Biosynthesis

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Extending the Biosynthetic Repertoire in Ribosomal Peptide Assembly

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Natural products are quite inspiring. To chemists, they inspire the development of new synthetic methods and the creation of ever more sensitive analytical techniques. Biologists, on the other hand, exploit natural products in the discovery of new molecular targets and drugs, as well as to learn more about the way cells or whole organisms communicate with each other. Natural products also motivate biochemists to explore new ways in which nature assembles complex organic molecules. Such products, in one form or another, have helped transform modern science.

In this post-genomic era, the scientific field of natural product biosynthesis has witnessed a constant flow of fascinating discoveries outlining new biochemical transformations in secondary metabolism. Most recently, cyclic peptide natural products have served as the chemical inspiration for the discovery of new biosynthetic processes associated with the posttranslational modification of ribosomally derived bacterial peptides. This is especially true in the cyanobacteria, which are notorious for their uncanny ability to synthesize a wide variety of structurally diverse peptidyl products.[1] Although they often employ nonribosomal peptide synthetase systems to capture a much wider array of substrates than the 20 proteinogenic amino acid building blocks that limit input into ribosomal peptides (RPs), two recent discoveries from the Schmidt[2] and Hertweck/Dittmann[3] research groups extend our understanding and appreciation of new enzymatic processes for diversifying ribosomally encoded peptides.

The general trend in the biosynthesis of bacteriocin RPs, many of which possess potent antimicrobal or toxic properties, involves the synthesis of an N-terminal-extended prepeptide that undergoes various modes of posttranslational modification followed by proteolytic cleavage to liberate the active peptide.[4] Well-known modifications involving the ligation of amino acid residues include the formation of disulfide linkages, lanthionine bridges such as in the lantibiotic nisin A,[5] heteroaromatic rings such as in microcin B17,[6] and macrolactam (amide) linkages such as in the lasso peptide microcin J25[7] (Scheme 1).

A few years ago, two independent studies surprisingly revealed that the patellamide class of ascidian-derived cyclic peptides was in fact derived ribosomally from the cyanobacterial symbiont *Prochloron didemni*.[8] These RPs, which include patellamide C (Scheme 1), were the first to combine structural features associated with the microcins: they harbored both heteroaromatic rings and were N to C cyclized. While the molecular basis for their assembly has been firmly established, and resulted in the combinatorial biosynthesis of structural libraries,[9] biochemical features associated with the individual enzymatic transformations have not yet been clarified, although it has been speculated that the macrocylization reaction may proceed spontaneously.[10] Recently, Schmidt and co-workers have extended their earlier observations and firmly established the identity of the "cyanobactins" as a major group of cyanobacterial RPs produced in symbionts and free-living organisms.[2] Newly inducted into this group are a number of prenylated cyclic peptides such as the patellins and the antitumor agent trunkamide (Scheme 1). Bioinformatic analysis of the *tru* biosynthetic gene cluster did not reveal canonical prenyltransferases, thus suggesting the presence of an orthogonal enzymatic pathway to peptidyl prenylation in the cyanobactins. The plasticity of the genetic system was clearly established through the heterologous expression and recombination of the *tru* genes in *Escherichia coli* for the production of the natural products, which nicely sets the stage for the future assembly of prenylated RP libraries.

The cyanobacterial toxins belonging to the microviridin family of tricyclic depsipeptides, such as microviridins B (Scheme 1) and J from *Microcystis* species, were recently reported by Hertweck, Dittmann, and co-workers to also be derived ribosomally.[3] Three intramolecular ω-ester and ω-amide linkages between side-chain residues distinguish these related natural products from other RPs. Inspection of the microviridin (*mdn*) biosynthetic loci from two producing strains revealed two genes (*mdnB* and *mdnC*) encoding ATP-dependent carboxylate-amine/thiol ligases adjacent to the microviridin precursor gene (*mdnA*). The gene products MdnB and MdnC surprisingly belong to the ATP-grasp fold superfamily, which includes D-alanine:D-alanine ligase, glutathione synthetase, biotin carboxylase, and succinate-CoA

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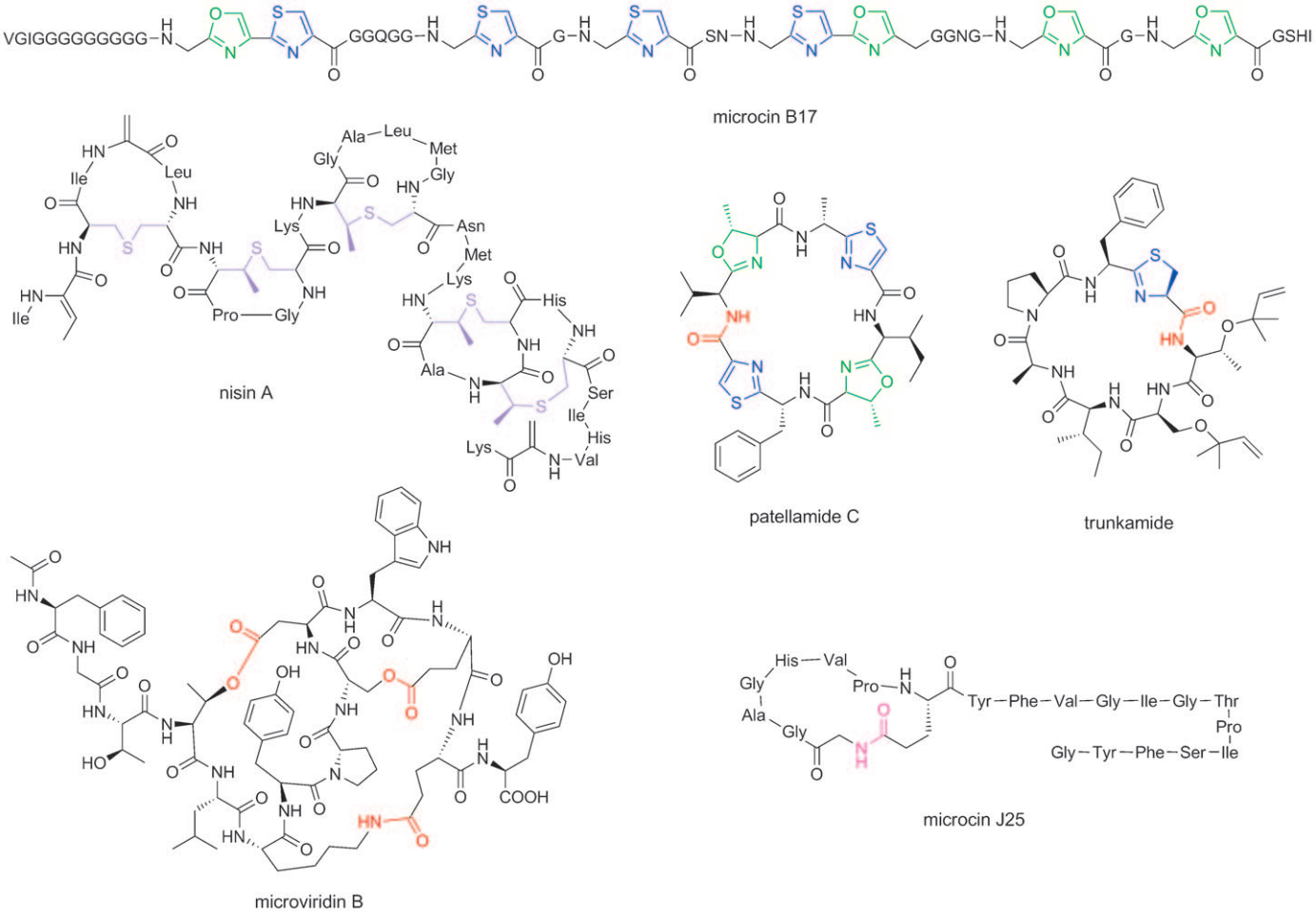

Scheme 1. Structures of ribosomally derived bacterial peptides representing diverse structural groups: microcins (microcin B17 and J25), lantibiotics (nisin A), cyanobactins (patellamide C and trunkamide), and microviridin B. Posttranslational modifications involving the conjugation of two amino acid residues are highlighted as follows: oxazoline and oxazole rings are shown in green, thiazoline and thiazole rings are shown in blue, (methyl)lanthionine bridges are shown in violet, amide linkages resulting from the macrocyclization of terminal residues are shown in orange and pink, and ester and ω-amide bonds involving side-chain residues are shown in red.

ligase.[11] Condensing enzymes belonging to this superfamily are well represented in primary metabolism and function by the ATP activation of carboxylates to acylphosphate intermediates, which are then prone to attack by nucleophiles to yield amide, ester, and thioester bonds (Scheme 2 A). This reaction is orthogonal to the dominant mode of ATP-mediated condensations that instead involves the cleavage of ATP to acyladenylates and pyrophosphate and is featured in the ribosomal and nonribosomal synthesis of peptide bonds.

The discovery of ATP-grasp enzymes associated with microviridin assembly is a distinctive biosynthetic feature of these RPs and is poorly represented outside primary metabolism, with only a couple of examples involving the assembly of the biopolymer multi-L-arginyl-poly-L-aspartic acid (cyanophycin)[12] and *N*-glycylclavaminic acid (Scheme 2 B).[13] Once again nature has recycled a key primary metabolic enzyme for the assembly of a natural product. The heterologous expression of the *mdnABC* gene cassette in *E. coli* confirmed that these three genes are minimally responsible for the synthesis of the correctly folded microviridin tricyclic depsispeptide core prior to cleavage of the *N*-terminal leader peptide.[3] The functional characterization and timing of the *mdn* ATP-grasp enzymes await further in vitro analyses. Although the formation of the microviridin ω-amide bond is analogous to that in the lasso peptides microcin J25[7] and capistruin[14] in which the macrolactam is formed by an ATP-dependent reaction between the α-amino group of Gly1 and the side-chain carboxyl groups of Glu/Asp residues, the enzymatic route in that case proceeds via an acyladenylate intermediate.

Biosynthetic lessons learned from the new additions of trunkamide and microviridins B and J to the RP family not only extend our basic knowledge of posttranslational options but also opens the door for the bioengineering of new chemical entities by mixing and matching RP modifying genes. Given the ease by which RP synthesis can be reprogrammed,[9,15] it will be interesting to follow how these systems are rationally manipulated and whether new drug leads can be designed.

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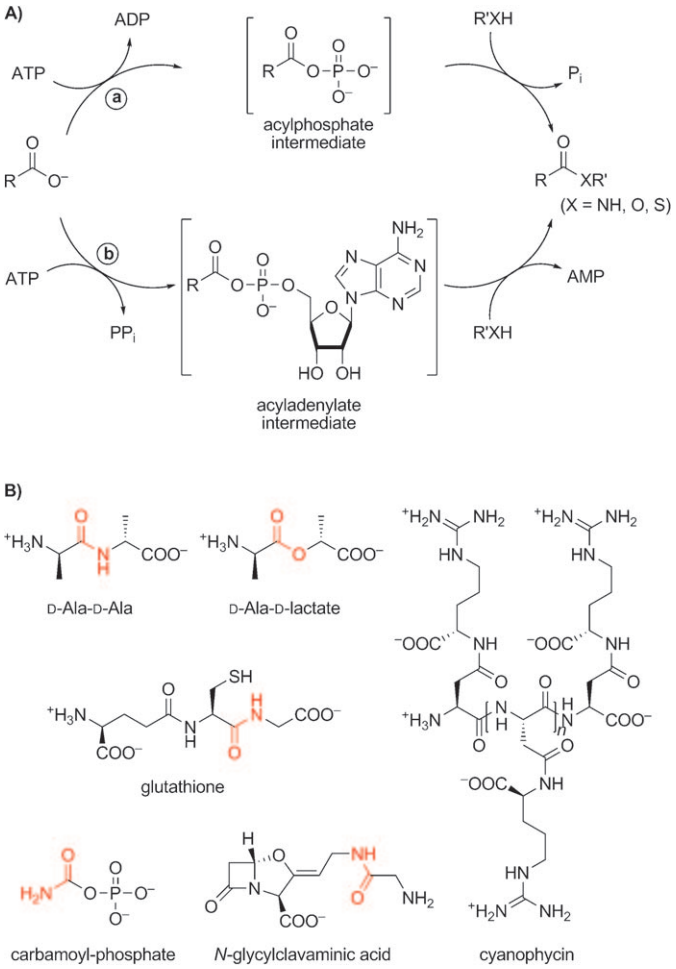

Scheme 2. Biosynthesis and structures of ATP-grasp ligase products. A) ATP-activation of carboxylic acids by ATP-grasp ligases proceeds via acylphosphate intermediates (path a) in contrast to acyladenylates (path b). P_i = inorganic phosphate, PP_i = pyrophosphate. B) Amide and ester linkages formed by ATP-grasp ligases are shown in red.

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