PH.D. THESIS

INVESTIGATION OF THE PHYSIOLOGICAL ROLE OF HIGH MOBILITY GROUP PROTEINS IN THE MODEL ORGANISM *ASPERGILLUS NIDULANS*

Zoltán Karácsony

Supervisor: Dr. Zsuzsanna Hamari

Biology Ph.D. Program

University of Szeged
Faculty of Sciences and Informatics
Department of Microbiology

Szeged

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INTRODUCTION

Aspergillus nidulans is one of the most frequently studied fungal model organism. This fungus has asexual, sexual and parasexual life cycle, and a haploid genome, therefore both classic and reverse genetic methods can be easily applied. The whole genome sequence of this fungus is already known, the genetic manipulation had been solved for a long time and wide repertoire of auxotroph mutants are available.

Several biological processes were studied in the *A. nidulans* as a model organism e.g. the regulation of life cycle, asexual and sexual reproduction, the organisation of mitochondrial genome, intron-mobility, organelle-associated processes, membrane-transport mechanisms, stress responses, apoptosis, protein evolution, chromatin function, metabolic pathways and their regulation and synthesis pathways of medically or industrially important secondary metabolites.

The High Mobility Group protein family is part of the group of chromatin associated, non-histone like proteins. This protein family was subjected to intense researches in the last decades. They have widespread functions in lower eukaryotes, moreover they play important role in human (e.g. in innate immunity and tumor formation), therefore they have a medical impact too. An increasing number of studies examine the chromatin organisation and function of *A. nidulans*. In these the nucleosome positioning in promoter regions, the role of histone acetyltransferases, -methyltransferases, -demethylases, chromatin remodelling complexes, heterochromatin protein 1 and H1 histone in the organisation and function of chromatin are studied. The chromatin-associated non-histone like High Mobility Group proteins, including the HMG-box (HMGB) proteins were poorly studied in *A. nidulans* and not a single architectural HMGB protein was identified and characterized in this species. In contrast, the examination of these proteins in *Saccharomyces cerevisiae* and *Candida albicans* has been started decades before. The aim of our work was the identification of the chromatin architectural HMGB proteins and the study of their physiological role in order to extend the knowledge about both *A. nidulans* and HMGB proteins.

AIMS OF THE WORK

No HMGB protein was identified in the well studied filamentous fungal model organism *A. nidulans* despite of the fact that the chromatin function and organisation of this species is well characterized. Therefore our goal was the identification of the HMGB proteins and the study of their physiological role.

For this purpose, we planned *in silico* identification and characterization of putative HMGB proteins. To investigate the physiological function of these proteins we planned to use classic and reverese genetic tools and the use of the modern molecular methods (gene expression measurement; construction of substitution and GFP fusion cassettes and transformation vectors; carrying out gene deletion; investigation of localization of GFP-fused proteins; study of sexual and asexual reproductions; monitoring the carbon- and nitrogen source utilisation of deleted strains; examination of response of deleted strains against low pH-, heat-, oxidative- and osmotic stress; determination of secondary metabolite production of deleted strains; study the complementation ability of GFP- and histidine-tagged fusion proteins).

Since the sequence of HmbB protein carries both nuclear and mitochondrial signal sequences, we focused our work particularly on the study of the *hmbBΔ* strain, since no mitochondrial HMGB protein with dual localization was recorded in the literature before this.

METHODS

Classic microbiological methods

- Media and growth conditions for *A. nidulans*
- Separation of germinating and non-germinating conidia of *hmbBΔ* strain
- Media and growth conditions *Escherichia coli*
- Germination test of spores
- Carbon- and nitrogen source assimilation tests, investigation of stress tolerance

Bioinformatics

- Identification of localization signal
- Phylogenetic analysis
- Protein structure determination

Nucleic acid-based techniques

- Total DNS isolation from *A. nidulans*
- RNS isolation from *A. nidulans*, cDNS synthesis
- Cloning, design and development of constructions
- Polymerase Chain Reaction
- Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)
- Southern-hybridization

Genetic manipulation of *A. nidulans*

- Transformation of *A. nidulans* for gene deletion or the expression of fusion proteins
- Homo- and heterothallic crossing of *A. nidulans*

Analytical mmethods

- GC-MS analysis of sugar and polyalcohol content of conidia
- Thin layer chromatography of sterigmatocystin
- Thin layer chromatography of trehalose

Assay of reactive oxygen species (ROS) and ROS-scavenging enzymes and metabolites

- Glutathione reductase (GR) assay
- Glutathione peroxidase (GPx) assay
- Glutatione S-transferase (GST) assay
- Glucose-6-phosphate dehydrogenase (G6PD) assay
- Superoxide dismutase (SOD) assay
- Catalase (CAT) assay
- Superoxide assay
- Dichlorofluorescein-diacetate (DCFDA)-oxidizing capacity assay
- Reduced and oxidized glutathione (GSH and GSSG) assay
- NADP and NADPH determination
- Total thiol determination

Western blot analysis

Microscopic examinations

- Examination of localization of GFP-fused proteins
- Viability tests after metabolic staining

RESULTS

Identification of chromatin-associated HMG-box proteins in *A. nidulans*

Seven HMG-box carrying proteins were identified in the genome of *A. nidulans* by *in silico* (BLAST) search. For this purpose the sequence of HMG-box domain of human HMGB1 was used as a reference. These genes are AN2885, AN1267, AN10103, AN3667, AN4734, AN3549 and AN1962. Only the AN4734 was characterized as the MatA (MAT2) mating type protein, the other proteins are uncharacterized. The sequence-specific/non-specific DNA-bindig of the protein can be predicted by the polarity of the amino acid residue preceding the second α-helix of the HMG-box domain. If this amino acid is polar, the protein binds the DNA in a sequence-specific manner. Polar residue can be found in the case of the MatA (AN4734, Asn), AN1962 (Asn), AN3549 (Lys) and AN3667 (His). The amino acid is

non-polar in the case of AN2885 (Phe), AN1267 (Val) and AN10103 (Pro) proteins. According to the polarity of the unique amino acid and to the mono-domain structure, AN2885, AN1267 and AN10103 genes are regarded as non-sequence specific, architectural HMG-box proteins. The proteins coded by these genes were named HmbA, HmbB and HmbC according to the nomenclature of *A. nidulans*.

The HmbA is a short protein, which contains one HMG-box domain like the Nhp6A/B proteins of *S. cerevisiae*, which have an architectural function in chromatin organisation.

According to our examinations the HmbB and its orthologue, the *P. anserina* mtHMG1 proteins carry both a nuclear and a mitochondrial signal sequence on the N-terminus. The 3D modelling of HmbB and mtHMG1 revealed that besides the conserved HMG-box domain, these proteins contain two HMG-boxes which can not be identified by sequence homology. These domains were named as „Shadow-HMG-box"-es.

The HmbC contains two HMG-boxes. According to *in silico* examinations, the closest characterized homologues of HmbC are the Hmo1 (*S.cerevisiae*) and the Sp-Hmo1 (*S. pombe*). These proteins (along with *S. cerevisiae* Hmo2) carry a somewhat conserved HMG-box domain on the N-terminus, and a considerably conserved HMG-box on the C-terminus, which is followed by a polar sequence. This structure was found in the case of HmbC. The N-teminal HMG-box of *S. cerevisiae* Hmo1 shows a dimerization function, nevertheless in the Hmo2 (paralogue of Hmo1) this domain binds DNA. The Hmo1 can act several ways e.g. it stimulates the Pol-I transcription activity in nucleolus. The Hmo2 binds to the double strand break sites, and as a component of INO80 complex, takes part in double strand break repair.

The physiological role of HmbA

By the comparison of *hmbAΔ* and *hmbA*$^{+}$ strains, we started to study the physiological function of HmbA protein. The growth rate of the deleted strain decreased significantly on MM medium containing glucose as carbon- and sodium nitrate as nitrogen source. The application of osmo-protectant agent (1 M sorbitol) had a curing effect and in that it increased the growth rate considerably. This result suggests that this phenotype may be related to deficiency in cell wall synthesis.

The *hmbAΔ* strain deficient in adaptation to acidic environment (pH 2.2). This phenotype can be partially complemented by supplementing the media with the osmo-

protectant (0.7 M KCl), which suggests that this phenotype may related to defective cell wall synthesis.

The deletion of *hmbA* resulted in the strong reduction of sterigmatocystin production and the stress-protectant agent trehalose in the mycelia, according to thin layer chromatography experiments.

The structure and localization of HmbB (Karácsony et al. 2014)

The HmbB protein shows some new structural and functional features. The gene coding this protein is present in almost the whole Pezizomycotina subphylum and absent in the Saccharomycotina group. Thus the ancestral gene of these proteins could be originated in the common ancestor of Taphrinomycotina and Pezizomycotina groups (Karácsony et al. 2014). The absence of the protein in the Saccharomycotina group may be explained also by its disappearance.

Our Western blot experiments showed that the mitochondrial localization of HmbB implies the proteolytic cleavage of the protein during the transport to the mitochondrial matrix. This proteolytic cleavage cut in the N-terminal ShHMG-box, however two HMG-boxes are sufficient for the bending of mtDNA. Following the cleavage of MTS (*Matrix Targeting Sequence*) two complete HMG-boxes, and two N-terminal alpha-helices with unknown function remain on HmbB protein. The truncated domain contains a nuclear signal sequence too.

According to our microscopic examinations, both the N-terminal (GFP-HmbB) and C-terminal (HmbB-GFP) GFP-fused HmB proteins were observed in the mitochondria, in all investigated developmental stages of the fungus. The fusion proteins were occasionally observed in the nuclei. We hypothesize that the dual localization of HmbB is based on the cleavage or non-cleavage of the MTS, which results in mitochondrial and nuclear isoforms. The intriguing observation, that that nuclear localization is limited to distinct hyphal comopartments suggests the presence of functionally specialized mycelial cells. This assumption needs further experiments to be proved.

The expression of the gene coding for HmbB (Karácsony et al. 2014)

We examined the expression of *hmbB* mRNA after 3, 8, 22, 36 and 48 hours of cultivation. The mRNA was present in large amount in the dormant conidia. The amount of

mRNA was decreased in the first 30 min of germination and increased again at the end of the isotropic growth phase (3 hours). This increasement continues during the mycelial growth phase (8, 22, 36 and 48 hours).

The role of HmbB in the establisment of normal cell morphology (Karácsony et al. 2014)

The deletion of *hmbB* resulted in the distortion of hyphal morphology. Round protrusions were formed on the mycelia of the deleted strain. This phenomenon may be explained by the defective synthesis of cell wall components or by the disfunction of signaling pathways which regulate cell wall integrity.

The role of HmbB in carbon- and nitrogen source assimilation and stress tolerance (Karácsony et al. 2014)

The growth rate of *hmbBΔ* was examined in media containig different carbon- and nitrogen sources. The deleted strain showed decreased growth compared to *hmbB*$^+$ in the case of galactose (-27%, $p<0.005$), lactose (-21%, $p<0.005$) and raffionose (-17%, $p<0.005$) carbon sources. The *hmbBΔ* strain showed decreased growth rate compared to wild type in the case of ammonium (-13%, $p<0.0005$) among the examined nitrogen sources.

We studied the stress tolerance of mutant strains against the following stresses: osmotic stress (1 M NaCl), heat stress (42 °C) and oxidative stress (0.4 mM menadione and 1.8 mM diamide). As the result of the deletion, the growth rate was slightly decreased under heat stress (-23%, $p<0.05$) and drastically decreased in the presence of 0.4 mM menadione (-85%, $p<0.0005$). We observed moderate resistance against diamide in the case of the deleted strains. As the result of the detailed examination of oxidative stress tolerance we found that the MIC_{50} for menadion was between 0.2 and 0.4 mM in the case of *hmbB*$^+$ and HZS.352 (reconstitution control) strains, while this value was between 0.05 and 0.01 mM in the case of *hmbBΔ* strains. The *hmbBΔ* strains showed increased tolerance against diamide at all examined concentrations, moreover 0.05 and 0.1 mM diamide slightly resultedd in increased (+9% and +12%, $p<0.05$ and $p<0.005$) growth rate. This result suggests that in the absence of HmbB the redox state of the cells was changed in a way which can be partially complemented by the oxidation of GSH (reduced glutathione) by diamide.

The effect of HmbB on the redox state (Karácsony et al. 2014)

Whilst the total glutathione level did not change in *hmbBΔ* conidia compared with wild type, we observed 20 times lower GSSG (oxidized glutathione) content in this strain, which indicates that both the reduction potential and the reducing capacity of the glutathione redox couple are markedly different from *hmbB*$^{+}$. Together with the observed lower levels of ROS (reactive oxygen species) content and SOD (superoxide dismutase) activity, we conclude that *hmbBΔ* conidia are in a highly reductive redox state. We propose that the highly reductive redox state of *hmbBΔ* conidia may be a contributing to the defective germination of sexual and asexual spores.

The higher GSSG content in *hmbBΔ* mycelia in comparison with *hmbB*$^{+}$ reflects a shift of the glutathione redox couple in the mutant toward an oxidative redox state, which is only partially compensated by the elevated level of GSH and thus by the increase of the reducing capacity of the redox couple. However, the detected shift in the glutathione redox system towards an oxidative redox state, the doubled superoxide content and the doubled SOD activity together indicate that *hmbBΔ* mycelia are subject to an endogenous oxidative strain. We hypothesize that the paradoxical menadione-sensitive and diamide-resistant phenotype of the *hmbBΔ* strain is due to the changes in the glutathione redox system and the endogenous oxidative stress.

During menadione treatment, the total glutathione level of *hmbBΔ* mycelia decreased by half, similar to that of *hmbB*$^{+}$ mycelia; however, the GSSG level unexpectedly remained unchanged in the mutant. Importantly, the GSSG level of the *hmbB*$^{+}$ mycelia decreased by 4.2-fold, which could be a compensatory mechanism for maintaining the redox potential of the glutathione redox couple. This compensatory mechanism is clearly dysfunctional in *hmbBΔ* mycelia, resulting in the shift of the glutathione redox couple toward a more oxidative redox state. The proposed higher oxidative strain in the mutant mycelia upon menadione treatment is also supported by experimental evidence. The G6PD (glucose-6-phosphate dehydrogenase) activity dropped to a 1.6 times lower rate, despite some components of the glutathione redox cycle being hyperactivated (8.5 times increase in GR (glutathione reductase) and GST (glutathione S-transferase) activities, 5.3 times increase in GPx (glutathione peroxidase) activity). As G6PD is responsible for the reduction of NADP to NADPH, with the latter being the thermodynamic driving force of the glutathione redox systems by maintaining the redox cycle, the G6PD activity may become a limiting factor in

the mutant mycelia. The decrease of the G6PD activity led to a reduced glutathione redox cycle capacity and therefore the *hmbBΔ* cells failed to efficiently replenish the reducing power.

Diamide exerts its biological effect mainly through the direct depletion of the GSH pool, thus changing the redox capacity of the glutathione redox couple. The observed resistance of the *hmbBΔ* mycelia upon diamide treatment may be related to its GSH content, which is remarkably kept at a steady level in this mutant, but severely depleted in *hmbB*$^{+}$ mycelia. We propose that the elevated GSH production, which is possibly a result of a compensatory response to the elevated GSSG level in the untreated *hmbBΔ* mycelia, is responsible for the diamide-resistant phenotype of the mutant.

The comparison of the NADPH and NADP content of swelling conidia and mycelia of *hmbB*$^{+}$ and *hmbBΔ* strains in the context of the glutathione recycling process revealed that the NADPH contents of *hmbBΔ* conidia and mycelia were paradoxical in the face of the observed NADP contents and G6PD activities. In conidia, the NADPH content was the same in *hmbB*$^{+}$ and *hmbBΔ* strains; however, the NADP level was 7.9 times higher and the G6PD activity was 4.4 times lower than in *hmbB*$^{+}$.

The NADPH level correlated well with the GSSG and GSH content and GR activity, supporting that the intracellular environment in conidia is reductive. Remarkably, we found that a wild type-like NADPH level was maintained in the swelling conidia, despite the G6PD activity being considerably lower. The most obvious explanations for this phenomenon may be the upregulation of metabolic processes other than the pentose phosphate pathway, which result in NADPH production [e.g. malic enzyme route, isocitrate route, glutamate route or the recently discovered 10-formyl-tetrahydrofolate pathway], and the enhancement of the mitochondria-dependent reduction of NADP by transhydrogenases and NADH. The opposite was observed in *hmbBΔ* mycelia. There, despite the 3.2 times higher level of NADP content and 3.5 times increase of the G6PD activity, the NADPH content was 1.4 times lower than in *hmbB*$^{+}$ mycelia. The depletion of the NADPH level contributes to the oxidative intracellular environment in *hmbBΔ* mycelia in conjunction with the high accumulation of GSSG and non-sufficient increase in GSH level. The reasons for the NADPH leakage may include the enhancement of NADPH-requiring metabolic processes and/or the mitochondrial P450 systems, which reduce oxygen by a single electron to produce the harmful superoxide radical. In fact, the superoxide level in the *hmbBΔ* mycelia was elevated in comparison with *hmbB*$^{+}$

and the source of the superoxide production is likely to be other than respiration as respiration of *hmbBΔ* mycelia is indistinguishable from that of *hmbB*$^{+}$. These observations may support the involvement of the P450 system in superoxide production and explain the unexpectedly low level of NADPH in *hmbBΔ* mycelia.

The transcription profiles of genes identified in high throughput transcriptomic/proteomic studies on menadione-treated mycelia changed significantly in *hmbBΔ* mycelia. Total GST activity was nearly doubled in *hmbBΔ* mycelia and we found that transcription levels of all the known or predicted GST-coding genes, except one (*gstB*), were upregulated in the *hmbBΔ* strain.

All the studied known or predicted glutaredoxins were upregulated in *hmbBΔ* mycelia. As glutaredoxins are powerful GSH-dependent disulfide reductants, it is reasonable to suggest that the elevated intracellular level of GSSG in *hmbBΔ* mycelia correlates with the upregulation of glutaredoxin genes. Moreover, the upregulation of glutaredoxin genes could indicates that the intracellular oxidative stress affects the intracellular redox-sensitive proteins (proteins with thiol groups) in *hmbBΔ* mycelia.

Interestingly, the transcription of those predicted GST and glutaredoxin genes with unknown physiological roles, which were chosen randomly from the AspGD database (AN0815, AN2948, AN5831, AN6158 and AN4215), showed significant upregulation in *hmbBΔ* mycelia. These results indicate that these genes are involved in the functioning of the glutathione couple system and in protection against oxidative damage, therefore being potentially interesting candidates for future studies of redox biological processes.

The total GR activity did not differ in *hmbBΔ* and *hmbB*$^{+}$ mycelia, although we measured a 2.8 times higher mRNA level of *glrA*. Either the elevated transcription activity does not result in a comparable increase of protein level and/or other factors (other GRs, post-translational modifications) could influence the GR activity.

The total SOD activity was doubled in *hmbBΔ* mycelia and we found that *sodA* expression showed a 2.6-fold higher level in the mutant mycelia in comparison with *hmbB*$^{+}$, whilst changes in *sodB* and *sodM* expression were not significant. We suggest that SodA is a dominant responsive protein upon the observed endogenous intracellular oxidative stress in the *hmbBΔ* mycelia.

As respiration is accompanied by the continuous production of harmful ROS, we hypothesized that the quantitative differences in the superoxide content between *hmbB*$^{+}$ and *hmbBΔ* samples (conidia and mycelia) are due to an alteration in the respiratory activity. Unexpectedly, the respiration of conidia and mycelia was very similar in the mutant and wild type. We propose that either HmbB functions do not influence respiratory activity or, if they do, this is compensated for by the action of other proteins. The fact that in spite of this phenomenon the absence of HmbB causes elevated superoxid level in mycelia, which may be explained by the increased operation of non-respiratory chain linked, superoxide producing systems (e.g. cytochrome P450 system).

The role of HmbB in the germination of sexual and asexual spores (Karácsony et al. 2014)

From the several mitochondria-related pleiotropic phenotypes (mycelial growth, morphology, conidial metabolism, tolerance against oxidative stress) observed in the case of *hmbBΔ* the most striking is the crucial importance of HmbB for both ascospore and conidiospore viability. The altered metabolism of sugars and polyalcohols in the absence of HmbB also suggests the disorder of conidiogenesis and germination in the deleted strain. Moreover the mtDNA content is greatly decreased in the deleted conidia, which correlates with the decreased germination ability (Karácsony et al. 2014). Thus a causal link between the absence of mitochondrial DNA and the inability of conidia to germinate seems reasonable. Conidia of *hmbB*Δ strains do not lack mitochondria. In fact they are metabolically active and have active mitochondria, as shown by staining with both MTT and mitomarker and respiration data. We can conclude that HmbB is crucial for DNA to be inserted in the mitochondria of mature conidia. The discrepancy between the levels of mtDNA and mtRNA transcripts in swelling conidia may be due to transcription occurring before the partition of mtDNA during conidiogenesis and its subsequent accumulation in the mature conidia. The distribution of HmbB-GFP in conidiophores suggests a succession of events, which could account for mtDNA loss during conidiogenesis in *hmbB*Δ strains. There is strong HmbB-GFP fluorescence in the vesicle and in the phialide, where we also see an accumulation of mitochondria. It is not unreasonable to suppose that during the process of conidia budding from the phialide, mtDNA is being replicated in the mitochondria included in the conidium and that HmbB is crucial for this process. There is no work extant on the fate of mitochondria during conidiogenesis in any species of *Aspergillus* except from some early electron microscopy work, which does not address specifically this issue but visualised mitochondria in conidia and

confirmed the accumulation of mitochondria in the vesicle. Nuclear division during conidial budding from phialide has been compared to that occurring in budding in *S. cerevisiæ*. We have established that the conidial viability *hmbB*Δ phenotype is autonomous, i.e., it cannot be complemented in heterokaryons (Karácsony et al. 2014). This is not surprising, as already vesicles of conidiating heterokaryons are almost without exception homokaryotic, and metula and phialide are necessarily so, the latter being actually uni-nucleate. The results are consistent with mitochondrial DNA segregation during conidiogenesis, but cannot establish when during conidiogenesis this process happens.

The role of HmbB on ascospore viability is analogous to its role in conidia. However, neither of the two HmbB-GFP fusions tested complement the loss of ascospore viability and consistently with this, HmbB-GFP is mostly absent in both asci and mature ascospores. It seems that tagging with GFP is incompatible with the presence of HmbB in ascospore mitochondria, the Hmb-GFP fusions behaving in this respect exactly as the HmbB deletion. This result, even if disturbing in the short run, points to a crucial difference in mitochondrial partition between the asexual and the sexual cycles.

The role of HmbB in sterigmatocystin biosynthesis (Karácsony et al. 2014)

We compared the secondary metabolite production of *hmbBΔ* and wild-type strains by thin layer chromatography. We found that deleted strain shows drastically decreased sterigmatocystin production, and the complete absence of the mycotoxin is can not be excluded (Karácsony et al. 2014). In the case of the histidine-tagged or the GFP-tagged HmbB expressing strains the sterigmatocystin production was re-established. To reveal the backround of the decreased sterigmatocystin production of *hmbBΔ*, we examined the expression of some genes of the sterigmatocystin gene cluster (*aflR* cluster-specific transcription factor, *stcO* ketoreductase and *stcU* oxidoreductase) by qRT-PCR, using the „*standard curve*" method. In the *hmbBΔ* strain all examined genes showed significantly increased expression. The results are surprising and do not provide a rationale for the impairment in sterigmatocystin biosynthesis. According to these results it can be proclaimed that the HmbB represses the transcription of the examined genes, and it can act positively on sterigmatocystin biosynthesis by an independent way.

Characterization of *hmbCΔ* strain

The routine examinations which were used in the case of *hmbAΔ* and *hmbBΔ* strains, did not reveal any phenotype for the *hmbCΔ* strains. Further investigations are needed to be done to identify the functions of HmbC.

SUMMARY

1. We identified 3 architectural HMG-box proteins (HmbA, HmbB and HmbC) in the genome of *A. nidulans*. Deletion strains were obtained by the deletion of the coding genes of these proteins and their physiological role were studied.

2. According to our results, the HmbA has a role in the maintenance of normal growth rate, in the adaptation to acidic pH, and in the synthesis of sterigmatocystin and trehalose.

3. The 3D structural studies of HmbB revealed two additional N-terminal HMG-box domains (which can not be identified by sequence homology) besides the conserved C-terminal HMG-box domain. The revealed domains were called as „Shadow-HMG-box"-es. The dual (mitochondrial and nuclear) localization of HmbB was proved by the use of GFP-fused HmbB protein expressing strains. The N-terminal cleavage of the HmbB protein was proved by Western blot experiments. The uncommon appearance of HmbB in nuclei suggests the presence of functionally specialized hyphal compartments in this fungus.

4. In the absence of HmbB the spore production and germination ability was drastically decreased in case of both sexual and asexual spores. Parallel with the decreased germination ability, the mtDNA copy number was decreased significantly in the *hmbBΔ* conidia. Despite of the lowered mtDNA content, the deleted conidia showed a wild-type like number of functioning mitochondria. The complementation experiments with the GFP-fused HmbB proteins pointed out that the segregation of mtDNA operates through different processes in conidia and ascospores.

5. The *hmbBΔ* strain was proved to be sensitive to menadione and tolerant to diamide oxidative stress agents. We propose that the endogenous oxidative stress of *hmbBΔ* mycelia, and the altered function of the components of glutathione regenerating cycle are the main reasons of the change in oxidative stress tolerance.

6. We proved that in the absence of HmbB *A. nidulans* can not produce sterigmatocystin despite of the increased transcription of the synthesis and regulator genes. The contradiction between the sterigmatocystin production and the expression data suggests, that the synthesis of this mycotoxin is regulated not only

at the level of transcription, but at post-transcriptional (translation/post-translation) level or by the regulation of the factors involved in the biosynthesis.

LIST OF PUBLICATIONS

Papers connected with the Ph.D. thesis :

Karácsony, Z., A. Gácser, C. Vágvölgyi, C. Scazzocchio, Z. Hamari, (2014) A dually located multi-HMG-box protein of *Aspergillus nidulans* has a crucial role in conidial and ascospore germination. *Mol. Microbiol.* **94:** 383-402. **IF.: 4.419**

Karácsony, Z., A. Gácser, C. Vágvölgyi, Z. Hamari, (2015) Further characterization of the role of the mitochondrial high-mobility group box protein in the intracellular redox environment of *Aspergillus nidulans*. *Microbiology.* **161:** 1897-1908. **IF.: 2.557**

Other papers:

Galgóczy, L., L. Kovács, **Z. Karácsony,** M. Virágh, Z. Hamari, C. Vágvölgyi (2013) Investigation of the antimicrobial effect of Neosartorya fischeri antifungal protein (NFAP) after heterologous expression in *Aspergillus nidulans*. *Microbiology.* **159:** 411-419. **IF.: 2.835**

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