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Outline of Research Result

Molecular mechanism of stress tolerance in conidia of *Aspergillus* species

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Research aims

Conidia are stress-resistant spores that are produced by many fungal species, including the genera *Aspergillus* and *Penicillium*. The ATF/CREB-type transcription factor AtfA has been characterized as a regulator of conidia stress tolerance in *A. nidulans* and *A. oryzae*.^{1,2)} Nonetheless, the role of ATF-type transcription factors in the pathogenic fungus *A. fumigatus* remains unclear. The conidium is an infectious agent that is transmitted to the human lungs from environmental niches. Therefore, a better understanding of the molecular mechanisms that regulate conidia stress tolerance would be beneficial, both for the prevention of aspergillosis and the development of new antifungals. In this study, we constructed an *A. fumigatus* $\Delta atfA$ deletion strain to characterize the role of AtfA in conidia stress tolerance.

Methods

A. fumigatus strain AfS35 (FGSC A1159) (*akuA::loxP*) was utilized to generate the following deletion strains used in this study: $\Delta atfA$, $\Delta saka$, $\Delta mpkC$, and $\Delta saka$, $\Delta mpkC$. For heat stress assays, diluted conidia suspensions were incubated at 55°C. For oxidative stress assays, H₂O₂ (Final 100 mM) was added to conidia suspensions. After 15 min, the conidia were appropriately diluted and spread onto normal medium agar. After 24 h, the number of colonies was counted. The polyol and trehalose contents of the conidia were measured by HPLC. To determine gene expression levels, quantitative real-time PCR (qRT-PCR) analysis was performed using the Life Technology 7300 system with SYBR Green detection. To obtain transcriptomes, Illumina Miseq and CLC Genomics Workbench were used.

Results

1. The role of AtfA in the stress resistance of *A. fumigatus* conidia

The conidia of the *atfA* deletion mutant (named $\Delta atfA$) exhibited remarkable sensitivity to both heat stress and oxidative stress, indicating that AtfA plays an indispensable role in the stress resistance of conidia. Carbohydrate quantification revealed that trehalose was more abundant in *A. fumigatus* conidia than other carbohydrates such as mannitol, arabinol, erythritol, and glycerol. In contrast, there was

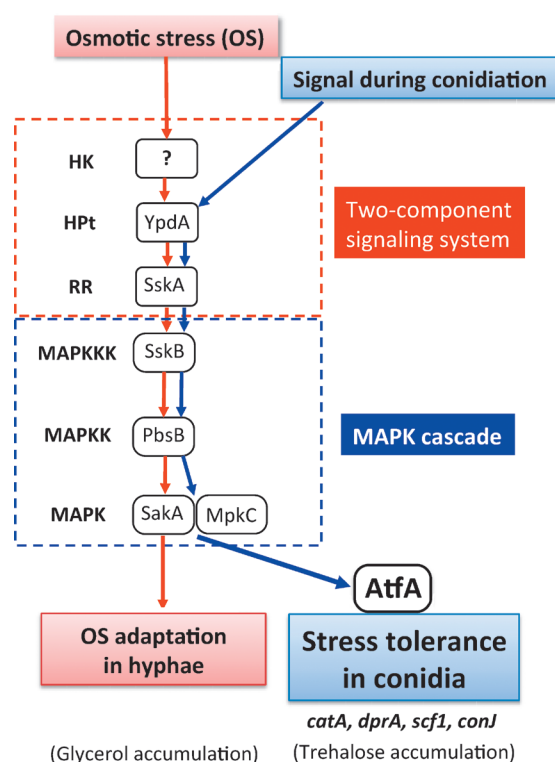


Fig. 1. The HOG pathway of *A. fumigatus*

significantly less trehalose in the conidia of the $\Delta atfA$ strain, suggesting that AtfA is involved in trehalose accumulation in *A. fumigatus* conidia. It was suggested that this decrease in trehalose levels results in the heat stress-sensitive phenotype of the $\Delta atfA$ conidia.

2. Transcriptome analysis of the asexual developmental stage of *A. fumigatus*

To gain a greater insight into the role of AtfA in conidia stress tolerance, we compared the transcriptomes of the WT and $\Delta atfA$ strains at the conidiation stage. We found that 738 genes were markedly induced at the conidiation stage. Of these, 54 were AtfA-dependent, including the genes that encode the conidia-specific catalase CatA, the dehydrin-like protein DprA, the HSP9/12 family protein Scf1, and the conidiation-specific protein ConJ. Because CatA and DprA were previously shown to be involved in the oxida-

tive stress resistance of conidia,^{3, 4)} the impaired expression of these genes during conidiation in the $\Delta atfA$ strain might be the cause of the observed sensitivity to oxidative stress.

3. The High Osmolarity Glycerol (HOG) pathway is involved in the stress tolerance of conidia

We questioned whether the *A. fumigatus* SakA MAP kinase is required for the stress tolerance of the conidia. Unexpectedly, conidia of the $\Delta sakA$ strain exhibited a level of stress tolerance comparable to that of the WT strain. Because *A. fumigatus* also encodes MpkC, a paralog of the SakA MAP kinase, we tested whether this protein confers stress tolerance to conidia in the presence or absence of SakA. Whereas the conidia of the $\Delta mpkC$ strain exhibited WT levels of tolerance to oxidative and heat stresses, the conidia of the $\Delta sakA, mpkC$ strain were sensitive to both stresses, suggesting that SakA and MpkC played a redundant or cooperative role in the conidial stress response.

Conclusion

In this study, we demonstrated the following points concerning the stress tolerance of *A. fumigatus* conidia: (1) trehalose is the predominant compatible solute; (2) the transcription factor AtfA governs the stress-tolerance of conidia; (3) the HOG pathway and, more specifically, (4) the SakA and MpkC MAPKs are required for conidia stress resistance.

References

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