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Screening for small molecules that activate fungal secondary metabolite production

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Research aims

Recent progress in fungal genome sequencing indicates fungi have a greater potential to biosynthesize secondary metabolites (SMs) than that speculated based on number of SMs isolated so far. Genes involved in SM biosynthesis in filamentous fungi are clustered in their genome, and their regulation is coordinated by complex regulatory systems consisting of global regulators and pathway-specific regulators.[1] Pathway-specific regulators, including C6-type transcription factors, have been considered as valid starting points for studying cryptic SM gene clusters.

The 120-kb subtelomeric region of chromosome 8p in *Aspergillus fumigatus* is responsible for three biosynthetic pathways of bioactive SMs, fumitremorgins,[2,3] fumagillin,[4] and pseurotin A[5] (Fig. 1). While more than 60% of the genes found in this region were predicted to be involved in SM biosynthesis, there is only one regulatory gene, *AFUA_8G00420*, in this region. However, it is unclear whether *AFUA_8G00420* regulates the biosynthesis of fumagillin or other SMs such as pseurotins and fumitremorgins. In this study, we studied the roles of *AFUA_8G00420* in SM production in *A. fumigatus*, and we constructed a reporter system to screen small molecules that activate fungal SM production.

Methods

To examine the roles of *AFUA_8G00420* in SM production in *A. fumigatus*, a knockout mutant (Δ*420*) was generated by replacing the entire coding region of the gene with a hygromycin B-resistance gene cassette. The fungal strains were cultivated in complete (0.5% yeast extract, 0.5% malt extract, 1% glucose, and 1% soluble starch) or glucose minimum medium at 28°C for 24 and 48 h to analyze their gene expression and metabolite production profiles. Expression analysis was carried out by RT-PCR. Ethyl acetate extracts of fungal cultures were analyzed by LC/MS.

Results

We compared SM gene expression in Δ*420* mutant with its parental wild-type strain. Expression of some of the *fma* genes such as *fmaAT* and *AFUA_8G00500* was lower in Δ*420* mutant, apparently because *AFUA_8G00420* was included in the fumagillin biosynthetic gene (*fma*) cluster. In-

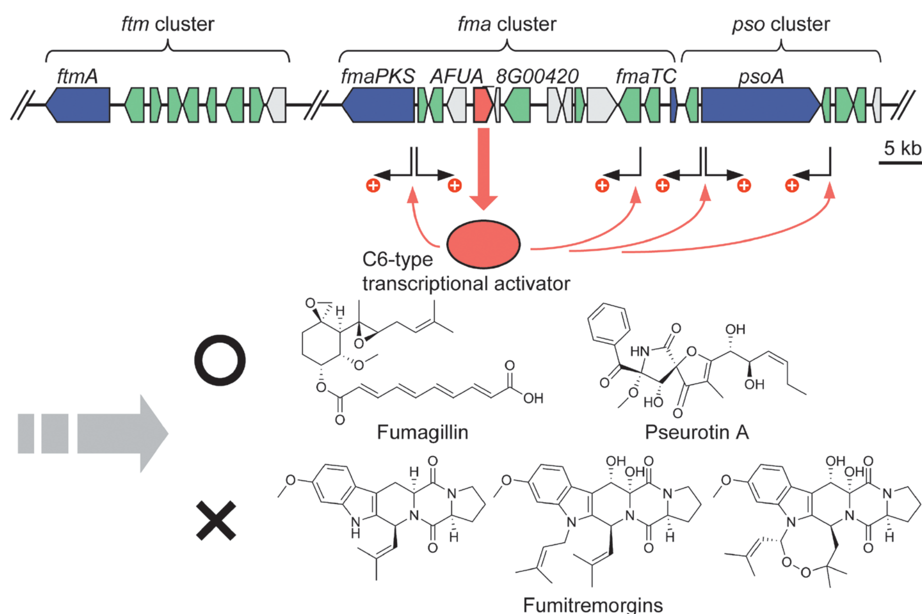

Fig. 1. Biosynthetic gene clusters for fumitremorgins, pseurotins, and fumagillin, located at the 120-kb region of chromosome 8p of *A. fumigatus*, and a pathway-specific regulator that controls the production of fumagillin and pseurotin.

terestingly, the mutant also showed lower expression of some *pso* genes than did the wild-type strain. We found no significant difference in *ftm* gene expression between the wild-type and Δ*420* mutant strains. To determine whether Δ*420* mutant strain produces fumitremorgins but not fumagillin and pseurotins, metabolite profiles of the *A. fumigatus* mutants were compared with those of the wild-type strains. The deletion of *AFUA_8G00420* caused a loss of production of fumagillin and pseurotin A, but not fumitremorgins, clearly demonstrating that *AFUA_8G00420* is involved in the regulation of the biosynthetic pathways of fumagillin and pseurotin A. These results agreed well with the expression profiling results of Δ*420* mutant.

Based on the results described above, a reporter system for the small molecule screen was constructed by fusing a reporter gene to the promoter regions of *AFUA_8G00420*-dependent, genes *fmaAT* and *AFUA_8G00530*.

Conclusion

Gene expression and metabolite production profiling of the knockout mutant of *AFUA_8G00420* demonstrated that the gene plays a role in the regulation of fumagillin and pseurotins, but is unlikely to be involved in fumitremorgin production in *A. fumigatus*. To the best of our knowledge, this is the first report of a C6-type transcriptional activator that regulates two discrete SM biosynthetic pathways in a filamentous fungus. Better understanding of the underlying mechanism of the unique crosstalk will allow us to exploit cryptic SM gene clusters through this family of transcription factors. Currently, we are employing this regulatory system and developing a screening system for small molecules that control fungal SM production.

References

1. Brakhage, A. A. (2013) Regulation of fungal secondary metabolism. *Nat Rev Microbiol* **11**: 21–32.
2. Li, S. M. (2011) Genome mining and biosynthesis of fumitremorgin-type alkaloids in ascomycetes. *J Antibiot* (Tokyo) **64**: 45–49.
3. Kato, N., *et al*. (2009) Identification of cytochrome P450s required for fumitremorgin biosynthesis in *Aspergillus fumigatus*. *Chembiochem* **10**: 920–928.
4. Lin, H. C., Chooi, Y. H., Dhingra, S., Xu, W., Calvo, A. M., and Tang, Y. (2013) The fumagillin biosynthetic gene cluster in *Aspergillus fumigatus* encodes a cryptic terpene cyclase involved in the formation of *β-trans*-bergamotene. *J Am Chem Soc* **135**: 4616–4619.
5. Maiya, S., Grundmann, A., Li, X., Li, S. M., and Turner, G. (2007) Identification of a hybrid PKS/NRPS required for pseurotin A biosynthesis in the human pathogen *Aspergillus fumigatus*. *Chembiochem* **8**: 1736–1743.