Biocombinatorial Engineering of Fungal PKS-NRPS Hybrids for Production of Novel Synthetic Natural Products

Natural products have had a huge impact on the history of drug development, and today, these compounds continue to be a key source of novel drug leads. Over the years, natural products have been exploited for their pharmacological and biological activities in the treatment of many diseases, as exemplified by many antimicrobial agents as well as anti-cancer agents, immunosuppressants and cholesterol-lowering drugs. A common approach for the discovery of new drug leads relies on screening of a large number of organisms, which is often followed by semi-synthetic modifications for final drug structure optimization. Within the last decade, an alternative approach for expanding natural product chemodiversity has been applied. This strategy, known as combinatorial biosynthesis, involves the re-engineering of biosynthetic pathways and ultimately the rational engineering of a novel product analogs. This field, however, has proved very challenging and many engineering efforts have resulted in enzymatic loss-of-function or reduced yields. Thus, the future success in combinatorial biosynthetic studies requires a thorough understanding of the structure and function of biosynthetic enzymes.

Secondary metabolites (SMs) from bacteria, plants and filamentous fungi constitute a large group of important natural products. In this thesis I explore the biosynthesis of several fungal SMs along with the enzymes responsible for their synthesis. Specifically, my research focuses on the expression and engineering of a certain type of fungal enzymes – natural fusions of polyketide synthases and nonribosomal peptide synthetases (PKS-NRPSs). The thesis is divided into two topics:

1) Expanding fungal chemodiversity through combinatorial biosynthesis
2) Two CRISPR-Cas9-based approaches to linking SMs to their genes in filamentous fungi

The first topic is covered in chapter 2 and 3. Chapter 2 includes a peer-reviewed research article published in PLOS ONE, while chapter 3 describes the results of a study, which will soon be submitted for publication. The second topic is covered in chapter 4 and 5, where chapter 4 includes a manuscript that has been submitted for publication, while chapter 5 describes the results from a study that is still under investigation. Chapter 6 represents a summary of additional results.

In the first study (chapter 2), I present the expression of two related PKS-NRPS hybrids, CcsA from Aspergillus clavatus and Syn2 from Magnaporthe oryzae, in Aspergillus nidulans, which resulted in production of two novel compounds, nidudaidin and niduporphin. Next, I describe the design and construction of functional chimeric enzymes and production of two novel compounds through the modular rearrangement of CcsA and Syn2. I also show that the PKS-NRPS intermodular region displays great tolerance with regards to sequence and length, and as a result demonstrates great flexibility in the exchange of whole modules.

In the second study (chapter 3), I venture into another case of combinatorial biosynthesis. The focus of this chapter is the nonaketide synthase LovB involved in the biosynthesis of lovastatin. By construction of two fusions of LovB to the NRPS module of a hybrid PKS-NRPS, it was shown that: 1) the C-terminal condensation (C) domain of LovB is incompatible with the remaining NRPS domains, and 2) the PKS-NRPS activity of LovB can be restored by replacing the C-terminal C domain with the full NRPS module. The study describes the first example of a PKS that has been re-engineered to function as a PKS-NRPS hybrid, and also highlights the flexibility in combining non-cognate PKS and NRPS modules. In the third study (chapter 4), I describe how CRISPR-Cas9, which was recently implemented for genome editing in filamentous fungi, can be used to link SMs to their genetic origin in a fungus where no genetic tools were previously available. Using CRISPR-Cas9, I identified a novel gene encoding a PKS-NRPS hybrid responsible for the production of a medically relevant compound in Talaromyces atroroseus. To the best of my knowledge, this study represents the first example of reverse engineering of a Talaromyces species.

In the fourth study (chapter 5), I used the CRISPR-Cas9 system for the construction of an Aspergillus aculeatus nonhomologous end-joining (NHEJ)-deficient strain, which subsequently facilitated efficient deletions of multiple genes using a conventional gene-targeting strategy. Acurin A and B, two stereoisomeric compounds that were isolated from extracts of A. aculeatus were linked to a common biosynthetic gene cluster. A combination of cluster-associated gene deletions and quantitative reverse transcription PCR (qRT-PCR) enabled delineation of the acurin biosynthetic gene cluster, and to propose a biosynthetic pathway for production of acurin.

In the last part (chapter 6), I summarize the results from the testing of additional PKS-NRPS chimeric variants, and demonstrate how the novel gene identified in T. atroroseus (chapter 4) was used in another successful case of PKS-NRPS module rearrangement. The vast amount of SMs isolated from filamentous fungi represents an enormous potential for further combinatorial biosynthetic studies. However, these metabolites must be linked to their genetic origin to allow this potential to be fully exploited. In summary, I will in this thesis, present examples of how CRISPR-Cas9 can be used as a new tool to link SMs to their genetic origin, and how this knowledge can be applied for the engineering of functional chimeric enzymes that can produce novel compounds in a predictable manner.

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