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Aspergillus nidulans における転写因子の融合タンパクを用いた休眠二次代謝遺伝子の活性化

糸状菌は多様な二次代謝産物を生産することが知られている。しかしながら、ほとんどの生合成遺伝子は実験室条件下では休眠状態であり、生産物が解明されていない。生合成遺伝子を活性化するアプローチの一つとして、遺伝子クラスターに含まれる Zn(II)2Cys6 型転写因子の発現量を増加させる方法があるが、この手法では、ごく一部の成功例を除き、二次代謝産物の生産量は変化せず失敗することが多い。そこで著者らは Zn(II)2Cys6 型転写因子のドメインを組み換えた融合タンパクを作製することで、二次代謝産物生合成遺伝子クラスターを活性化できないか試みた。Zn(II)2Cys6 型転写因子は、DNA 結合ドメインと転写活性化ドメインの二つのドメインを含んでいる。著者らは、生産物未知の遺伝子クラスターに含まれる転写因子の、転写活性化ドメインを組み換えることで、(+)-Asperlin の生合成遺伝子クラスターの同定に成功しており、Zn(II)2Cys6 型転写因子の融合タンパクによる休眠遺伝子の活性化が可能であることが示唆された。

紹介論文

Hybrid Transcription Factor Engineering Activates the Silent Secondary Metabolite Gene Cluster for (+)-Asperlin in Aspergillus nidulans

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要旨

Fungi are a major source of valuable bioactive secondary metabolites (SMs). These compounds are synthesized by enzymes encoded by genes that are clustered in the genome. The vast majority of SM biosynthetic gene clusters are not expressed under normal growth conditions, and their products are unknown. Developing methods for activation of these silent gene clusters offers the potential for discovering many valuable new fungal SMs. While a number of useful approaches have been developed, they each have limitations, and additional tools are needed. One approach, upregulation of SM gene cluster-specific transcription factors that are associated with many SM gene clusters, has worked extremely well in some cases, but it has failed more often than it has succeeded. Taking advantage of transcription factor domain modularity, we developed a new approach. We fused the DNA-binding domain of a transcription factor associated with a silent SM gene cluster with the activation domain of a robust SM transcription factor, AfoA. Expression of this hybrid transcription factor activated transcription of the genes in the target cluster and production of the antibiotic (+)-asperlin. Deletion of cluster genes confirmed that the cluster is responsible for (+)-asperlin production, and we designate it the aln cluster. Separately, coinduction of expression of two aln cluster genes revealed the pathway intermediate (2Z,4Z,6E)-octa-2,4,6-trienoic acid, a compound with photoprotectant properties. Our findings demonstrate the potential of our novel synthetic hybrid transcription factor strategy to discover the products of other silent fungal SM gene clusters.