From Synthetic DNA to Complex Natural Products

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Natural products of microbial origin continue to be an important source of pharmaceuticals and agrochemicals exhibiting potent activities and often novel modes of action. Due to their inherent structural complexity chemical synthesis is often hardly possible leaving fermentation as only viable production route. In addition, the pharmaceutical properties of natural products often need to be optimized for application by sophisticated medicinal chemistry and/or biosynthetic engineering. The latter requires a detailed understanding of the biosynthetic process and genetic tools to modify the producing organism which are often unavailable. Consequently, heterologous expression of complex natural product pathways has been in the focus of development over recent years.

However, piecing together DNA cloned from natural sources into large constructs and achieving efficient expression in heterologous circuits represent formidable tasks involving several limitations: The construction of expression vectors for complex pathways is technically challenging and the functionality of native DNA sequences is often restricted in heterologous hosts, just to name a few. Such limitations can be addressed by synthetic biology approaches involving the redesign and subsequent gene synthesis of complex biosynthetic pathways. Native DNA sequences are modified according to constructional and functional requirements predetermined to the pathway and the expression system of choice. The codon composition and other functional elements are adapted to the target host and, simultaneously, genetic elements (e.g. unique restriction sites) are engineered into the sequence in order to permit pathway assembly and future interchangeability of pathway elements.

Our recent approaches to establish synthetic DNA platforms for the heterologous production of bioactive natural products from myxobacteria will be discussed. Synthetic gene clusters of 20 to 50 kb in size were constructed to achieve heterologous production in the myxobacterial model strain *Myxococcus xanthus* and non-related hosts *Pseudomonas putida* and *Yarrowia lipolytica*.