent cultures were used for microarray experiments, RT-qPCR tests
143 and for physiological characterizations.

144

145 *Reverse-transcription quantitative real-time polymerase chain reaction (RT-qPCR)* 146 *assays*

147 Total RNA was isolated from lyophilized mycelia according to Chomczynski
148 (1993) [34] and RT-qPCR experiments were carried out as described earlier [23]. The
149 applied primer pairs are presented in Supplementary Table 1. Relative transcription
150 levels were quantified with the $\Delta\Delta\text{CP}$ value (mean \pm S.D. calculated from 4 biological
151 replicates). $\Delta\Delta\text{CP}$ was defined as $\Delta\text{CP}_{\text{treated}} - \Delta\text{CP}_{\text{control}}$, where $\Delta\text{CP}_{\text{treated}} = \text{CP}_{\text{reference gene}}$
152 $- \text{CP}_{\text{tested gene}}$ measured in stress treated cultures, $\Delta\text{CP}_{\text{control}} = \text{CP}_{\text{reference gene}}$ - $\text{CP}_{\text{tested gene}}$
153 measured in untreated cultures and CP values stand for the RT-qPCR cycle numbers of
154 crossing points. As reference gene, *actA* (AN6542) was used [35]. RT-qPCR
155 experiments were carried out in the Genomic Medicine and Bioinformatics Core
156 Facility, University of Debrecen, Debrecen, Hungary. RT-qPCR data showed strong
157 correlation with microarray data in both strains (Figs. 1A and 1B).

158

159 *Enzyme activity assays*

160 Specific enzyme activities were determined from cell free extracts prepared by
161 X-pressing [36] according to the protocol of Chiu et al., (1976) [37] (glutathione
162 peroxidase; GPx), Pinto et al., (1984) [38] (glutathione reductase; GR), Roggenkamp et

163 al., (1974) [39] (catalase), Emri et al., (1994) [36] (glucose-6-phosphate dehydrogenase;
164 G6PD) and Bruinenberg et al., (1983) [40] (nitrate reductase; NR). Protein content of
165 the samples was determined with Bradford reagent [41].

166

167 *Sterol content determination*

168 Total sterol measurement was performed according to Arthington-Skaggs et al.,
169 (1999) [42] using lyophilized mycelia. Samples were saponificated with 25 w/v % KOH
170 dissolved in 65 v/v % ethanol for 1 h at 85 °C and sterols were extracted with *n*-heptane.
171 The sterol content of the heptane phase was determined spectrophotometrically using
172 standard curve made with ergosterol. All samples were taken at 4 h after stress
173 treatments.

174

175 *Extracellular siderophore production*

176 Siderophore content was determined as described earlier [43] using fermentation
177 broths or concentrated (10x) fermentation broths prepared by lyophilization as samples.

178

179 *Evaluation of the microarray data*

180 Normalized DNA chip data (Gene Expression Omnibus; accession number
181 GSE63019) were obtained from the experiments described earlier [23] using Agilent
182 60-mer oligonucleotide high density arrays (4 × 44 K; design number 031140; Kromat
183 Ltd., Budapest, Hungary). Total RNA samples were isolated from lyophilized mycelia
184 originated from untreated and stress treated cultures. RNA samples gained from three
185 independent experiments were pooled in 1:1:1 ratio and these mixtures were used for
186 DNA chip experiments.

187 Genes represented by oligomer probes on the DNA chip but modified (splitted,
188 merged) or deleted from the genome during most recent revisions (AspGD;
189 <http://www.aspergillusgenome.org>) were omitted from the evaluation, and the modified
190 gene list was used in further analyses. Stress responsive genes (genes up-regulated or
191 down-regulated by the stress treatment) were selected by the D1 metric (a derivative of
192 the J5 test [44,45]) with threshold 3. Co-regulated genes (core oxidative stress response
193 genes; [23]) were defined as genes showing unidirectional stress responsive behavior in
194 all the three stresses applied. Uniquely regulated genes were defined as genes up-
195 regulated or down-regulated only in one out of the three applied stresses. AtfA-
196 dependent genes were regarded as genes where up-regulation (down-regulation) was

197 detected in the control strain but no regulation or regulation on the opposite direction
198 was observed in the *AtfA* mutant regardless the $SI_{\text{treated,control}}/SI_{\text{treated,AtfA}}$ ratio (SI stands
199 for the normalized microarray signal intensity).

200 Gene enrichment analysis was carried out with the AspGD Gene Ontology Term
201 Finder (<http://www.aspergillusgenome.org/cgi-bin/GO/goTermFinder>) applying default
202 settings, using the appropriate background gene set (*i.e.* the modified gene list of the
203 DNA chip) and biological process ontology GO terms. The FungFun2 package
204 (<https://elbe.hki-jena.de/fungifun/fungifun.php>), with default settings and the
205 appropriate background gene set, was also used to test the enrichment of genes related
206 to FunCat categories in selected gene groups [46]. Only hits with p-value < 0.05 were
207 taken into consideration during the evaluation process.

208 In addition to the sets of stress responsive, uniquely regulated, co-regulated and
209 *atfA*-dependent genes, groups of functionally related genes were also generated and
210 studied by extracting information from the *Aspergillus* Genome Database
211 (<http://www.aspergillusgenome.org>) unless otherwise indicated. Typically, these gene
212 groups contain all genes described by the mentioned GO terms or by their child terms.
213 The following gene groups were generated and used in the further evaluation of the
214 transcriptomic data:

215 - “Ribosome biogenesis”, “mitotic cell cycle”, “iron-sulfur cluster assembly”,
216 and “ER to Golgi vesicle-mediated transport” genes.

217 - “Two-component signal transduction system” genes. These groups contain all
218 genes directly related to these FunCat terms according to the FungiFun2 server
219 (<https://elbe.hki-jena.de/fungifun/fungifun.php>).

220 - “Antioxidant enzyme” genes. This gene group includes genes encoding known
221 and likely antioxidant enzymes (*Aspergillus* Genome Database; [47]).

222 - “Siderophore biosynthesis” genes. This group of genes contains all genes
223 directly involved in the “siderophore biosynthetic process”, “positive regulation of
224 siderophore biosynthetic process” and in the “N',N'',N'''-triacetylfusarinine C
225 biosynthetic process”.

226 - “Nitrate utilization” genes. This group contains all genes directly related to the
227 “nitrate transmembrane transporter activity”, “nitrite uptake transmembrane transporter
228 activity”, “nitrate reductase (NADPH) activity”, “nitrite reductase [NAD(P)H] activity”,
229 “nitrate assimilation”, “regulation of nitrate assimilation”.

230 - “Squalene - ergosterol pathway” genes. This gene group contains the
231 orthologues of *A. fumigatus* genes [48] encoding enzymes involved in ergosterol
232 biosynthesis from squalene.

233 - “Signal transduction” genes. This group contained solely those stress
234 responsive genes, which belonged to the “signal transduction” GO term or to its child
235 terms.

236

237 **Results**

238 *Genome-wide transcriptional changes caused by atfA deletion*

239 Global transcriptional changes in *A. nidulans* under MSB, tBOOH and diamide
240 exposures were detected and compared (Figs 2A and 2B). Altogether, the up-regulation
241 of 785 genes as well as the down-regulation of 772 genes showed AtfA-dependence in
242 at least one stress condition in our experiments (Fig.2C). The most AtfA-dependent
243 genes were found among the MSB stress-dependent genes: out of the 1557
244 aforementioned genes, 883 (57 %) were AtfA-dependent under MSB stress (Fig. 2C).
245 The majority of the AtfA-dependent genes showed AtfA-dependence only under one
246 stress treatment (Fig. 2C): only 11 genes (0.7 %) showed AtfA-dependence under all the
247 three stress conditions tested. It also meant that AtfA affected the transcription of
248 different genes under different stress conditions (Fig. 2C).

249 The numbers of co-regulated genes (which were regarded as core oxidative
250 stress response genes earlier [23]) were $79 + 73 = 152$ and $53 + 163 = 216$ in the control
251 and the $\Delta atfA$ strain, respectively (Figs. 2A and 2B), which numbers represent only 6-10
252 % of the stress responsive genes. Deletion of *atfA* did not decrease the total number of
253 co-regulated stress responsive genes and the overlap between the two co-regulated gene
254 groups was relatively small (Figs. 2A, 2B and 2D). In other words, deletion of *atfA*
255 prevented the co-regulation of genes (altogether 88 genes) but also resulted in a number
256 of new co-regulations (altogether 152 genes) (Fig. 2D).

257 Changes in the regulation of AtfA-dependent genes elicited by deleting the *atfA*
258 gene itself are summarized in Supplementary Table 2. These data provided us with the
259 following pieces of information: (i) Lots of genes (1045 genes) lost their stress
260 responsiveness in the $\Delta atfA$ mutant, while lots of other genes (704 genes) became stress
261 responsive in this strain. (ii) Many co-regulated genes (88 genes) lost their co-regulated
262 nature while others (152 genes) became co-regulated. (iii) Lots of tBOOH stress

263 specific genes (altogether 312 genes) gained MSB stress dependence in the $\Delta atfA$ strain.
264 Although the deletion of *atfA* elicited further changes in the stress responsiveness of
265 other stress dependent genes as well the numbers of affected genes were typically much
266 lower and varied only between 52 (diamide-dependent genes, which gained tBOOH
267 dependence) and 120 (tBOOH-dependent genes, which gained diamide dependence), as
268 a function of the actual oxidative stress treatments employed (Supplementary Table 2).

269

270 *Functional categorization of stress responsive genes*

271 Gene enrichment analysis of stress responsive genes resulted in several
272 significant shared GO and FunCat terms which are presented in Supplementary Table 3
273 and a list of selected terms is shown in Supplementary Table 4. Gene enrichment
274 analysis of the *AtfA*-dependent genes resulted in several very different biological
275 process terms which are not related tightly to oxidative stress response (Table 1,
276 Supplementary Table 3).

277

278 *AtfA-dependence of selected gene groups*

279 *AtfA*-dependent and *AtfA*-independent regulations of 10 gene groups were
280 traversed by us and our findings are presented here in details.

281

282 1. “Ribosome biogenesis” genes

283 “Ribosome biogenesis” genes were significantly enriched under all three stresses
284 in both the control and the $\Delta atfA$ mutant strains when down-regulated genes were
285 analyzed (Supplementary Table 5). Interestingly, different genes were down-regulated
286 under MSB than under diamide stress and therefore the number of co-regulated genes
287 was low (1 gene). Deletion of *atfA* significantly increased the number of down-
288 regulated genes under MSB stress (Supplementary Table 5). Several genes which were
289 tBOOH or tBOOH-diamide stress dependent became MSB stress dependent as well and
290 as a consequence the number of co-regulated genes increased from 1 to 43 genes (Figs.
291 3A and 3B).

292

293 2. “Mitotic cell cycle” genes

294 Down-regulated “mitotic cell cycle” genes were significantly enriched in all
295 three stress treatments in the control strain (Supplementary Table 5). Deletion of *atfA*

296 significantly decreased the number of down-regulated genes under MSB, tBOOH and
297 diamide stresses (Supplementary Table 5).

298

299 3. *Genes encoding antioxidant enzymes*

300 “Antioxidant enzyme” genes were significantly enriched in both strains in all
301 three stress exposures when up-regulated genes were studied (Supplementary Table 5).
302 Deletion of *atfA* had only minor effect on the transcription of these genes
303 (Supplementary Table 5). These up-regulations were confirmed in both strains by RT-
304 qPCR in case of several genes (Table 2). Moreover, elevated specific GR, GPx and
305 catalase activities were measured in both strains after stress treatments (Table 3).

306

307 4. *Genes involved in siderophore biosynthesis*

308 “Siderophore biosynthesis” genes were significantly enriched in the up-regulated
309 tBOOH stress dependent gene groups of both the control and the $\Delta atfA$ mutant strains
310 (Supplementary Table 5). Neither the deletion of *atfA* nor MSB and diamide treatments
311 had significant effects on this gene group (Supplementary Table 5). Up-regulation of
312 *hapX* and *sidA* under tBOOH stress in both strains was also supported by RT-qPCR data
313 and these two genes showed up-regulation under MSB stress as well in the control
314 strain (Table 2). Interestingly, extracellular siderophore accumulations were not
315 detected in any of the cultures (data not shown).

316

317 5. *“Iron-sulfur cluster assembly” genes*

318 The up-regulated “iron-sulfur cluster assembly” genes were significantly
319 enriched under MSB stress in the control strains (Supplementary Table 5). Deletion of
320 *atfA* significantly decreased the number of up-regulated “iron-sulfur cluster assembly”
321 genes from 9 to 4 under MSB stress (Supplementary Table 5). The behavior of these
322 genes (AtfA-dependent regulation under MSB stress) was justified by testing the
323 transcription of selected 11 genes with RT-qPCR: All the 11 genes were up-regulated
324 under MSB stress in the control strain but only 5 of them showed up-regulation in the
325 $\Delta atfA$ mutant (Table 2). In case of tBOOH and diamide stresses 11 and 9 genes showed
326 up-regulation in the control strain, respectively and 8 genes had elevated mRNA level in
327 the mutant strain under both stress conditions (Table 2).

328

329 6. *“Two-component signal transduction system” genes*

330 The enrichment of the up-regulated “two-component signal transduction system”
331 genes was significant only under MSB stress in the control strain; and deletion of *atfA*
332 decreased the number of up-regulated genes from 4 to zero (Supplementary Table 5).
333 The AtfA-dependent up-regulation of these genes under MSB stress treatment was also
334 demonstrated in RT-qPCR measurements: all the tested 7 genes were up-regulated
335 under MSB stress in the control strain and only 1 of them showed elevated transcription
336 in the mutant (Table 2).

337

338 7. Nitrate utilization genes

339 The enrichment of these genes (altogether 14 genes) was significant only in case
340 of the MSB stress (control strain) when the down-regulated genes were studied
341 (Supplementary Table 5) and deletion of *atfA* decreased the number of down-regulated
342 genes from 4 to zero. It is noteworthy that the cluster containing the genes, *niaD*, *niiA*,
343 *crnA* (encoding nitrate reductase, nitrite reductase and nitrate/nitrite transporter,
344 respectively; [49]) showed significantly reduced transcription in all three stress
345 treatments in the control strain and this down-regulation was clearly AtfA dependent in
346 case of MSB treatment according to the RT-qPCR measurements (Table 2). Moreover,
347 significantly reduced nitrate reductase activities were detected in all stress treatments
348 but not under MSB stress in the $\Delta atfA$ strain (Table 3).

349

350 8. “ER to Golgi vesicle-mediated transport” genes

351 “ER to Golgi vesicle-mediated transport” genes were significantly enriched
352 under MSB stress in the control strain when down-regulated genes were analyzed
353 (Supplementary Table 5). Deletion of *atfA* significantly decreased the number of down-
354 regulated genes from 12 to 4 under MSB stress (Supplementary Table 5).

355

356 9. Squalane - ergosterol biosynthetic pathway genes

357 Although a few genes showed down-regulation under stress treatments, their
358 enrichment was not significant in any case (Supplementary Table 5). Sterol
359 measurement demonstrated that the sterol content was significantly decreased in the
360 tBOOH treated cultures of both strains (Supplementary Table 6).

361

362 10. “Signal transduction” genes

363 Many signal-transduction-related genes (37 and 18 in the control and the mutant
364 strains, respectively) were stress responsive in our experiments (Supplementary Table
365 5), and most of them were uniquely regulated under one stress condition (Figs. 3C and
366 3D, Supplementary Table 5). Besides the up-regulation of *tcsA*, *hk2*, *hk-8-2*, *hk-8-3* two-
367 component signal transduction system genes, which was characteristic for MSB treated
368 control cultures, the up-regulations of *pdeA* (coding for a low affinity cAMP
369 phosphodiesterase [50]) and *lreB* (encoding a protein involved in blue-light responsive
370 differentiation and secondary metabolite production [51]) were also observed in
371 diamide treated $\Delta atfA$ and control cultures. In addition, the up-regulations of *hsp90* heat
372 shock protein and AN4419 (putatively encoding a tyrosine phosphatase) were detected
373 typically under tBOOH stress (Supplementary Table 5). Deletion of *atfA* significantly
374 decreased the number of down-regulated signal transduction genes from 11 to 1 under
375 MSB stress (Supplementary Table 5). The majority of the 26 AtfA-dependent signal-
376 transduction-related genes (17 genes) lost their MSB stress dependence in the $\Delta atfA$
377 deletion strain (Fig. 3E).

378

379 Discussion

380 In a previous study we generated an *A. nidulans* $\Delta atfA$ mutant and the
381 appropriate control strain [23]. The mutant strain showed elevated oxidative stress
382 sensitivity on surface cultures in the presence of MSB, tBOOH, diamide and H₂O₂ [23].
383 To gain information on the physiological changes in *A. nidulans* under oxidative stress
384 as well as on the role of AtfA in the regulation of oxidative stress response, DNA chip
385 experiments were conducted using submerged liquid cultures. According to these data,
386 the stress responses were unexpectedly different in each oxidative stress treatment in
387 both strains, which were characterized with few co-regulated and high number of
388 uniquely regulated genes. Moreover, the number of co-regulated genes sharply
389 decreased when the number of studied stress initiating agents was increased, suggesting
390 that the existence of a *Saccharomyces cerevisiae*-type (general) environmental stress
391 response is very unlikely in *A. nidulans* [23]. Deletion of *atfA* affected mRNA
392 accumulation of an unexpectedly high number of genes after MSB exposure but the
393 transcription of several genes showed AtfA-dependence under the other stress
394 conditions and even in untreated cultures [23]. Further analysis of stress responsive
395 genes detected under MSB, tBOOH and diamide stresses in the control strain and in a

396 *ΔatfA* mutant strain was carried out in order i) to understand why the oxidative stress
397 responses were very different at the level of transcriptome, ii) to identify gene
398 groups/biological processes, which are under the control of AtfA in oxidative stress
399 exposed cultures, and iii) transcriptome data were also used to set up hypotheses
400 describing how AtfA contributes to the regulation of these gene groups.

401

402 *Oxidative stress response elements revealed by transcriptomic data*

403 Oxidative stress inhibited both the mitotic cell cycle and ribosome biogenesis
404 (mRNA translation) in all three stress treatments studied (Supplementary Table 5).
405 Their inhibition is a typical element of stress responses under strong stresses in fungi
406 [10-11, 52]. Stress-exposed cells can save lots of energy and materials in this way,
407 which can be used to cope with the stress condition itself and, moreover, it prevents
408 damages or even cell death caused by improper translation of proteins or erroneous cell
409 cycle [52].

410 Up-regulation of genes encoding antioxidative enzymes is among the most
411 typical and characteristic stress response steps under oxidative stress [8-11]. This
412 phenomenon was also observed in all three stress treatments we employed
413 (Supplementary Table 5, Tables 2 and 3).

414 Although the efficient activity of the thioredoxine, glutaredoxine and glutathione
415 systems needs a high-level and continuous supplementation of NADPH [53] no up-
416 regulation of oxidative pentose phosphate shunt, which is one of the most important
417 NADPH producing pathways in fungi grown on glucose carbon source, was observed
418 (Supplementary Table 3, Table 3). This observation is unexpected because the
419 inductions of genes encoding G6PDH and 6-phosphogluconate dehydrogenase
420 (6PGDH) are among the commonest oxidative stress response steps in yeasts [9, 52]. In
421 addition, up-regulation of GsdA (G6PDH) was also observed in proteomic analysis of
422 long-term MSB treated *A. nidulans* cultures [54] meanwhile no elevated specific G6PD
423 and 6PGDH activities were detected in a high β -lactam producer *Penicillium*
424 *chrysogenum* strain under oxidative stress [55-56]. We can hypothesize that an
425 increased flux of the oxidative pentose phosphate shunt may have been reached by
426 regulatory mechanisms other than the transcriptional regulation of the genes encoding
427 pathway specific enzymes, *e.g.* through decreasing the metabolite flux through the
428 glycolytic pathway as it has been described in several organisms [57]. Alternatively, the
429 up-regulation of some other NADPH producing processes, which were not identified in

430 these experiments, *e.g.* NADP isocitrate dehydrogenase [58] or the interconversion of
431 glyceraldehyde-3-phosphate and glycerol as described in yeasts [59], as well as the
432 reduction of NADPH consumption dispensable in stress-exposed cultures may also have
433 provided stress exposed *A. nidulans* cells with satisfactory quantities of NADPH to
434 minimize the deleterious effects of oxidative stress exposures.

435 Regarding the NADPH-consuming processes, which are not directly coupled to
436 oxidative stress defense, the nitrate reduction cluster (*niaD*, *niiA*, *crnA*; [49]) was repressed
437 under all stress conditions (Supplementary Table 5, Tables 2 and 3). The oxidative stress
438 dependent inactivation of nitrate reduction was also detected previously in *P. chrysogenum*
439 [60]. It is reasonable to assume that the reduced metabolization of nitrate helps cells to
440 provide them with enough NADPH to neutralize the deleterious effects of the oxidative stress
441 generating agents but other explanations should also be considered. For example, the reduced
442 metabolization of nitrate can also be a simple consequence of the reduced growth recorded in
443 stress exposed cultures [23] or can also prevent the formation of various harmful reactive
444 nitrogen compounds, *e.g.* nitric monoxide [61]. Importantly, the genes of nitric oxide-
445 metabolizing proteins (*fhbA* and *fhbB*) are co-regulated with the nitrate reduction cluster
446 genes in this fungus [62].

447 Oxidative stress caused profound alterations in the primary metabolism as well.
448 For example, the transcriptions of several genes related to both amino acid biosynthesis
449 and degradation were altered (Supplementary Tables 3 and 4), which was likely the
450 consequence of the cutback of *de novo* protein synthesis, which obviously perturbed the
451 homeostasis of amino acids.

452 Although the aforementioned changes were observed in all three (in the case of
453 amino-acid metabolism two of three) stresses, it did not mean that the up-regulation or
454 down-regulation of these processes were necessarily ensured by an outstandingly high
455 number of co-regulated genes. The most characteristic example is the behavior of
456 “ribosome biogenesis” genes: [Out of the 110 down-regulated genes only one showed](#)
457 [down-regulation under all the three stress conditions studied, however the number of](#)
458 [down-regulated genes was considerable in each individual stress treatment \(Fig. 3A\).](#)
459 [This observation is a good example of that even if the overall changes in the stress](#)
460 [response processes are similar to each other the responses recorded at the level of the](#)
461 [expression of individual stress genes may follow unique, stress-type-specific patterns.](#)
462 [Not surprisingly, several biological processes were identified, which were characteristic](#)

463 for one stress condition only, which is also in line with the observed differences
464 between the transcriptional changes detected at the level of individual genes.

465 Up-regulation of peroxisome related processes (“protein localization to
466 peroxisome”, “peroxisomal transport” and “fatty acid β -oxidation”) was observed only
467 under tBOOH stress, which is in good accordance with the well known lipid damaging
468 nature of this stressor [63]. A reduced production of sterols has been reported as a
469 typical event of oxidative stress in order to maintain the fluidity of membranes under
470 lipid peroxidation [64]. Although a few genes showed down-regulation, enrichment of
471 the down-regulated ergosterol biosynthesis genes - in our case - was not detected under
472 the studied stress conditions (Supplementary Table 5). However, the detected reduced
473 sterol content of tBOOH treated cells demonstrated that alterations in ergosterol
474 synthesis can be an important oxidative stress response in *A. nidulans* even if these
475 changes are not regulated or at least were not detectable at the level of transcriptome.

476 Up-regulation of several “siderophore biosynthesis” genes was also
477 characteristic for the tBOOH induced oxidative stress response. Emerging data suggest
478 that siderophores can have other physiological functions aside from iron uptake or
479 storage. Peralta *et al.* demonstrated that enterobactin, a siderophore produced by
480 *Escherichia coli*, protects cells from oxidative stress and this protection is independent
481 of its iron scavenging activity [65]. Moreover, it was also suggested that reduced
482 siderophore content enhances oxidative stress sensitivity of *A. fumigatus* [66].
483 Unfortunately, we failed to detect siderophores from the fermentation broth of tBOOH
484 treated cultures at 4 h after stress treatment. Hence, further studies are needed to reveal
485 the significance of the transcriptional changes observed with “siderophore biosynthesis”
486 genes.

487 Down-regulation of ER-specific processes under MSB stress (“protein
488 localization to endoplasmic reticulum”, “ER to Golgi vesicle-mediated transport”) was
489 also remarkable. Recent studies demonstrated that oxidative protein folding in ER and
490 ER associated NADPH oxidases are important sources of reactive oxygen species
491 including superoxide [67-68] and, as a consequence, the down-regulation of ER-related
492 processes can be a relevant response to the elevated intracellular superoxide levels
493 elicited by MSB. Up-regulation of genes involved in FeS cluster assembly
494 (Supplementary Table 5) was also among the foreseeable elements of MSB stress
495 responses [69-70] and it is in good accordance with the widely known sensitivity of the
496 FeS cluster proteins to increasing intracellular superoxide levels [71]. Not surprisingly,

497 down-regulation of tricarboxylic acid cycle, which contains several FeS cluster proteins,
498 was also observed specifically under MSB stress (Supplementary Table 5).

499 The observed stress-type-specific differences between the detected global
500 transcriptional changes are in good accordance with the stress-type-dependent
501 regulations of various signal transduction genes (Fig. 3C). These data convincingly
502 demonstrate that MSB, tBOOH and diamide induced quite different stress responses in
503 *A. nidulans* and the differences observed either in the groups of stress responsive genes
504 or in the biological processes set into operation under various types of oxidative stress
505 treatments cannot be explained merely with a few signaling pathways responding
506 uniformly to each oxidative stress condition tested. Instead, the regulations of these
507 pathways followed different patterns under different oxidative stress conditions.
508 Although many of the above mentioned oxidative stress dependent biological processes
509 were under the control of AtfA these AtfA-dependent regulations also showed high
510 stress-type specificity. The AtfA-dependent biological processes include the down-
511 regulation of mitotic cell cycle (under all the three studied stress conditions), the MSB
512 stress specific up-regulation of FeS cluster assembly and the MSB stress specific down-
513 regulation of nitrate reduction, tricarboxylic acid cycle or ER to Golgi vesicle-mediated
514 transport. The diversity of the AtfA-dependent biological processes together with the
515 high number of AtfA-dependent down-regulated genes (besides the up-regulated ones)
516 supports the view that the majority of the observed changes are only indirect
517 consequences of *atfA* deletion. Interestingly the up-regulation of antioxidant enzymes
518 did not show AtfA-dependence; however, several studies have demonstrated the
519 AtfA/Atf1 dependent induction of genes encoding catalases or GPx [22, 24, 72]. Most
520 likely the up-regulation of these genes is under the control of several transcription
521 factors which can substitute one and other under certain conditions.

522

523 *Possible role of AtfA in the regulation of oxidative stress response*

524 In order to elucidate how AtfA regulates oxidative stress response we set up a
525 hypothesis based on the following assumptions generated by transcriptomic data:

526 Assumption 1. AtfA regulates (directly and indirectly) many genes encoding
527 elements of the stress signaling network. This assumption explains why *atfA* deletion
528 affected a great number of genes with versatile functions and how AtfA can contribute
529 to both the up-regulation and down-regulation of these genes.

530 Altogether 26 genes encoding or putatively encoding signal transduction
531 proteins showed AtfA-dependent expression in our experiments (Fig. 3E,
532 Supplementary Table 5). Among them, some members of the “two-component signal
533 transduction system” gene group (*tcsA*, *phkB*, *hk2*, *hk-8-2*, *hk-8-3* and *hk-8-6*) (Table 2,
534 Supplementary Table 5) are particularly interesting. Many of them (*phkB*, *hk-8-2*, *hk-8-3*)
535 together with others (*tcsB*, *hk-8-1*, *phkA*, *nikA*, *hk-8-4*) also showed AtfA-dependent
536 regulation when the transcriptomes of the control and the Δ *atfA* mutant strains were
537 compared in unstressed cultures [23]. Meanwhile, some members of this gene group,
538 *e.g.* *nikA*, *ypdA*, *tcsA*, *tcsB*, are important upstream elements of the HogA/SakA
539 signaling pathway in *A. nidulans*, which regulates oxidative stress response *via* AtfA
540 itself [73-74]. In their most recent publication, Pereira Silva *et al.* [31] found that MpkC
541 and SakA, which regulate the expressions of *atfA* and *atfB*, also influence the
542 transcriptions of “two-component signal transduction system” genes, which are
543 important for their own activation in *A. fumigatus*. The most “two-component signal
544 transduction system” genes (7 genes) were up-regulated under MSB stress suggesting
545 that this positive feed back regulation is particularly important under this type of
546 oxidative stress (Table 2, Supplementary Table 5). Five of these genes were AtfA-
547 dependent (Table 2, Supplementary Table 5), which can be one possible reason for why
548 the highest changes observed in the transcriptome were detected under MSB stress (Fig.
549 2C; [23]).

550 Assumption 2. AtfA interacts with other elements of the stress signaling network
551 and/or with other transcriptional regulators. These interactions may modify the
552 biological activity of either AtfA or the interacting elements or both. This assumption is
553 essential when we want to explain the stress-type dependence of the action of AtfA
554 (Figs. 2C, 3E, Table 1, Supplementary Table 2). Importantly, stress-type-dependent
555 regulations by FgAtf1 of the wheat pathogen fungus *F. graminearum* have also been
556 observed in the formation of antioxidative enzymes [25].

557 Although both the interacting partners of AtfA and the nature of their
558 interactions have remained yet to be elucidated it is well known that orthologs and
559 paralogs of AtfA can physically interact with other bZIP transcription factors or even
560 with other signal transduction pathway proteins, *e.g.* Atf1 of *S. pombe* forms
561 heterodimer with Pcr1, another pZIP-type transcription factor, and physically interacts
562 with Cid2 poly(A) polymerase, while AtfB of *A. parasiticus* also forms heterodimer

563 with AP-1, another bZIP protein [30, 75-77]. Moreover, it has also been suggested that
564 AtfA may physically interact with AtfB (AN8643) in *A. nidulans* as well [12].

565 Assumption 3. AtfA (directly or indirectly) hinders the activity of signaling
566 network elements and/or other transcriptional regulators. It is an important assumption
567 when we would like to explain the behavior of co-regulated genes. For example, the
568 number of co-regulated genes did not change significantly however the spectrum of
569 them altered markedly in the absence of AtfA (Figs. 2A, 2B and 2D).

570 This nature of AtfA was most obvious under MSB stress where it likely
571 prevented the response of tBOOH stress specific genes. As a consequence of this AtfA-
572 mediated asymmetric cross-talk between MSB-elicited and tBOOH-elicited stress
573 responses, several AtfA-dependent genes lost their MSB stress responsiveness (883
574 genes; Fig. 2C, Supplementary Table 2) while several tBOOH stress responsive genes
575 became MSB stress responsive (312 genes in total; Supplementary Table 2) in the *ΔatfA*
576 mutant. Importantly, cross-talk between stress signaling pathways (cationic stress vs.
577 oxidative stress) has been delineated at the level of Hog1 MAPK and Cap1 bZIP
578 transcription factor in the opportunistic human pathogen *C. albicans* by Brown *et al.*
579 [78]. Further research, including interactome studies, is needed to elucidate the possible
580 interacting partners of the bZIP-type transcription factor AtfA under MSB and tBOOH
581 stresses.

582

583 **Conclusions**

584 We set up a mechanistic model to explain the effects of *atfA* gene deletion on the
585 transcriptomic changes observed in oxidative stress exposed vegetative tissues of *A.*
586 *nidulans*. According to this model, AtfA can modulate significantly the working of the
587 regulatory network under oxidative stress besides activating directly certain oxidative
588 stress response genes. This model is based on the following premises and assumptions:
589 i) AtfA regulates positively elements of the signaling network, *e.g.* “two-component
590 signal transduction system” genes, which amplify considerably the number and
591 diversity of AtfA-dependent stress response genes. ii) The AtfA-dependent up-
592 regulation of the “two-component signal transduction system” is particularly important
593 under MSB stress and the absence of this positive feed-back regulation explains the
594 detected outstanding transcriptional changes caused by the deletion of *atfA*. iii) AtfA
595 interacts with elements of the signaling network, which leads to the stress-specific
596 regulation of stress response genes. iv) AtfA (directly or indirectly) prevents the

597 [activation of tBOOH specific genes under MSB stress](#), which contribute to the
598 prevention of any significant decrease in the number of co-regulated genes in the *AtfA*
599 mutant. We hope that his model will help us to gain a deeper insight in the background
600 of the AtfA-dependent regulations and help to understand the sometimes contradictory
601 observations in various fungal species.

602

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606

607 **Conflict of interest**

608 The authors declare that there is no conflict of interest regarding the publication of this paper.

609

610 **References**

611

612 1. M. L. Rabinovich, A. V. Bolobova and L. G. Vasilchenko, “Fungal decomposition of
613 natural aromatic structures and xenobiotics: A review”, *Prikladnaya Biokhimiya i*
614 *Microbiologiya*, vol. 40, pp. 1-17, 2004.

615

616 2. J. C. Fountain, B. Scully and X. Z. Ni *et al.*, “Environmental influences on maize -
617 *Aspergillus flavus* interactions and aflatoxin production”, *Frontiers in Microbiology*,
618 vol. 5, article 40, 2014.

619

620 3. A. D. Dantas, A. Day, M. Ikeh *et al.*, “Oxidative stress responses in the human fungal
621 pathogen, *Candida albicans*”, *Biomolecules*, vol. 5, pp. 142-165, 2015.

622

623 4. N. A. Brown and G. H. Goldman, “The contribution of *Aspergillus fumigatus* stress
624 responses to virulence and antifungal resistance”, *Journal of Microbiology*, vol. 54, pp.
625 243-253, 2016.

626

627 5. M. Breitenbach, M. Weber, M. Rinnerthaler, *et al.*, “Oxidative stress in fungi: Its
628 function in signal transduction, interaction with plant hosts, and lignocellulose
629 degradation”, *Biomolecules*, vol. 5, pp. 318-342, 2015.

630

- 631 6. M. A. Papadakis, and C. T. Workman, “Oxidative stress response pathways: Fission
632 yeast as archetype”, *Critical Reviews in Microbiology*, vol. 41, pp. 520-535, 2015.
633
- 634 7. H. Taymaz-Nikerel, A. Cankorur-Cetinkaya and B. Kirdar, “Genome-wide
635 transcriptional response of *Saccharomyces cerevisiae* to stress-induced perturbations”,
636 *Frontiers in Bioengineering and Biotechnology*, vol. 4, article 17, 2016.
637
- 638 8. S. B. Farr and T. Kogoma, “Oxidative stress responses in *Escherichia coli* and
639 *Salmonella typhimurium*”, *Microbiological Reviews*, vol. 55, pp. 561-585, 1991.
640
- 641 9. M. B. Toledano, A. Delaunay, B. Biteau, *et al.*, “Oxidative stress responses in yeast”,
642 *Topics in Current Genetics*, vol. 1, pp. 241-304, 2003.
643
- 644 10. K. A. Morano, C. M. Grant and W. S. Moye-Rowley, “The response to heat shock
645 and oxidative stress in *Saccharomyces cerevisiae*”, *Genetics*, vol. 190, pp. 1157-1195,
646 2012.
647
- 648 11. J. A. Imlay, “The molecular mechanisms and physiological consequences of
649 oxidative stress: lessons from a model bacterium”, *Nature Reviews Microbiology*, vol.
650 11, pp. 443-454, 2013.
651
- 652 12. F. Lara-Rojas, O. Sanchez, L. Kawasaki, *et al.*, “*Aspergillus nidulans* transcription
653 factor AtfA interacts with the MAPK Saka to regulate general stress responses,
654 development and spore functions”, *Molecular Microbiology*, vol. 80, pp. 436-454, 2011.
655
- 656 13. M. Sanso, I. Vargas-Perez, P. Garcia, *et al.*, “Nuclear roles and regulation of
657 chromatin structure by the stress-dependent MAP kinase Sty1 of *Schizosaccharomyces*
658 *pombe*”, *Molecular Microbiology*, vol. 82, pp. 542-554, 2011.
659
- 660 14. W. P. Wahls and G. R. Smith, “A heteromeric protein that binds to a meiotic
661 homologous recombination hot spot: correlation of binding and hot spot activity”,
662 *Genes and Development*, vol. 8, pp. 1693-1702, 1994.
663

- 664 15. T. Takeda, T. Toda, K. Kominami *et al.*, “*Schizosaccharomyces pombe atf1(+)*
665 encodes a transcription factor required for sexual development and entry into stationary
666 phase”, *The EMBO Journal*, vol. 14, pp. 6193-6208, 1995.
667
- 668 16. K. Shiozaki and P. Russell, “Conjugation, meiosis, and the osmotic stress response
669 are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast”, *Genes
670 and Development*, vol. 10, pp. 2276-2288, 1996.
671
- 672 17. S. Jia, K. Noma, and S. I. Grewal, “RNAi-independent heterochromatin nucleation
673 by the stress-activated ATF/CREB family proteins”, *Science*, vol. 304, pp. 1971-1976,
674 2004.
675
- 676 18. D. Hagiwara, Y. Asano, T. Yamashino, *et al.*, “Characterization of bZip-type
677 transcription factor AtfA with reference to stress responses of conidia of *Aspergillus
678 nidulans*”, *Bioscience Biotechnology and Biochemistry*, vol. 72, pp. 2756-2760, 2008.
679
- 680 19. D. Hagiwara, S. Suzuki, K. Kamei, *et al.*, “The role of AtfA and HOG MAPK
681 pathway in stress tolerance in conidia of *Aspergillus fumigatus*”, *Fungal Genetics and
682 Biology*, vol. 73, pp. 138-149, 2014.
683
- 684 20. D. Hagiwara, H. Takahashi, Y. Kusuya *et al.*, “Comparative transcriptome analysis
685 revealing dormant conidia and germination associated genes in *Aspergillus* species: an
686 essential role for AtfA in conidial dormancy”, *BMC Genomics*, vol. 17, article 358,
687 2016.
688
- 689 21. K. Sakamoto, K. Iwashita, O. Yamada *et al.*, “*Aspergillus oryzae atfA* controls
690 conidial germination and stress tolerance”, *Fungal Genetics and Biology*, vol. 46, pp.
691 887-897, 2009.
692
- 693 22. A. Balázs, I. Pócsi, Z. Hamari *et al.*, “AtfA bZIP-type transcription factor regulates
694 oxidative and osmotic stress responses in *Aspergillus nidulans*.”, *Molecular Genetics
695 and Genomics*, vol. 283, pp. 289-303, 2010.
696

- 697 23. T. Emri, V. Szarvas, E. Orosz, *et al.*, “Core oxidative stress response in *Aspergillus*
698 *nidulans*”. *BMC Genomics*, vol. 16, article 478, 2015.
699
- 700 24. R. Jaimes-Arroyo, F. Lara-Rojas, O. Bayram *et al.*, “The SrkA kinase is part of the
701 SakA mitogen-activated protein kinase interactome and regulates stress responses and
702 development in *Aspergillus nidulans*”, *Eukaryotic Cell*, vol. 14, pp. 495-510, 2015.
703
- 704 25. T. Van Nguyen, C. Kroger, J. Bonnighausen, *et al.*, “The ATF/CREB transcription
705 factor Atf1 is essential for full virulence, deoxynivalenol production, and stress
706 tolerance in the cereal pathogen *Fusarium graminearum*”, *Molecular Plant-Microbe*
707 *Interactions*, vol. 26, pp. 1378-1394, 2013.
708
- 709 26. X. Z. Qi, L. J. Guo, L. Y. Yang, *et al.*, “Foatf1, a bZIP transcription factor of
710 *Fusarium oxysporum* f. sp. *ubense*, is involved in pathogenesis by regulating the
711 oxidative stress responses of Cavendish banana (*Musa* spp.)”, *Physiological and*
712 *Molecular Plant Pathology*, vol. 84, pp. 76-85, 2013.
713
- 714 27. N. Temme, B. Oeser, M. Massaroli *et al.*, “BcAtf1, a global regulator, controls
715 various differentiation processes and phytotoxin production in *Botrytis cinerea*”,
716 *Molecular Plant Pathology*, vol. 13, pp. 704-718, 2012.
717
- 718 28. M. Guo, W. Guo, Y. Chen *et al.*, “The basic leucine zipper transcription factor
719 Moatf1 mediates oxidative stress responses and is necessary for full virulence of the rice
720 blast fungus *Magnaporthe oryzae*”, *Molecular Plant-Microbe Interactions*, vol. 23, pp.
721 1053-1068, 2010.
722
- 723 29. E. Nathues, S. Joshi, K. B. Tenberge *et al.*, “CPTF1, a CREB-like transcription
724 factor, is involved in the oxidative stress response in the phytopathogen *Claviceps*
725 *purpurea* and modulates ROS level in its host *Secale cereale*”, *Molecular Plant-*
726 *Microbe Interactions*, vol. 17, pp. 383-393, 2004.
727
- 728 30. L. V. Roze, A. Chanda, J. Wee, *et al.*, “Stress-related transcription factor AtfB
729 integrates secondary metabolism with oxidative stress response in aspergilli”, *Journal of*
730 *Biological Chemistry*, vol. 286, pp. 35137-35148, 2011.

731

732 31. L.P. Silva, P. A. de Castro, T. F. Dos Reis *et al.*, “Genome-wide transcriptome
733 analysis of *Aspergillus fumigatus* exposed to osmotic stress reveals regulators of
734 osmotic and cell wall stresses that are SakA^{HOG1} and MpkC dependent”, *Cellular*
735 *Microbiology*, vol. 19, doi:10.1111/cmi.12681, 2017.

736

737 32. K. Yamashita, A. Shiozawa, S. Watanabe *et al.*, “ATF-1 transcription factor
738 regulates the expression of *ccg-1* and *cat-1* genes in response to fludioxonil under OS-2
739 MAP kinase in *Neurospora crassa*”, *Fungal Genetics and Biology*, vol. 45, pp. 1562-
740 1569, 2008.

741

742 33. R. W. Barratt, G. B. Johnson and W. N. Ogata, “Wild-type and mutant stocks of
743 *Aspergillus nidulans*”, *Genetics*, vol. 52, pp. 233-246, 1965.

744

745 34. P. Chomczynski, “A reagent for the single-step simultaneous isolation of RNA,
746 DNA and proteins from cell and tissue samples”, *Biotechniques*, vol. 15, pp. 532-534,
747 536-537, 1993.

748

749 35. Z. Kovács, M. Szarka, S. Kovács *et al.*, “Effect of cell wall integrity stress and
750 RlmA transcription factor on asexual development and autolysis in *Aspergillus*
751 *nidulans*”, *Fungal Genetics and Biology*, vol. 54, pp. 1-14, 2013.

752

753 36. T. Emri, G. Bartók and A. Szentirmai, “Regulation of specific activity of glucose-6-
754 phosphate-dehydrogenase and 6-phosphogluconate dehydrogenase in *Penicillium*
755 *chrysogenum*”, *FEMS Microbiology Letters*, vol. 117, pp. 67-70, 1994.

756

757 37. D. T. Y. Chiu, F. H. Stults and A. L. Tappel, “Purification and properties of rat lung
758 soluble glutathione peroxidase”, *Biochimica et Biophysica Acta*, vol. 445, pp. 558-566,
759 1976.

760

761 38. M. C. Pinto, A. M. Mata and J. Lopezbarea, “Reversible inactivation of
762 *Saccharomyces cerevisiae* glutathione-reductase under reducing conditions”, *Archives*
763 *of Biochemistry and Biophysics*, vol. 228, pp. 1-12, 1984.

764

- 765 39. R. Roggenkamp, H. Sahm and F. Wagner, "Microbial assimilation of methanol
766 induction and function of catalase in *Candida boidinii*", *FEBS Letters*, vol. 41, pp. 283-
767 286, 1974.
- 768
- 769 40. P. M. Bruinenberg, J.P. Van Dijken and W. A. Scheffers, "An enzymatic analysis of
770 NADPH production and consumption in *Candida utilis*", *Journal of General*
771 *Microbiology*, vol. 129, pp. 965-971, 1983.
- 772
- 773 41. M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram
774 quantities of protein utilizing the principle of protein-dye binding", *Analytical*
775 *Biochemistry*, vol. 2, pp. 248–254, 1976.
- 776
- 777 42. B. A. Arthington-Skaggs, H. Jradi, T. Desai, *et al.*, "Quantitation of ergosterol
778 content: novel method for determination of fluconazole susceptibility of *Candida*
779 *albicans*", *Journal of Clinical Microbiology*, vol. 37, pp. 3332-3337, 1999.
- 780
- 781 43. V. Tóth, K. Antal, G. Gyémánt *et al.*, "Optimization of coprogen production in
782 *Neurospora crassa*", *Acta Biologica Hungarica*, vol. 60, pp. 321-328, 2009.
- 783
- 784 44. S. Patel and J. Lyons-Weiler, "caGEDA: a web application for the integrated
785 analysis of global gene expression patterns in cancer" *Applied Bioinformatics*, vol. 3,
786 pp. 49-62, 2004.
- 787
- 788 45. R. Jordan, S. Patel, H. Hu, *et al.*, "Efficiency analysis of competing tests for finding
789 differentially expressed genes in lung adenocarcinoma" *Cancer Informatics*, vol. 6, pp.
790 389-421, 2008.
- 791
- 792 46. S. Priebe, C. Kreisel, F. Horn, *et al.*, "FungiFun2: a comprehensive online resource
793 for systematic analysis of gene lists from fungal species", *Bioinformatics*, vol. 31, pp.
794 445-446, 2015.
- 795
- 796 47. F. Bakti, A. Király, E. Orosz *et al.*, "Study on the glutathione metabolism of the
797 filamentous fungus *Aspergillus nidulans*", *Acta Microbiologica et Immunologica*
798 *Hungarica*, *accepted*. 2017.

799

800 48. L. Alcazar-Fuoli and E. Mellado, "Ergosterol biosynthesis in *Aspergillus fumigatus*:
801 its relevance as an antifungal target and role in antifungal drug resistance", *Frontiers in*
802 *Microbiology*, vol. 3, article 439, 2013.

803

804 49. I. L. Johnstone, P. C. McCabe, P. Greaves *et al.*, "Isolation and characterisation of
805 the *crnA-niiA-niaD* gene cluster for nitrate assimilation in *Aspergillus nidulans*", *Gene*,
806 vol. 90, pp. 181-192, 1990.

807

808 50. A. Lafon, K. H. Han, J. A. Seo *et al.*, "G-protein and cAMP-mediated signaling in
809 aspergilli: a genomic perspective", *Fungal Genetic and Biology*, vol. 43, pp. 490-502,
810 2006.

811

812 51. J. Purschwitz, S. Müller, C. Kastner *et al.* "Functional and physical interaction of
813 blue- and red-light sensors in *Aspergillus nidulans*" *Current Biology*, vol. 18, pp. 255-
814 259, 2008.

815

816 52. A. P. Gasch, "The environmental stress response: a common yeast response to
817 diverse environmental stresses", *Topics in Current Genetics*, vol. 1, pp. 11-70, 2003.

818

819 53. A.P. Fernandes and A. Holmgren, "Glutaredoxins: glutathione-dependent redox
820 enzymes with functions far beyond a simple thioredoxin backup system", *Antioxidants*
821 *and Redox Signaling*, vol. 6, pp. 63-74, 2004.

822

823 54. T. Pusztahelyi, É. Klement, E. Szajli *et al.*, "Comparison of transcriptional and
824 translational changes caused by long-term menadione exposure in *Aspergillus*
825 *nidulans*", *Fungal Genetics and Biology*, vol. 48, pp. 92-103, 2011.

826

827 55. T. Emri, I. Pócsi and A. Szentirmai, "Glutathione metabolism and protection against
828 oxidative stress caused by peroxides in *Penicillium chrysogenum*", *Free Radical*
829 *Biology and Medicine*, vol. 23, pp. 809-814, 1997.

830

- 831 56. T. Emri, I. Pócsi and A. Szentirmai, “Analysis of the oxidative stress response of
832 *Penicillium chrysogenum* to menadione”, *Free Radical Research*, vol. 30, pp. 125-132,
833 1999.
- 834
- 835 57. M. Ralser, M. M. Wamelink, A. Kowald, *et al.*, “Dynamic rerouting of the
836 carbohydrate flux is key to counteracting oxidative stress”, *Journal of Biology*, vol. 6,
837 pp. 10, 2007.
- 838
- 839 58. M. Singh, N. S. Scrutton and M. C. Scrutton, “NADPH generation in *Aspergillus*
840 *nidulans* - Is the mannitol cycle involved?” *Journal of General Microbiology*, vol. 134,
841 pp. 643-654, 1988.
- 842
- 843 59. A. K. Pahlman, K. Granath, R. Ansell, *et al.*, “The yeast glycerol 3-phosphatases
844 gpp1p and gpp2p are required for glycerol biosynthesis and differentially involved in
845 the cellular responses to osmotic, anaerobic, and oxidative stress”, *Journal of Biological*
846 *Chemistry*, vol. 276, pp. 3555-3563, 2001.
- 847
- 848 60. T. Emri, L. Sámi, A. Szentirmai, *et al.*, “Co-ordination of the nitrate and nitrite
849 assimilation, the glutathione and free radical metabolisms, and the pentose phosphate
850 pathway in *Penicillium chrysogenum*”, *Journal of Basic Microbiology*, vol. 39, pp. 109-
851 115, 1999.
- 852
- 853 61. C. Meyer, U. S. Lea, F. Provan, *et al.*, “Is nitrate reductase a major player in the
854 plant NO (nitric oxide) game?” *Photosynthesis Research*, vol. 83, pp. 181-189, 2005.
- 855
- 856 62. T. Schinko, H. Berger, W. Lee, *et al.* “Transcriptome analysis of nitrate assimilation in
857 *Aspergillus nidulans* reveals connections to nitric oxide metabolism” *Molecular*
858 *Microbiology*, vol. 78, pp. 720–738, 2010.
- 859
- 860 63. M. J. Davies, “Detection of peroxy and alkoxy radicals produced by reaction of
861 hydroperoxides with rat liver microsomal fractions” *Biochemical Journal*, vol. 257, pp.
862 603–606, 1989.
- 863

864 64. F. M. Montanes, A. Pascual-Ahuir and M. Proft, “Repression of ergosterol
865 biosynthesis is essential for stress resistance and is mediated by the Hog1 MAP kinase
866 and the Mot3 and Rox1 transcription factors”, *Molecular Microbiology*, vol. 79, pp.
867 1008-1023, 2011.

868

869 65. D. R. Peralta, C. Adler, N. S. Corbalán, *et al.*, “Enterobactin as part of the oxidative
870 stress response repertoire” *PLoS One*, vol. 11, e0157799, 2016.

871

872 66. M. Brandon, B. Howard, C. Lawrence, *et al.*, “Iron acquisition and oxidative stress
873 response in *Aspergillus fumigatus*” *BMC System Biology*, Vol. 9, 19, 2015.

874

875 67. S. X. Tan, M. Teo, Y. T. Lam, *et al.*, “Cu, Zn superoxide dismutase and NADP(H)
876 homeostasis are required for tolerance of endoplasmic reticulum stress in
877 *Saccharomyces cerevisiae*”, *Molecular Biology of the Cell*, vol. 20, pp. 1493-1508,
878 2009.

879

880 68. M. Rinnerthaler, S. Buttner, P. Laun *et al.*, “Yno1p/Aim14p, a NADPH-oxidase
881 ortholog, controls extramitochondrial reactive oxygen species generation, apoptosis, and
882 actin cable formation in yeast”, *Proceedings of the National Academy of Sciences of the*
883 *United States*, vol. 109, pp. 8658-8663, 2012.

884

885 69. R. V. Perez-Gallardo, L. S. Briones, A.L. Diaz-Perez *et al.*, “Reactive oxygen
886 species production induced by ethanol in *Saccharomyces cerevisiae* increases because
887 of a dysfunctional mitochondrial iron-sulfur cluster assembly system”, *FEMS Yeast*
888 *Research*, vol. 13, pp. 804-819, 2013.

889

890 70. A. Popovic-Bijelic, M. Mojovic, S. Stamenkovic *et al.*, “Iron-sulfur cluster damage
891 by the superoxide radical in neural tissues of the SOD1(G93A) ALS rat model”, *Free*
892 *Radical Biology and Medicine*, vol. 96, pp. 313-322, 2016.

893

894 71. J. A. Imlay, “Iron-sulphur clusters and the problem with oxygen”, *Molecular*
895 *Microbiology*, vol. 59, pp. 1073-1082, 2006.

896

- 897 72. C. W. Nakagawa, K. Yamada and N. Mutoh, “Role of Atf1 and Pap1 in the
898 induction of the catalase gene of fission yeast *Schizosaccharomyces pombe*”, *Journal of*
899 *Biochemistry*, vol. 127, pp. 233-238, 2000.
900
- 901 73. D. Hagiwara, Y. Asano, J. Marui *et al.*, “Transcriptional profiling for *Aspergillus*
902 *nidulans* HogA MAPK signaling pathway in response to fludioxonil and osmotic
903 stress”, *Fungal Genetics and Biology*, vol. 46, pp. 868-878, 2009.
904
- 905 74. M. Miskei, Z. Karányi and I. Pócsi, “Annotation of stress-response proteins in the
906 aspergilli”, *Fungal Genetic and Biology*, vol. 46, pp. S105-S120, 2009.
907
- 908 75. A. W. Reinke, J. Baek, O. Ashenberg, *et al.*, “Networks of bZIP protein-protein
909 interactions diversified over a billion years of evolution”, *Science*, vol. 340, pp. 730-
910 734, 2013.
911
- 912 76. T. V. Vo, J. Das, M. J. Meyer *et al.*, “A Proteome-wide fission yeast interactome
913 reveals network evolution principles from yeasts to human”, *Cell*, vol. 164, pp. 310-
914 323, 2016.
915
- 916 77. M. Sanso, M. Gogol, J. Ayte, *et al.*, “Transcription factors Per1 and Atf1 have
917 distinct roles in stress- and Sty1-dependent gene regulation”, *Eukaryotic Cell*, vol. 7, pp.
918 826-835, 2008.
919
- 920 78. A. J. Brown, S. Budge, D. Kaloriti, *et al.*, “Stress adaptation in a pathogenic
921 fungus”, *Journal of Experimental Biology*. vol. 217, pp. 144-155, 2014.
922

923 **Table 1** Gene enrichment analysis of AtfA-dependent genes

Analyzed gene group	Significant shared GO and FunCat terms	Stress dependence
<i>AtfA-dependent up-regulated genes</i>		
	alpha-amino acid biosynthetic process (GO) degradation of isoleucine, methionine, valine, arginine (FunCat)	tBOOH tBOOH
	peroxisomal transport (FunCat)	tBOOH
	fatty acid metabolic process (GO)	tBOOH
<i>AtfA-dependent down-regulated genes</i>		
	mitotic cell cycle (GO)	MSB, tBOOH, diamide
	mitotic sister chromatid segregation (GO)	MSB, tBOOH, diamide
	cytokinesis (GO)	MSB, tBOOH
	ribosome biogenesis (GO)	tBOOH
	translation (GO)	MSB, tBOOH
	tricarboxylic acid cycle (FunCat)	MSB
	aerobic respiration (FunCat)	tBOOH
	homeostasis of metal ions (Na, K, Ca etc.) (FunCat)	diamide
	ER to Golgi vesicle-mediated transport (GO)	MSB

924 The full lists of the significant shared biological process terms are available in Supplementary
 925 Table 3.

Gene ID	Gene name	Known/putative function	Stress conditions					
			Control strain			<i>ΔatfA</i> mutant		
			MSB	tBOOH	Diamide	MSB	tBOOH	Diamide
<i>“Antioxidant enzyme” genes</i>								
AN9339	<i>catB</i>	catalase	1.4 ± 0.9*	2.4 ± 1.2*	1.2 ± 0.8*	2.9 ± 1.0*	3.1 ± 1.2*	1.6 ± 0.8*
AN10220	<i>ccp1</i>	cytochrome c peroxidase	5.3 ± 1.1*	3.0 ± 1.2*	5.1 ± 1.4*	1.8 ± 0.7*	4.7 ± 1.1*	1.7 ± 0.9*
AN0932	<i>glrA</i>	glutathione reductase	4.8 ± 1.4*	1.3 ± 0.8*	3.4 ± 0.8*	1.7 ± 1.1*	1.6 ± 1.1*	1.2 ± 0.7*
AN2846	<i>gpxA</i>	glutathione peroxidase	2.5 ± 1.3*	4.2 ± 2.0*	3.5 ± 1.1*	1.8 ± 1.0*	1.8 ± 0.9*	2.8 ± 1.0*
AN7567		glutaredoxin	1.3 ± 0.9*	2.5 ± 1.5*	3.5 ± 0.7*	1.1 ± 0.7*	1.6 ± 1.1*	1.9 ± 0.9*
AN5831		glutathione transferase	5.3 ± 1.4*	3.1 ± 2.0*	2.3 ± 1.6*	3.3 ± 1.5*	2.0 ± 1.0*	1.6 ± 0.9*
AN3581	<i>trxR</i>	thioredoxin reductase	4.5 ± 1.0*	2.9 ± 1.4*	2.8 ± 1.4*	4.1 ± 1.2*	3.1 ± 1.0*	4.9 ± 1.2*
AN8692	<i>prxA</i>	thioredoxin-dependent peroxidase	3.4 ± 1.0*	3.8 ± 0.8*	3.9 ± 1.4*	3.8 ± 1.4*	2.7 ± 0.8*	3.9 ± 0.9*

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“Siderophore biosynthesis” genes

AN5823	<i>sidA</i>	L-ornithine N5-monooxygenase	2.4 ± 0.9*	1.2 ± 0.6*	-2.5 ± 1.1*	0.6 ± 0.8	1.2 ± 0.7*	-0.2 ± 0.5
AN8251	<i>hapX</i>	bZIP transcription factor	2.1 ± 0.8*	1.2 ± 0.7*	0.8 ± 0.5*	0.6 ± 0.6	1.5 ± 0.6*	0.8 ± 0.5*

“Iron-sulfur cluster assembly” genes

AN10584		cysteine desulfurase	2.5 ± 0.8*	1.6 ± 0.9*	2.2 ± 1.0*	0.4 ± 0.5	0.8 ± 0.5*	0.2 ± 0.6
AN2508		cysteine desulfurase	2.0 ± 0.8*	1.3 ± 0.7*	0.1 ± 0.5	0.0 ± 0.6	0.2 ± 0.5	0.4 ± 1.0
AN4655		iron-sulfur transferase	1.9 ± 0.8*	2.2 ± 1.0*	2.1 ± 0.9*	2.3 ± 0.9*	3.0 ± 0.7*	2.2 ± 1.1*
AN0447		role in iron-sulfur cluster assembly	3.2 ± 0.8*	0.8 ± 0.6*	3.1 ± 1.1*	0.6 ± 0.7	0.9 ± 0.8*	1.6 ± 0.7*
AN1407		role in iron-sulfur cluster assembly	2.2 ± 0.7*	1.2 ± 0.9*	2.9 ± 1.1*	0.6 ± 0.8	0.6 ± 0.7	2.6 ± 0.7*
AN2155		role in iron-sulfur cluster assembly	3.1 ± 0.8*	1.2 ± 0.6*	3.3 ± 1.4*	0.9 ± 0.7*	1.4 ± 0.8*	2.2 ± 0.7*
AN3632		role in iron-sulfur cluster assembly	2.9 ± 0.8*	2.0 ± 0.9*	0.5 ± 0.9	1.1 ± 0.6*	2.7 ± 1.1*	0.8 ± 0.6
AN5953		role in iron-sulfur cluster assembly	1.8 ± 0.8*	1.3 ± 0.7*	1.6 ± 1.0*	0.70 ± 1.1	1.3 ± 0.8*	2.6 ± 0.8*

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AN8485		role in iron-sulfur cluster assembly	$2.5 \pm 1.0^*$	$3.0 \pm 1.2^*$	$1.4 \pm 0.7^*$	$1.5 \pm 0.5^*$	$1.3 \pm 0.6^*$	$1.6 \pm 0.5^*$
AN10012		role in iron-sulfur cluster assembly	$3.1 \pm 1.0^*$	$0.8 \pm 0.6^*$	$1.2 \pm 0.5^*$	0.3 ± 0.5	0.4 ± 0.6	$1.4 \pm 0.9^*$
AN11060		role in iron-sulfur cluster assembly	$3.1 \pm 0.9^*$	$0.8 \pm 0.7^*$	$1.2 \pm 0.9^*$	$2.1 \pm 1.1^*$	$0.9 \pm 0.7^*$	$2.5 \pm 0.9^*$

“Two-component signal transduction system” genes

AN5296	<i>tcsA</i>	histidine kinase	$2.7 \pm 0.8^*$	$3.1 \pm 1.0^*$	$1.9 \pm 0.9^*$	0.6 ± 0.6	$1.9 \pm 0.8^*$	-0.1 ± 0.5
AN1800	<i>tcsB</i>	histidine kinase	$4.2 \pm 1.1^*$	$2.2 \pm 0.7^*$	$1.3 \pm 0.7^*$	$2.4 \pm 1.2^*$	$1.4 \pm 0.8^*$	-0.3 ± 0.7
AN3101	<i>phkB</i>	histidine kinase	$1.5 \pm 0.8^*$	-0.5 ± 0.5	$-1.3 \pm 0.4^*$	-0.5 ± 0.6	-0.6 ± 0.6	$-1.7 \pm 0.8^*$
AN7945	<i>hk2</i>	histidine kinase	$4.2 \pm 1.1^*$	-0.4 ± 0.5	-0.1 ± 0.7	0.2 ± 0.7	0.6 ± 0.7	0.4 ± 0.8
AN4113	<i>hk-8-2</i>	histidine kinase	$2.6 \pm 0.5^*$	-0.4 ± 0.6	0.4 ± 0.6	-0.3 ± 0.7	$-1.2 \pm 0.7^*$	$-1.1 \pm 0.6^*$
AN6820	<i>hk-8-3</i>	histidine kinase	$2.9 \pm 1.0^*$	$-0.9 \pm 0.4^*$	$-2.0 \pm 1.1^*$	0.3 ± 0.6	0.4 ± 0.6	$0.9 \pm 0.7^*$
AN2363	<i>hk-8-6</i>	histidine kinase	$3.5 \pm 1.3^*$	$1.8 \pm 0.8^*$	-0.3 ± 0.6	0.6 ± 0.6	0.2 ± 0.7	$-0.9 \pm 0.5^*$

Continued on next page.

“Nitrate utilization” genes

AN1006	<i>niaD</i>	nitrate reductase	$-1.0 \pm 0.5^*$	$-2.2 \pm 1.1^*$	$-4.3 \pm 1.2^*$	0.5 ± 0.6	$-2.2 \pm 0.9^*$	$-1.9 \pm 1.1^*$
AN1007	<i>niiA</i>	nitrite reductase	$-1.5 \pm 0.7^*$	$-1.4 \pm 0.7^*$	$-3.2 \pm 1.0^*$	0.2 ± 0.6	$-1.9 \pm 1.0^*$	$-2.1 \pm 1.2^*$
AN1008	<i>crnA</i>	nitrate transporter	$-4.8 \pm 1.2^*$	$-1.1 \pm 0.7^*$	$-2.1 \pm 0.9^*$	$0.9 \pm 0.8^*$	$-2.4 \pm 1.1^*$	-0.5 ± 0.5

Other genes

AN1168	<i>cch1</i>	calcium ion transporter	0.1 ± 0.6	$0.8 \pm 0.7^*$	$-1.3 \pm 0.6^*$	-0.6 ± 0.7	$1.3 \pm 0.8^*$	-0.4 ± 0.7
AN1628	<i>enaB</i>	calcium ion transporter	-1.5 ± 1.1	$2.5 \pm 1.2^*$	$-1.2 \pm 0.7^*$	0.4 ± 0.6	$1.2 \pm 0.5^*$	0.2 ± 0.7
AN4920	<i>pmcB</i>	calcium ion transporter	0.7 ± 0.8	$1.9 \pm 0.9^*$	$2.1 \pm 1.1^*$	$-1.3 \pm 0.6^*$	$1.1 \pm 0.5^*$	$1.4 \pm 0.7^*$
AN8842	<i>mid1</i>	calcium ion transporter	0.5 ± 0.7	$0.8 \pm 0.7^*$	0.3 ± 0.6	$-1.3 \pm 0.7^*$	$1.3 \pm 0.6^*$	-0.2 ± 0.6

940 Relative transcription levels were quantified with the $\Delta\Delta\text{CP}$ value. Mean \pm S.D. values are presented. The *actA* (AN6542) gene was used as
941 reference gene.

942 * - Significantly differ from zero according to Student's t-test ($p < 0.05$, $n = 4$).

944 **Table 3** Specific enzyme activities and sterol contents of the cultures

945

Cultures	NR (mkat/kg protein)	G6PDH (mkat/kg protein)	GR (mkat/kg protein)	GPx (mkat/kg protein)	Catalase (kat/kg protein)	Sterol content ($\mu\text{g}/\text{mg}$)
Control strain untreated	2.6 \pm 0.3	8.0 \pm 1	3.8 \pm 0.5	0.40 \pm 0.04	0.20 \pm 0.02	5.8 \pm 0.6
Control strain MSB	1.6 \pm 0.3*	8.5 \pm 1	4.8 \pm 0.6*	0.51 \pm 0.05*	0.38 \pm 0.03*	5.7 \pm 0.2
Control strain tBOOH	0.3 \pm 0.1*	8.3 \pm 0.9	4.4 \pm 0.6*	0.57 \pm 0.05*	0.40 \pm 0.03*	3.3 \pm 0.2*
Control strain diamide	0.6 \pm 0.1*	7.8 \pm 1	4.5 \pm 0.5*	0.77 \pm 0.08*	0.30 \pm 0.03*	7.0 \pm 0.7
$\Delta atfA$ strain untreated	2.8 \pm 0.3	7.4 \pm 0.9	3.4 \pm 0.4	0.33 \pm 0.04	0.18 \pm 0.02	6.8 \pm 0.7
$\Delta atfA$ strain MSB	3.1 \pm 0.4*	8.0 \pm 1	4.6 \pm 0.5*	0.46 \pm 0.05*	0.43 \pm 0.04*	5.7 \pm 0.4
$\Delta atfA$ strain tBOOH	0.3 \pm 0.1*	7.7 \pm 0.8	4.8 \pm 0.5*	0.58 \pm 0.06*	0.44 \pm 0.04*	2.7 \pm 0.2*
$\Delta atfA$ strain diamide	0.7 \pm 0.1*	8.1 \pm 1.2	4.6 \pm 0.4*	0.44 \pm 0.05*	0.43 \pm 0.04*	7.3 \pm 0.3

946

947 Mean \pm S.D. values are presented.

948 * - Significantly different from the value measured in the appropriate untreated cultures according to the Student's t-test ($p < 0.05$, $n = 3$).

949

950 **Legends to figures**

951

952 **Fig. 1** Correlation between microarray and RT-qPCR data in case of the control (A) and
953 the *ΔatfA* (B) strain.

954

955 **Fig. 2** Venn-diagram of stress responsive genes.

956 A: Distribution of stress responsive (up-regulated/down-regulated) genes among the 3
957 oxidative stresses in the control strain.

958 B: Distribution of stress responsive (up-regulated/down-regulated) genes among the 3
959 oxidative stresses in the *ΔatfA* strain.

960 C: Distribution of AtfA-dependent genes (showing up-regulation/down-regulation in the
961 control strain) according to their stress dependence lost in the mutant strain.

962 D: Distribution of co-regulated genes between the two strains.

963 Stress responsive, up-regulated, down-regulated, AtfA-dependent and co-regulated
964 genes are defined in the Materials and methods section.

965

966 **Fig. 3** Stress type dependence of “ribosome biogenesis” and “signal transduction”
967 genes.

968 A-B: Distribution of down-regulated “ribosome biogenesis” genes among the 3 stresses
969 in the control and the *ΔatfA* strain, respectively.

970 C-D: Distribution of up-regulated/down-regulated “signal transduction” genes among
971 the 3 stresses in the control and the *ΔatfA* strain, respectively.

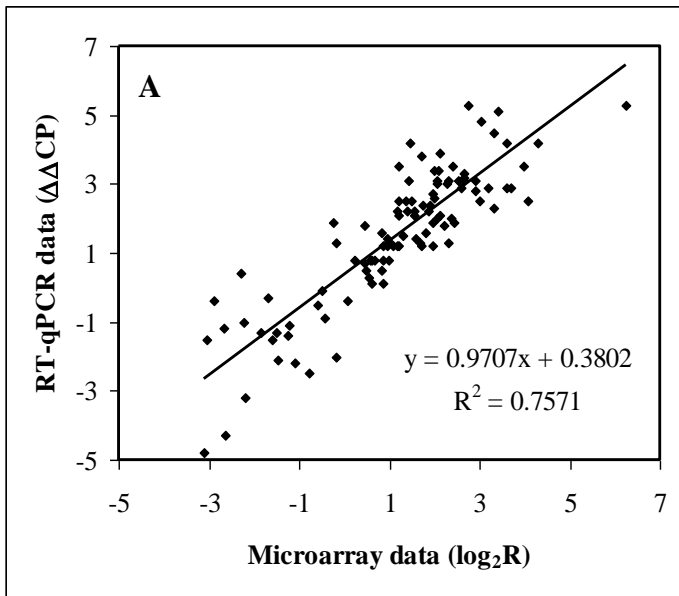
972 E: Distribution of AtfA-dependent “[signal transduction](#)” genes (showing up-
973 regulation/down-regulation in the control strain) according to their stress dependence
974 lost in the mutant strain.

975

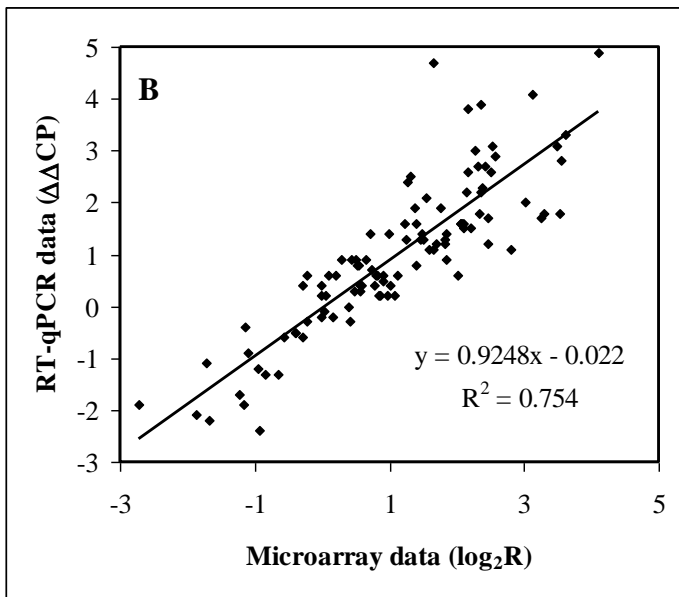
976

977 **Fig. 1**

978



979

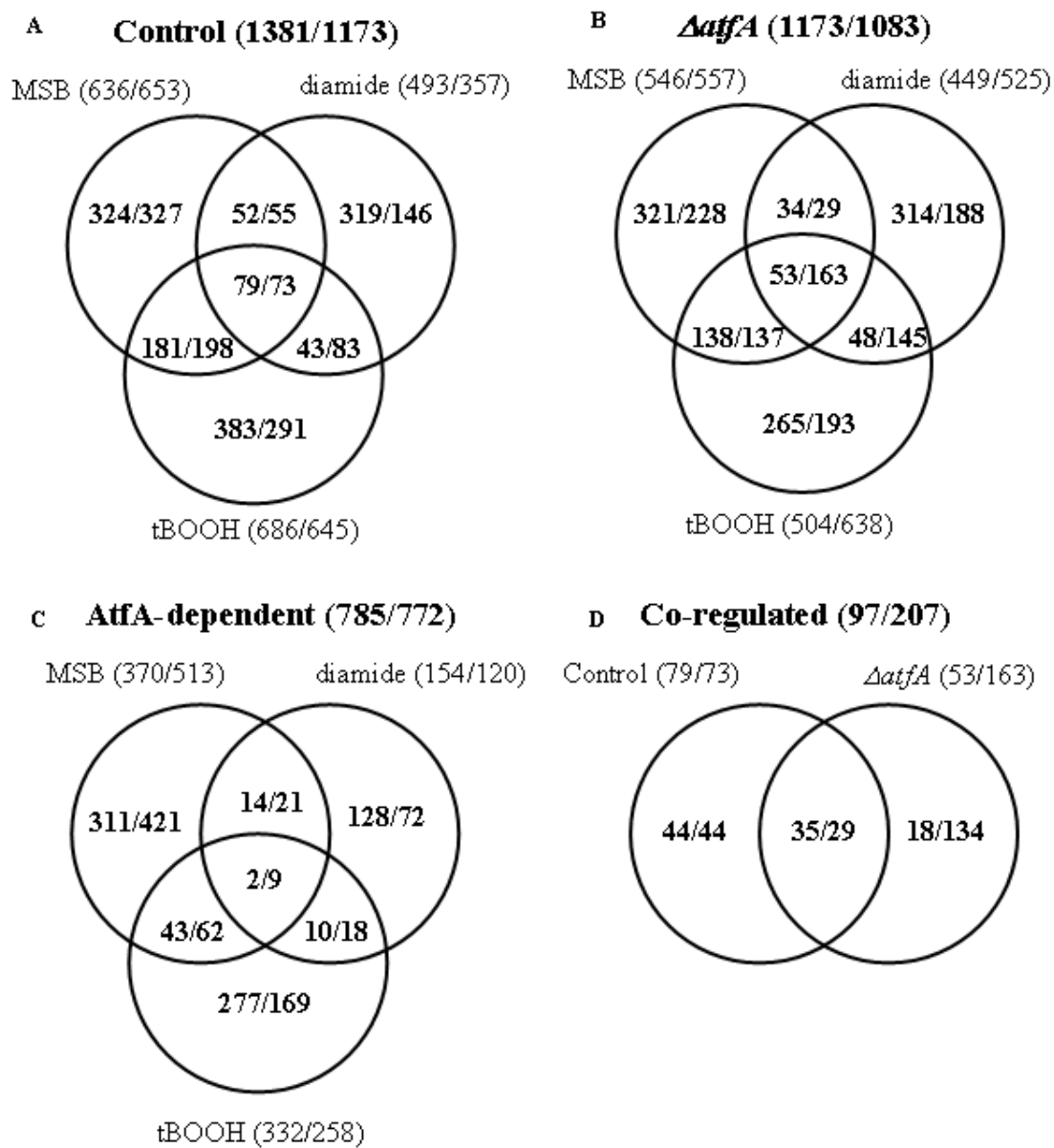


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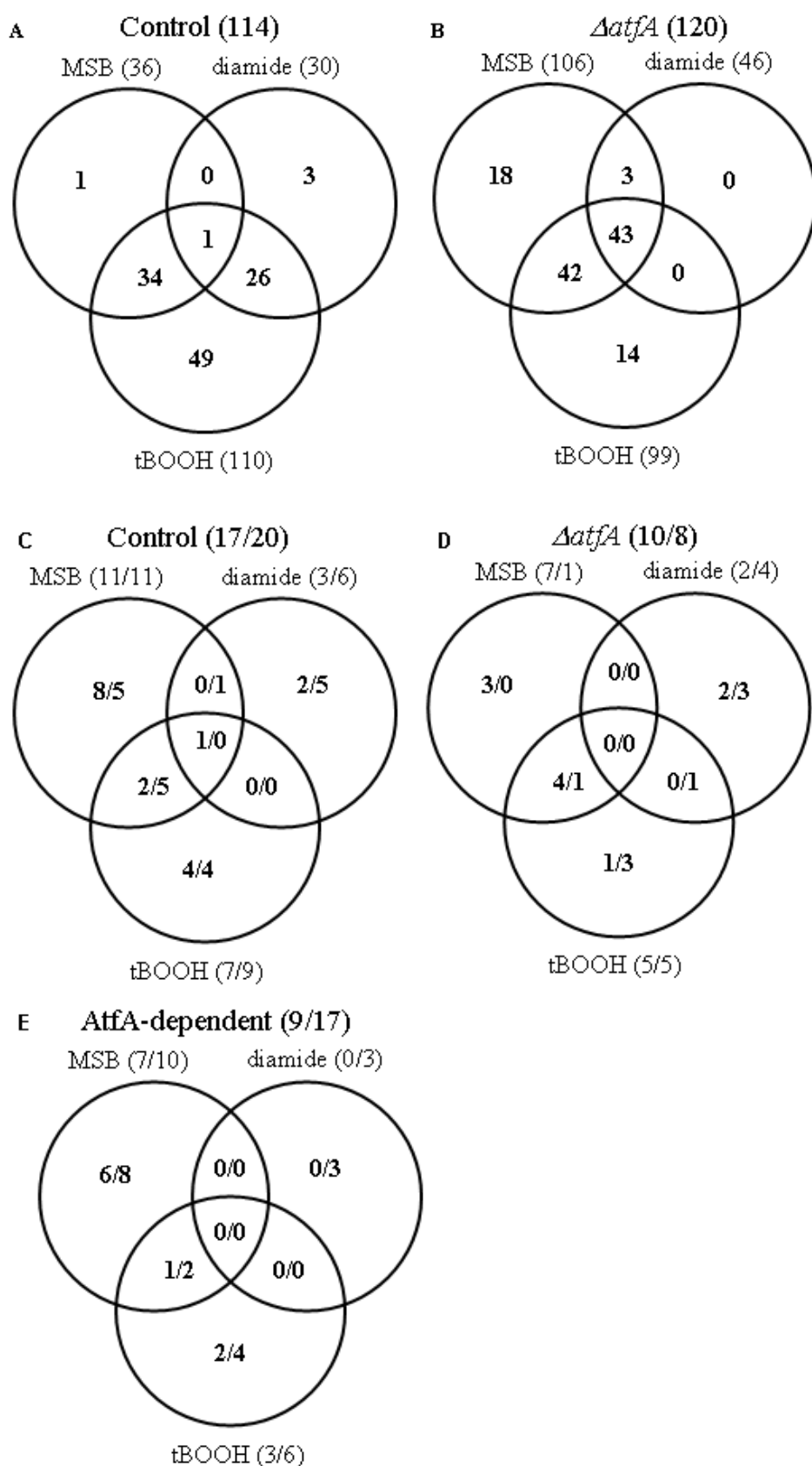
982 **Fig. 2**

983



984

985



990 **Supplementary materials**

991

992 **Supplementary Table 1** - Suppl Table 1.doc

993 List of primer pairs used in this study.

994

995 **Supplementary Table 2** - Suppl Table 2.xls

996 Number and stress responsiveness of genes showing altered regulation by deleting *atfA*.

997

998 **Supplementary Table 3** - Suppl Table 3.xls

999 Gene enrichment analysis of stress responsive genes.

1000 Sheet 1 - Control strain (up- and down-regulated gene groups)

1001 Sheet 2 - *ΔatfA* mutant strain (up- and down-regulated gene groups)

1002 Sheet 3 - AtfA-dependent genes (up- and down-regulated gene groups)

1003 AspGD Gene Ontology Term Finder ([http://www.aspergillusgenome.org/cgi-](http://www.aspergillusgenome.org/cgi-bin/GO/goTermFinder)

1004 [bin/GO/goTermFinder](http://www.aspergillusgenome.org/cgi-bin/GO/goTermFinder)) applying default settings and biological process ontology GO

1005 terms as well as the FunFun2 package (<https://elbe.hki-jena.de/fungifun/fungifun.php>),

1006 with default settings and FunCat categories were used. Only hits with p-value < 0.05

1007 were taken into consideration during the evaluation process.

1008

1009 **Supplementary Table 4** - Suppl Table 4.doc

1010 Selected significant shared GO, FunCat and KEGG pathway terms and their stress

1011 dependence under MSB, tBOOH or diamide induced stresses.

1012

1013 **Supplementary Table 5** - Suppl Table 5.xls

1014 Microarray data of genes belonging to selected gene groups.

1015 Composition of the gene groups are defined in the Materials and methods section.

1016 Microarray data are expressed as $\log_2 R$ values. R is equal to $SI_{\text{treated}}/SI_{\text{untreated}}$ and SI

1017 values stand for the normalized microarray signal intensities.

1018