Review

CellPress

Nucleoside antibiotics: biosynthesis, regulation, and biotechnology

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The alarming rise in antibiotic-resistant pathogens has coincided with a decline in the supply of new antibiotics. It is therefore of great importance to find and create new antibiotics. Nucleoside antibiotics are a large family of natural products with diverse biological functions. Their biosynthesis is a complex process through multistep enzymatic reactions and is subject to hierarchical regulation. Genetic and biochemical studies of the biosynthetic machinery have provided the basis for pathway engineering and combinatorial biosynthesis to create new or hybrid nucleoside antibiotics. Dissection of regulatory mechanisms is leading to strategies to increase the titer of bioactive nucleoside antibiotics.

Nucleoside antibiotics

Nucleoside antibiotics are a large family of microbial natural products derived from nucleosides and nucleotides. Because nucleosides and nucleotides play essential roles in most of the fundamental cellular metabolism, nucleoside antibiotics exhibit a broad spectrum of biological activities, such as antibacterial, antifungal, antiviral, insecticidal, immunostimulative, immunosuppressive, and antitumor activities [1]. Based on their biological functions, they can be classified into three major groups. The antibacterial nucleoside antibiotics target bacterial cell wall biosynthesis: they are competitive inhibitors of bacterial phospho-N-acetylmuramyl-pentapeptide translocase (translocase I, denoted MraY), which initiates the lipid cycle of peptidoglycan biosynthesis [2]. The antifungal nucleoside antibiotics also target cell wall biosynthesis: they act as competitive inhibitors of fungal chitin synthases. They can also function as inhibitors of protein biosynthesis. The antiviral nucleoside antibiotics block protein biosynthesis by inhibiting peptidyl transferase. Over the past two decades, several excellent reviews have focused on the structure, biosynthesis, and biological activity of nucleoside antibiotics [1–3]. Here we highlight recent findings on their biosynthesis and its complex regulation and summarize progress in the use of this information to generate new nucleoside antibiotics and to increase production levels.

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Keywords: nucleoside antibiotics; biosynthesis; regulation; genetic engineering.

0966-842X/
 http://dx.doi.org/10.1016/j.tim.2014.10.007

Biosynthesis of nucleoside antibiotics

Nucleoside antibiotics are endowed with unique structural features, suggesting the occurrence of unusual enzymatic reactions during the biosynthetic process. With the rapid development of methodology and the availability of an increasing number of gene clusters, biosynthetic studies have accelerated at both genetic and biochemical levels, providing the basis for pathway engineering and combinatorial biosynthesis of nucleoside antibiotics.

Biosynthesis of antibacterial nucleoside antibiotics: pacidamycin, liposidomycin, tunicamycin, and capuramycin

This group, which is also referred to as uridine-based nucleosides, can be further divided into four subfamilies: uridyl peptide antibiotics, uridyl lipopeptide antibiotics, uridyl lipodisaccharide antibiotics, and uridyl glycosylpeptide antibiotics. The uridyl peptide antibiotics encompass pacidamycin, napsamycin, mureidomycin, and sansanmycin. They share a common structural scaffold, a unique 3′-deoxy-4′,5′-enamino-uridine nucleoside linked to a pseudotetra- or pentapeptide backbone (Figure 1), and exhibit selective antibacterial activity against *Pseudomonas aeruginosa*, a common nosocomial pathogen that is intrinsically resistant to various clinically used antibiotics. Sansanmycin also displays inhibition against multidrug-resistant *Mycobacterium tuberculosis* strains [4]. The uridyl lipopeptide antibiotics are represented by liposidomycin, caprazamycin, muraymycin, muraminomicin, and A-90289. They are structurally characterized by a 5′-*C*-glycyluridine (GlyU), an aminoribofuranoside, a diazepanone, and variable fatty acid side chains (Figure 1). The uridyl lipodisaccharide antibiotics are represented by tunicamycins comprising an unusual 11-carbon aminodialdose core, uracil, N-acetylglucosamine (GlcNAc), and variable fatty acyl moieties (Figure 1). The uridyl glycosylpeptide antibiotics include capuramycin and related compounds (A-503083, A-102395, and A-500359), which are characterized by a 5′-carbamoyluridine (CarU), an unsaturated hexuronic acid, and an aminocaprolactam ring (Figure 1).

Comparison of gene clusters shows high sequence similarities of the corresponding proteins involved in the biosynthesis of pacidamycin, napsamycin/mureidomycin, and sansanmycin [5–8]. Most genetic and biochemical evidence comes from pacidamycin biosynthesis. Biosynthesis of the peptide backbone has been investigated extensively by Christopher Walsh and colleagues. Apart from the two non-proteinogenic amino acids meta-tyrosine (*m*-Tyr)

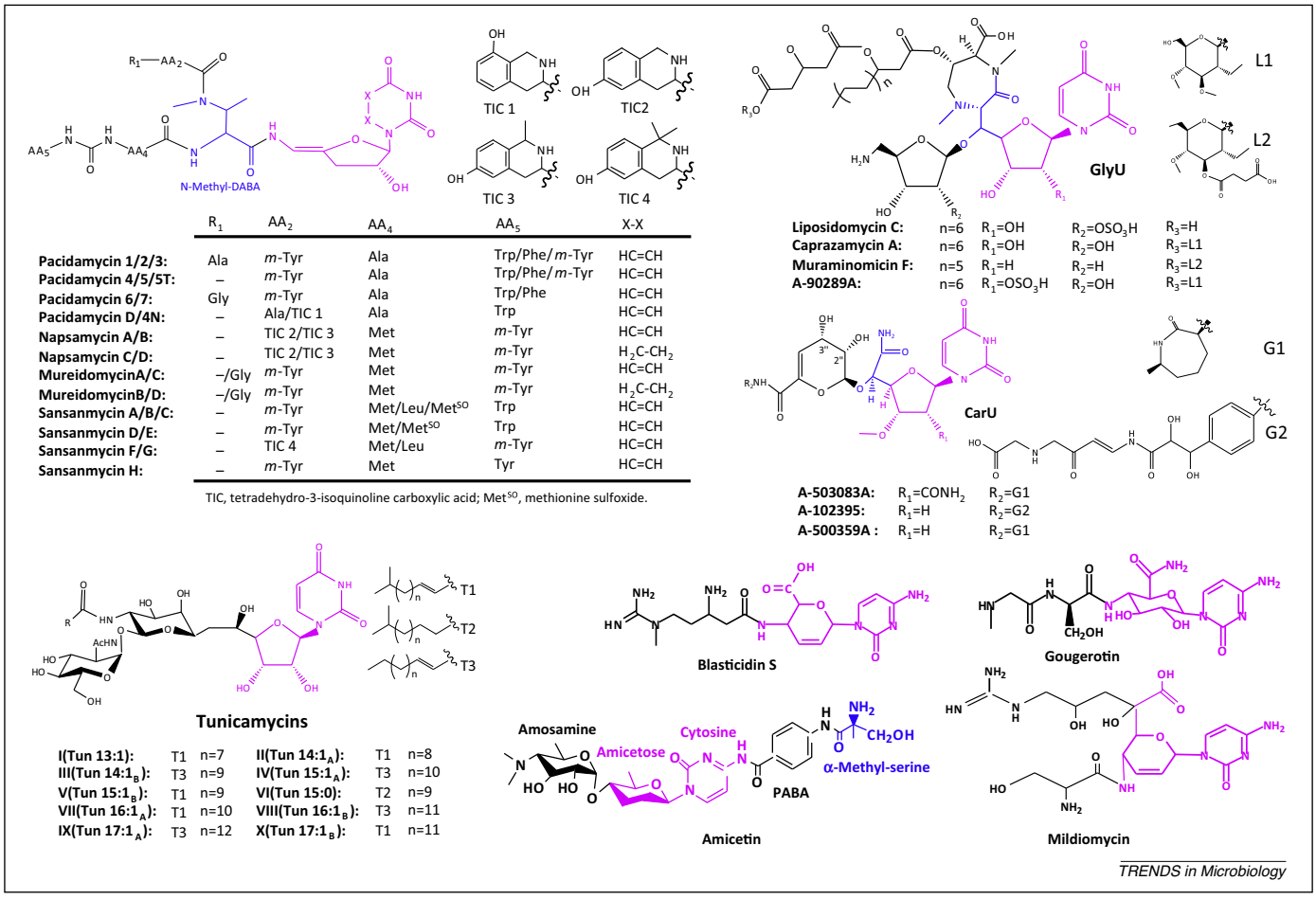

Figure 1. Chemical structures of representative nucleoside antibiotics. Parts shaded in pink indicate the nucleoside moieties. CarU, 5′-carbamoyluridine; DABA, 2,3-diaminobutyric acid; GlyU, 5′-*C*-glycyluridine; PABA, *p*-aminobenzoic acid.

and 2,3-diaminobutyric acid (DABA), other amino acids are thought to originate from primary metabolism. DABA is postulated to be synthesized from L-threonine and ammonia [6]. *m*-Tyr is generated from L-phenylalanine by a novel iron (II)-dependent phenylalanine-3-hydroxylase (PacX) [9]. These amino acids are linked by nine proteins that constitute the non-ribosomal peptide synthetase (NRPS) assembly line [10]. One exceptional feature of this assembly is that it does not start at the N-terminal residue AA_1 and proceed to the C-terminal residue AA_5. Instead, assembly is initiated by the activation and methylation of the core residue DABA and the chain is then built from the middle outward in both directions [11]. It should be noted that the transferase PacB is responsible for the transfer of the alanyl residue from alanyl-tRNA to the N terminus of the tetrapeptide intermediate yielding the pentapeptide scaffold [12]. This enzyme is unusual compared with typical NRPSs in that it catalyzes peptide bond formation in a tRNA-dependent way, hijacking an aminoacyl-tRNA from the primary metabolic pathway to the secondary antibiotic biosynthetic pathway. Rebecca Goss and colleagues have shown that the biogenesis of the unique 3′-deoxy-4′,5′-enamino-uridine proceeds through three steps (Figure 2A). Uridine is first oxidized by flavin-dependent dehydrogenase (Pac11/PacK) to uridine-5′-aldehyde (UA), which is then subjected to 3′,4′-dehydration and 5′-transamination through the action of a Cupin family enzyme (Pac13/PacM) and pyridoxal-5′-phosphate (PLP)-dependent aminotransferase (Pac5/PacE) [13]. The free-standing condensation protein PacI is thought to then catalyze the release of the assembled peptide and its linkage with the nucleoside scaffold to build pacidamycins [10].

Six genes (*lipK*, *lipL*, *lipO*, *lipP*, *lipM*, and *lipN*) are essential for the formation of the aminoribosyl moiety in lipopeptide antibiotics (Figure 2B). The pathway is initiated by oxidative dephosphorylation of uridine-5′-monophosphate (UMP) to UA, catalyzed by a non-heme iron (II)-dependent dioxygenase (LipL) [14]. This is in contrast to the first step in the biosynthesis of the pacidamycin nucleoside (3′-deoxy-4′,5′-enamino-uridine), where uridine is oxidized to UA. Subsequently, UA is converted to 5′-amino-5′-deoxyuridine through the action of the L-methionine:UA aminotransferase (LipO). 5′-Amino-5′-deoxyuridine then serves as the substrate for a phosphorylase (LipP) to generate 5′-amino-5′-deoxy-α-D-ribose-1-phosphate. This sugar-1-phosphate is then processed by two enzymes in a manner that parallels typical glycosylation events: the 5-amino-5-deoxy-α-D-ribose-1-phosphate is activated by a nucleotidylyltransferase (LipM) as the nucleotide-5′-diphosphate (NDP)-sugar [uridine-5′-diphosphate (UDP)-5′-amino-5′-deoxy-α-D-ribose] and the sugar