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Promiscuity Breeds Diversity: The Role of Polyketide Synthase in Natural Product Biosynthesis

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Secondary metabolites are often highly biologically active molecules and are widely used from antibacterial to anticancer drugs. In this issue of *Chemistry and Biology*, Zaehle and coworkers describe the gene cluster and biosynthesis of the polyketide terrein, a secondary metabolite produced by the soil-borne fungus *Aspergillus terreus*.

The discovery and subsequent medicinal use of antibacterial compounds such as sulfonamides and penicillin represent a major revolution in medicine and public health and thereby a milestone in human history. Many bacterial infections, which, in previous times, often caused the death of the patients and even resulted in several worldwide epidemies, suddenly could be cured. Whereas sulfonamides are of a chemical origin, penicillin is a typical fungal product. Many microorganisms produce a large variety of so-called *secondary metabolites*, which do not play an obvious role in their primary metabolism but may serve important functions for the survival in their natural habitats. Meanwhile, thousands of such compounds have been described, and some of them are of tremendous value for us as antibiotics (β-lactam antibiotics, erythromycin, and tetracyclin), immunosuppressives (rapamycin), anticancer (epothilone B) or anti-cholesterol drugs (lovastatin). Indeed, there is a great interest to find novel compounds to combat multidrug-resistant bacteria (von Nussbaum et al., 2006).

Huge compound screening programs have been employed in the past to identify novel molecules and then test their pharmacological potential. This period was followed by a period where rational design was favored and screening for novel compounds was somehow neglected. Nowadays ''omics''approaches give new impetus to drug discovery and compound characterization. The article by Zaehle et al. (2014) in this issue of *Chemistry & Biology* is a beautiful example of how genomics, genetics, molecular biology, biochemistry, synthetic biology, and toxicology are nowadays combined for the analysis of fungal secondary metabolites.

The molecule studied in the article by Zaehle et al. (2014), terrein, belongs to the group of polyketides in whose biosynthesis a polyketide synthase is the key enzyme. Polyketide synthases (PKSs) are large, multidomain enzymes or enzyme complexes that produce an extraordinary complex variety of natural products starting from small acids, such as acetyl-CoA, propionyl-Co, buturyl-CoA, and derivatives (Khosla et al., 2014). With similarities to fatty acid synthase, the short building blocks condense, accompanied by decarboxylation, to complicated molecules. Despite many similarities to fatty acid synthesis, a significant difference is the fact that, during fatty acid biosynthesis, the final molecule is largely reduced, whereas the building blocks of polyketides may remain in the oxidative state and can thus be easily further modified. Another difference concerns the building blocks itself. Although each PKS is thought to be specific for one specific polyketide, there are cases where different starting blocks or building blocks for the extension can be used. Thus, the promiscuity of a PKS to produce different chain lengths along with the reactivity of the end products of the PKS reaction results in the enormous variety of natural secondary metabolites.

Thousands of secondary metabolites have been described to date. Molecular analysis of their biosynthesis revealed that the genes are normally clustered in the genome (Keller et al., 2005). Hundreds of such gene clusters have been identified, meanwhile, in the course of genome annotations. However, the link between the compounds and the biosynthetic gene clusters responsible for their biosyntheses is largely missing. In addition, many gene clusters are not expressed under laboratory conditions and need to be activated in order to identify further secondary metabolites (Wiemann and Keller, 2014). One approach to assign a function to a certain PKS or the entire gene cluster is a gene deletion or silencing strategy with subsequent metabolic profiling of the mutant strains (Saha et al., 2012). This has been applied by Zaehle et al. (2014) to identify the gene cluster responsible for terrein biosynthesis in the common soil-borne fungus *Aspergillus terreus* (Figure 1). The compound itself was chemically described 80 years ago, and the structure was resolved in 1954 (Grove, 1954; Barton and Miller, 1955). Previous classical stable-isotope labeling experiments suggested that five acetate molecules condensed to form the molecule, although the formula implies that it is composed of only four acetates (eight C-atoms). This riddle has been solved by Zaehle et al. (2014). Deletion of several of the *terA* gene cluster genes in combination with ^{13}C-glucose feeding experiments allowed the authors to identify 6-hydroxymellein (ten C atoms) as an intermediate. The alternation of labeled and unlabeled carbon atoms in 6-hydroxmellein can be explained by the condensation of one acetyl- and

four malonyl-Co residues. In terrein, two adjacent carbon atoms were nonlabeled, suggesting decarboxylation and ring contraction of 6-hydroxymellein. This is, thus, a beautiful example of how genetics in combination with isotope-labeling and structural chemistry methods, such as high-resolution mass spectrometry and nuclear magnetic resonance, complement each other to unravel biochemical pathways.

In a synthetic biology approach, the authors expressed the PKS in a different filamentous fungus, *A. niger*, and were able to identify several products formed by this PKS, although only one of them is apparently further converted to the final product, terrein. The fate of the other two PKS products remains elusive. However, it is possible that these two molecules are further transformed into other secondary metabolites, and, thus, this in the first view nonproductive catalysis could contribute to the diversity of secondary metabolites.

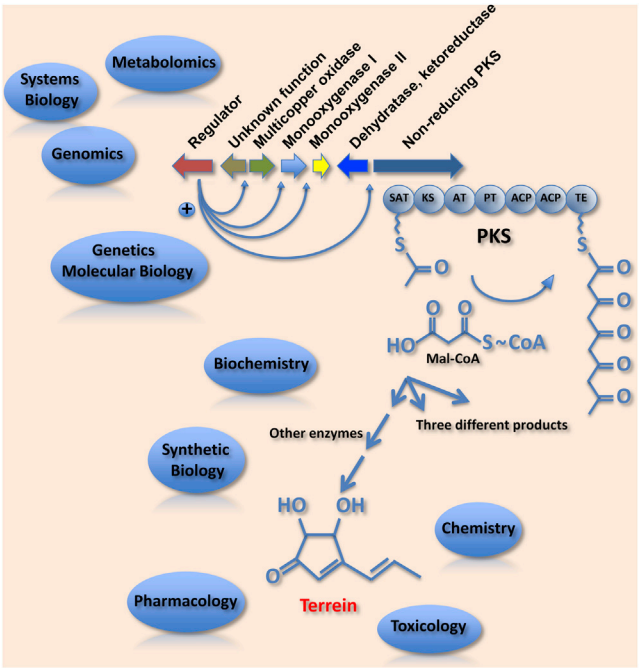

Figure 1. The Minimal Terrein Biosynthesis Gene Cluster of *Aspergillus terreus* Represents the Typical Arrangement for Secondary Metabolite Genes

A pathway-specific regulator controls the expression of the other genes involved in the biosynthesis. The central enzyme of many secondary metabolites is the multidomain-containing polyketide synthase consisting of a starter acyltransferase (SAT), a ketoacyl synthase (KS), an acyltransferase (AT), a product template (PT), two acyl carrier protein domains (ACP), and a thioesterase domain (TE). In the case of terrein biosynthesis two, three, or four malonate-CoA molecules can be added as building blocks, resulting in three different products. Multidisciplinary approaches are now well established to discover novel secondary metabolites and characterize their structure and function.

Last but not least, the article adds to the knowledge of potential functions of secondary metabolites in nature. Although it was described that terrein has antibacterial, antifungal, anti-inflammatory, antioxidative, antiproliferative, proapoptotic properties, it was only in 2007 that the plant-growth inhibiting function of terrein was recognized. Zaehle et al. (2014) now show that this polyketide causes lesions on fruit surfaces. This phytotoxic activity could be of vital importance in the struggle for nutrients in nature and thus could add to the scarce knowledge of the biological function of secondary metabolites.

The identification of the terrein biosynthesis gene cluster has several important aspects. Because *A. terreus* is a common soil-borne fungus, contaminates feed and food, and is also an emerging pathogen, the monitoring of the potentially toxic terrein is highly desirable. The knowledge of the terrein gene cluster opens new avenues for RNA-based detection methods for gene expression long before the compound can be detected (Schmidt-Heydt and Geisen, 2007). In addition, the knowledge of the genes encoding the enzymes for terrein biosynthesis allows further synthetic biology approaches to modify the final product and thereby perhaps change the biological activities. Next steps should, of course, be the elucidation of the exact mode of action and the molecular target to explain the observed activities and evaluate the potential uses of terrein or derivatives thereof.

Whereas serendipity led to the discovery of the first secondary metabolite with high commercial value, penicillin, recent approaches aim at systematic screening programs, the waking up of secondary metabolite gene clusters, and designer drugs by rationally reprogramming through combinatorial manipulation (Chiang et al., 2013; Unkles et al., 2014). However, as yet shown for several polyketide synthases, enzyme promiscuity may be important in the playground of nature, and thus understanding promiscuity may help to develop novel man-made drugs.

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