

PREFACE

In 2009, I edited two volumes of *Methods in Enzymology* (Volumes 458 and 459) entitled “Complex enzymes in microbial natural product biosynthesis.” The project was motivated by two main factors. The first was the development, over the previous few years, of a novel toolbox of practical techniques for the study of natural product biosynthesis, involving a fusion of chemistry, genetics, enzymology, and structural studies, thereby bringing within reach an understanding of the “programming” of complex, multifunctional enzyme systems that had not been attainable previously and opening the possibility of creating “unnatural natural products” by genetic engineering. The second was the increasing need for novel bioactive natural products, especially antibiotics and anticancer drugs, and the new possibilities for addressing this need by carrying out “chemistry through genetics” and by studying the gamut of potential natural products revealed by the sequencing of microbial genomes. Three years later, these driving forces are still very much alive, hence the motivation to extend the project.

As well as including overview articles, the two 2009 volumes covered many of the hotspots in peptide and polyketide research, plus aminocoumarin compounds and some aspects of carbohydrate-based natural products. Therefore, the main emphasis this time is on chemical classes that were not included in the previous volumes, notably terpenoids and alkaloids, as well as further coverage of peptides and inclusion of Type III polyketides, which did not make it into the previous volumes. Interesting tailoring reactions, which often give natural products their biological activity by adding functional groups to the carbon skeletons assembled by complex enzyme systems, are also included.

Less obvious, in a series dedicated to enzymology, is the inclusion of sections dealing with the isolation and study of novel classes of organisms and of organisms from novel habitats. Other chapters describe heterologous pathway expression and methods for waking up sleeping gene clusters. The reasoning is that getting hold of the enzymes is an essential prerequisite for their study. Apart from its intrinsic scientific interest, this is a growth area in relation to natural product discovery and is revealing an Aladdin’s cave of novel metabolism, much of it unexpressed under the conditions employed in traditional natural product screening campaigns.

In contrast to the previous two-volume set, which was focused on microorganisms, this time I have widened the coverage to include plants. Plants have long been known to produce an enormous number of important natural products, but study of their enzymology at the detailed molecular biological level lagged behind that of microbial products, largely for technical reasons. With the rise of plant genomics, made possible by the recent development of next-generation sequencing technologies and the analysis of transcriptomes by RNAseq, which together bring the large genomes of higher plants within reach, this deficiency has been redressed and there are now many examples of penetrating analysis of plant metabolites. Where appropriate, chapters on microbial systems—both bacterial and fungal—are grouped with chapters on plant metabolites so that interesting comparisons can be made. In a further difference from last time, the main criterion for inclusion in the new volumes is good biochemical and/or genetic understanding of a biosynthetic pathway, combined with interesting chemistry and/or unusual producing organisms. Thus, the choice is not confined to “complex enzymes” *per se*.

Especially in the descriptions of methods to study plant systems, there is overlap between a few of the chapters, even if they deal with the analysis of different classes of metabolites. I should like to regard this as a strength rather than a defect in editing! These technologies are still developing, so having more than one set of protocols to explore may be helpful to those who wish to extend the techniques to classes of compounds that are not explicitly covered in these volumes.

Volume A opens with a major section on terpenoids. Members of this huge class of natural products are derived from five-carbon isoprene units, ranging in number from hemiterpenes with one unit, monoterpenes with two, sesquiterpenes with three, diterpenes with four, tetraterpenes with eight, and polyterpenes with many. At one time thought to be rare or even absent from prokaryotes, they are now known to be important in bacteria as well as in the eukaryotic fungi. Coverage of the terpenoids in Volume A reflects this wide distribution and importance, with eight chapters devoted to various aspects of the study of terpenoid compounds. Several of the chapters introduce novel, cutting-edge technology. Appropriately for *Methods in Enzymology*, Chapter 1 describes novel enzymology: the steady-state kinetic characterization of plant sesquiterpene synthases by gas chromatography–mass spectrometry (GC–MS). Likewise, Chapter 4 represents a fine example of enzymology, defining and describing the specialized polyterpenoid synthase that makes natural rubber. Other chapters in this section deal with the genetic

engineering of terpenoids and their expression in heterologous hosts, while providing a mine of information on the biosynthesis of the individual compounds, ranging from fungal mycotoxins to bacterial menaquinones.

The next group of chapters in Volume A is devoted to the alkaloids and glucosinolates. Alkaloids represent perhaps the longest known group of plant natural products. They are highly diverse in their structures and biological activities but are united by the presence of a basic nitrogen atom at some position in the molecule. Chapters 9 and 10 describe techniques for the discovery and analysis of monoterpene-derived indole alkaloids, which include the crucial antitumor Vinca alkaloids as well as several other classes of molecules with important medicinal uses, while Chapter 11 deals with the L-tyrosine-derived benzyloquinoline alkaloids from opium poppy and related species, again compounds of extreme pharmacological interest, including morphine. Chapter 12 introduces the ergot alkaloids, classically associated with plants but actually made by the fungi that parasitize them, notably species of *Claviceps*, but now known to be made by a wider range of fungi. This section ends with a chapter on the amino acid-derived glucosinolates of plants, notable as beneficial dietary components found in brassicas, and their heterologous expression in *Nicotiana* and in yeast.

As mentioned above, polyketide synthases dominated the 2009 volumes in this series, but did not include the Type III systems, best known for the biosynthesis of anthocyanin pigments in higher plants but responsible for a wide range of other important compounds and now well established also as the producers of metabolites of microorganisms, both bacterial and fungal. They differ from the Type I and II synthases in consisting of small homodimeric proteins rather than large multifunctional enzymes with a multitude of separate active sites, making them in some ways easier to study biochemically but more cryptic in their programming. Chapters 14–16 describe the analysis and manipulation of these important enzyme systems.

Peptide-derived natural products are perhaps best known as being derived by nonribosomal assembly-line mechanisms very distinct from those depending on the ribosome. Several examples were covered in the 2009 volumes, along with a single class of compounds—the lantibiotics—resulting from ribosomal biosynthesis followed by extensive posttranslational modifications. Volume B opens with four further examples of ribosomally derived metabolites: the thiopeptide antibiotics produced by *Streptomyces* and *Bacillus* species, microviridin made by cyanobacteria, the plant cyclotides, and the cyclic peptide toxins of mushrooms, including the infamous amatoxins. This section ends with a special activity of a novel

nonribosomal peptide synthetase, the Pictet-Spengler mechanism involved in the biosynthesis of tetrahydroisoquinoline antitumor antibiotics.

The next section of Volume B contains three chapters describing very diverse enzymology. The P–C bonds in phosphonate and phosphinate natural products endow them with a high level of stability and the ability to mimic phosphate esters and carboxylates, so their biosynthesis is particularly intriguing. The radical SAM enzymes carry out remarkable chemical transformations by releasing an active radical via the cleavage of S-adenosyl-L-methionine; the second chapter in this section describes novel methods for their purification and characterization. The third chapter describes methods for probing the biosynthesis of novel high-carbon sugar nucleosides containing up to 11 contiguous carbons.

Very often, natural product biosynthesis proceeds by the assembly of a core backbone—perhaps a polyketide, peptide, or terpene—followed by reactions that add functional groups that endow the molecules with their specific biological activities. Volume B continues with a section containing nine chapters devoted to such important tailoring reactions. It begins with one of the most famous classes of tailoring enzymes, the heme-dependent cytochromes P450, followed by the less well known nonheme iron-dependent enzymes. Then come two chapters on the halogenating enzymes of microorganisms and plants, first those that introduce fluorine and then chlorinating and brominating enzymes. Next comes prenylation, here represented by fungal enzymes of the dimethylallyltryptophan superfamily. This chapter relates to the biosynthesis of ergot alkaloids and so could have been placed in the alkaloid section of Volume A but is included in the tailoring enzyme section of Volume B because of the widespread importance of prenylation in determining the biological activity of molecules. Acylation is another crucial tailoring step in conferring biological activity on natural products; the section includes a chapter on one of the most important classes of acylating enzymes of plants, the serine carboxypeptidase-like acyltransferases. The actinomycete-derived enediynes are some of the most remarkable natural products both structurally and for their extreme cytotoxicity. Two chapters in this section derive from aspects of their biosynthesis, but they are included for the much wider applicability of the resulting enzymology. Chapter 15 deals with 4-methylideneimidazole-5-one (MIO)-containing aminomutases that catalyze β -amino acid formation, and Chapter 16 deals with tailoring enzymes acting on carrier-protein-tethered substrates, an approach that promises to open new vistas in the engineering of designer natural products. The section ends with a chapter on glycosylation. This

topic received substantial coverage in the 2009 volumes but has recently been refined by the development of high-throughput colorimetric assays for nucleotide sugar formation and glycosyl transfer.

Volume C is devoted to methods for the discovery of novel secondary metabolite-synthesizing gene clusters and their analysis. First comes a group of five chapters describing novel sources of natural products or novel methods for their discovery. This is a growth area in the field of natural product research and could have included a much larger number of studies. Three of the selected chapters deal with the isolation of endophytic microorganisms from the tissues of traditional Chinese medicinal plants, with methods for handling cyanobacteria in relation to natural product discovery, and with the fascinating ecosystem represented by the nests of leaf-cutting ants that farm fungi as a food source and depend on beneficial microbes—mostly actinomycetes in the known examples—to protect their fungus gardens from parasitic fungi. The other two chapters in this section cover small molecule-mediated interactions within and between microbial colonies, an aspect of microbial ecology that is revealing interesting new metabolites in increasing numbers.

The next section containing three chapters represents another growth area in natural product research, namely, computational and bench-level approaches to the analysis of the gene clusters that contain the sets of genes encoding natural product biosynthetic genes. It has been a long-established paradigm that such gene sets are clustered together on bacterial genomes, with spectacular examples first discovered in the actinomycetes and later extended to myxobacteria and other groups of differentiating bacteria. Cotranscription of operons of clustered genes for primary metabolism in bacteria was an early discovery of the golden age of bacterial genetics, providing one driving force for clustering, though rarely do the secondary metabolic gene clusters represent a single operon. It came as a mild surprise that clustering is also the rule in the eukaryotic fungi, in which operons are not found. Recently, clustering has also been found to be a feature of some but not all secondary metabolic pathways in higher plants, though the clusters are very different from those of microorganisms in containing long stretches of untranslated DNA between the protein-encoding genes.

A powerful approach to the functional analysis of gene clusters is to express them in nonnative hosts, where they may be reassembled and/or engineered, sometimes to make unnatural products. A group of six chapters deal with various systems for such analysis, ranging from the use of virus vectors for heterologous expression in plants to systems for heterologous expression in streptomycetes, filamentous fungi, and, especially, yeast.

The final section of Volume C reflects the crucial discovery that the genomes at least of microorganisms contain far more clusters of genes potentially encoding natural product biosynthesis than are expressed under a given set of conditions. The challenge is to find generally applicable methods to wake up such "sleeping" gene sets and so give access to a range of potentially valuable compounds that would otherwise go unexplored. Two chapters in this section describe diverse approaches in filamentous fungi and two in *Streptomyces* species. The section ends with a chapter on the intriguing and important problem of "persisters" that represent a subpopulation of a bacterial pathogen in which the whole cell is "sleeping." They evade killing by antibiotics but may be outwitted by judicious treatments, some of which block the normal wake-up process.

It goes without saying that the value of any edited work depends on the renown of the invited authors and their willingness to write chapters. I was particularly impressed by the enthusiastic response of nearly all my invitees and the quality of the submitted manuscripts. Indeed, such was the enthusiasm of the authors that an original two-volume project expanded to the final three-volume version. My grateful thanks go to all of you as well as to the small army of coauthors who were recruited to the task. I am very grateful also to colleagues who made suggestions for the content of these volumes, especially Greg Challis, Wilfred van der Donk, Sarah O'Connor, Paul O'Maille, Ben Shen, and Anne Osbourn. I also thank Shaun Gamble of Elsevier, who never failed to offer timely advice and reassurance during the entire project.

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