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Review article
Pregledni članak

rRNA METHYLTRANSFERASES AND THEIR ROLE IN RESISTANCE TO ANTIBIOTICS

rRNK METILTRANSFERAZE I NJIHOVA ULOGA U REZISTENCIJI NA ANTIBIOTIKE

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Summary: Methyltransferases (MTases), a large protein superfamily, commonly use S-adenosyl-L-methionine (SAM) as the methyl group donor. SAM-dependant MTases methylate both nucleic acids (DNA, RNA) and proteins, and thus modulate their activity, function and folding. Methylation of G1405 or A1408 nucleotides of 16S rRNA in aminoglycoside-producing microorganisms confers the resistance to their own toxic product(s). This mechanism of resistance has been considered as unique to antibiotics producers until recently. Since 2003, methylation of 16S rRNA as a mechanism of resistance is increasingly emerging in pathogenic bacteria. This represents a major threat towards the usefulness of aminoglycosides in the clinical practice. A potential solution to the problem involves the design of novel compounds that would act against new ribosomal targets. The second approach to the issue includes the development of resistance MTases' inhibitors, with the idea to prevent them from modifying the bacterial rRNA, and thus reinstate the therapeutic power of existing aminoglycosides. As the latter approach has considerable potential, it is obvious that fundamental research related to protein expression, in-depth understanding of the mechanism of action and resolving a tertiary structure of 16S rRNAs MTases are prerequisites for application in medicine.

Keywords: aminoglycosides, methyltransferases, resistance, ribosome

Kratak sadržaj: Metiltransferaze (MTaze), koje čine veliku proteinsku superfamiliju, kao donatora metil grupe najčešće koriste S-adenozil-L-metionin (SAM). SAM-zavisne MTaze metiluju nukleinske kiseline (DNK, RNK) i proteine, modulišući tako njihovu aktivnost, funkciju i strukturnu organizaciju. Metilacija G1405 ili A1408 baza u 16S rRNK mikroorganizama koji proizvode aminoglikozide obezbeđuje rezistenciju na sopstvene toksične proizvode. Ovaj mehanizam rezistencije je donedavno bio opisan samo kod proizvođača antibiotika. Od 2003. godine i kod patogenih bakterija beleži se neprestan porast rezistencije na aminoglikozide putem ovog mehanizma, što predstavlja veliku pretnju efikasnoj upotrebi aminoglikozida u kliničkoj praksi. Jedno od mogućih rešenja problema leži u razvoju novih jedinjenja koja bi efikasno delovala na nova mesta u okviru ribozoma. Drugi pristup rešavanju ovog problema uključuje razvoj inhibitora MTaza odgovornih za rezistenciju, sa idejom da se onemogući modifikacija bakterijske rRNK i na taj način vrati terapeutska efikasnost postojećim aminoglikozidima. Fundamentalna istraživanja vezana za proteinsku ekspresiju, potpuno razumevanje mehanizma rezistencije kao i razrešenje tercijarne strukture proteina su neophodan preduslov za primenu inhibitora 16S rRNK MTaza u medicini.

Ključne reči: aminoglikozidi, metiltransferaze, rezistencija, ribozom

Introduction

Methyltransferases (MTases), which are a large, diverse and biologically important protein superfamily, most commonly use the ubiquitous S-adenosyl-L-methionine (SAM) molecule as a methyl group donor. SAM-dependant MTases methylate a broad array of substrates – nucleic acids, proteins, polysaccharides, lipids, and a range of small molecules, after their primary synthesis, in both prokaryotic and eukaryotic cells (1). Methylating nucleic

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acids (DNA, RNAs) and proteins, MTases modulate their activity, function and folding; hence, they guide the cell's fate. Methyl group transfer is an alkylation reaction, and the atomic targets for the reaction can be carbon, oxygen, nitrogen, sulfur or even halides (1–3).

The methylation of DNA directs bacterial destiny by controlling numerous processes, including chromosome replication, mismatch repair, transcriptional regulation, and regulation of transposition. One of the main functions of DNA methylation in bacteria is to protect the cell from the effect of foreign DNA. Bacterial restriction modification systems discriminate between endogenous and foreign DNA, which is not protected by methylation (4). Furthermore, DNA methylation is crucially involved in the control of replication fidelity, a process that directs faithful transmission of genetic material during cell division. Repair system corrects mismatches that occur as replication errors in the newly synthesized unmethylated strand, ensuring accurate DNA replication (5). In addition, a growing number of reports suggest that DNA methylation may be a versatile regulator of virulence gene expression in pathogenic bacteria during the course of infection (6, 7).

Translation of the genetic information encoded in a DNA molecule into a functional protein is executed by the ribosome. The ribosome is nature's largest, most complex and most ancient enzyme that consists, even in the simplest organisms, of more than fifty different proteins (r-proteins) and three ribosomal RNAs (5S, 16S and 23S rRNA in prokaryotes). Preservation of its function has been coupled with an overall conservation of structure, and ribosomes in organisms as phylogenetically distinct as bacteria and archaea show a remarkable degree of resemblance. Furthermore, numerous evidence indicates that eukaryotic ribosomes, including those in human cells, are also similar in structure to their prokaryotic counterparts. This complex, dynamic macromolecular ribonucleoprotein machine translates, in a multi-stage process, the genetic information encoded in messenger RNA (mRNA) into proteins (8). Correct assembly, that is subjected to intricate control and aided by a multitude of assembly factors, is prerequisite for its proper functioning (9). While the biological significance of r-protein modifications by methylation is poorly understood (10), the importance of methylations of rRNAs that carry out 'housekeeping' roles essential for the general functioning of rRNA, during protein synthesis, has been recognized. Bacterial rRNAs can contain over thirty 'housekeeping' modifications, all of which are added post-transcriptionally (11). The sites of 'house-keeping' methylations have been most accurately mapped in *Escherichia coli*. Its ribosome contains 24 methylated nucleotides – 10 in the 16S rRNA (small ribosome subunit, i.e. 30S) and 14 in the 23S rRNA (large ribosome subunit, i.e. 50S) (12).

It is noteworthy that for the proper reading of the genetic code, post-transcriptional methylation of transport RNA (tRNA) that can occur at the bases flanking the anticodon, and in some cases even within the anticodon itself, is important. It is believed that modifications in positions flanking the anticodon affect the function of tRNA through relevant alternation of the »rigidity« and »flexibility« of tRNA, which impinge directly on proper base pairing required for codon recognition (1). Remarkably, it has been recently reported that DNA MTase plays hitherto un known role(s) in the post-transcriptional control of gene expression (i.e. mRNA) of a transcriptional regulator important for *Salmonella enterica* pathogenicity (13).

Being of fundamental importance for cell viability, it is not surprising that many antibiotics target ribosomes (14). The main contact sites for the antibiotics are on the rRNA, rather than on the ribosomal protein components (15), which is consistent with the view that the rRNAs carry out the primary functions of the ribosome, while the r-proteins have supporting roles (16, 17). Several classes of antibiotics, such as aminoglycosides, tetracycline, macrolides, lincosamides and streptogramin B, target the rRNA-rich surfaces on the 30S and 50S ribosomal subunits, interfering with their functions in protein synthesis (14). Not surprisingly, therefore, changes that confer antibiotic resistance to these chemotherapeutics mainly consist of nucleotide methylations or base substitutions (15).

Antibiotics, resistance and methylation

Actinomycetes, ubiquitous Gram-positive bacteria, are prolific producers of antibiotics and other secondary metabolites. After the discovery of streptomycin and other potent antibiotics, isolated from soil actinomycetes, antibiotics were defined as »chemical substances of microbial origin, that possess antibiotic powers«, on the assumption that these compounds were exhibiting their natural roles (18). Their bona fide environmental activities are not known, although there is evidence that the majority of low-molecular-weight organic compounds made and secreted by microorganisms play roles as cell-signaling molecules in the environment (19).

In order to avoid the toxic effects of antibiotics, antibiotic-producing microorganisms have developed specific mechanisms to overcome the toxicity of their own product(s). Several mechanisms of resistance to antibiotics have been recognized to be engaged in producers' protection, including target site modification through mutation or modifications, e.g. rRNA methylation. Resistance by rRNA methylation is a frequent mechanism among macrolide (20, 21), and aminoglycoside (22, 23) antibiotic-producing actinomycetes strains.

23S rRNA methyltransferase

Several groups of antibiotics inhibit protein synthesis by interfering with the function of the 50S ribosomal subunit, and resistance could be achieved by the specific methylation of 23S rRNA (24). The first example described in the literature is resistance to thiostrepton, a thiopeptide antibiotic produced by *Streptomyces azureus*, which inhibits a number of ribosomal functions linked to GTPase activity. The Tsr MTase from the producer methylates the 2'-O-ribose of A1067 in the 23S rRNA within the drug binding site (25, 26). Another group of 50S-targeting antibiotics, orthosomycin antibiotics, bind at a site close to where the initiation factor IF2 interacts and resistance to this group of antibiotics has been developed in livestock isolates due to the methylation of G2470 (27), while the antibiotic producing strain immunizes itself with two MTases acting within the antibiotic binding region – one methylates G2535, and the other targets 2'-O-ribose of U2479 (28). The third group of antibiotics is a chemically diverse group that comprises macrolide, lincosamide, and streptogramin B (MLS_B) compounds. The MLS_B antibiotics bind to overlapping sites within the 50S ribosomal subunit tunnel close to the peptidyl transferase center, either directly inhibiting catalysis at the peptidyl transferase center, and/or acting as a physical barrier to the growth of the peptide chain within the tunnel (24). Macrolides have yet another inhibitory effect in their repertoire and block the manufacture of new 50S ribosomal subunits by binding to precursor particles as they are assembled from their r-protein and rRNA components (29).

Specific methylation of the N6 position of nucleotide A2058 in the peptidyl transferase loop within domain V of 23 S rRNA is catalyzed by members of the Erm family of MTases (24, 30, 31). A2058 and nucleotides that are nearby in the primary and higher-order rRNA structures are involved in the binding of erythromycin and other MLS_B antibiotics (32–35). Mutations at these nucleotides or modification by Erm MTases confer antibiotic resistance (36–38), presumably by reducing the strength of the drug–rRNA interaction (39). All structural features that are required for methylation by the Erm MTases are contained within domain V of the rRNA (40–42).

Erm MTases differ according to whether they add one or two methyl groups to A2058 (43, 44). The first class includes those that monomethylate adenine, e.g. Lrm from *Streptomyces lividans* (45), Clr from *Streptomyces caelestis* (46), and TlrD (ErmN) from *Streptomyces fradiae* (21). The second class includes those that predominantly dimethylate adenine, e.g. ErmC from *S. aureus* (47), ErmE from *Saccharopolyspora erythrea* (*Streptomyces erythraeus*) (46), and TlrA (ErmSF) from *S. fradiae* (48). Monomethylation confers the so-called MLS_B type I phenotype, with high resistance to lincosamides, low-to-moderate resistance to macrolide and streptogramin B antibiotics (43), but no resistance to ketolides such as telithromycin (49). Erm dimethyltransferases confer the MLS_B type II phenotype, with high resistance to all MLS_B antibiotics and to telithromycin (43), and this is a more common resistance mechanism in bacterial pathogens. Dimethylation at the N6 of A2058 occludes the main contact site for all MLS_B drugs and constitutes the most effective form of resistance against these drugs. It is surprising therefore that, in addition to an Erm dimethyltransferase, some bacteria have their own idiosyncratic resistance mechanisms. For instance, the tylosin-producing actinomycete *Streptomyces fradiae* has a combination of Erm mono- and dimethyltransferases (21, 48) in addition to a third methyltransferase ($RlmA^{II}$) that is specific for the N1 of nucleotide G748 (50). The Erm dimethyltransferase is first expressed when tylosin levels are relatively high. Up to this point, tylosin resistance is conferred by the synergistic action of the Erm monomethyltransferase and $RlmA^{II}$, with neither methylation on its own causing any appreciable reduction in tylosin binding (49). The N1 of G748 points into the lumen of the 50S ribosomal subunit tunnel facing nucleotide A2058 approximately 15 Å away and the resistance mechanism is explained by the position of tylosin in its binding site (51). It is not clear why *S. fradiae* has retained this array of rRNA MTases, and the methylations could possibly have other functions, such as facilitating the passage of the nascent peptide through the tunnel.

16S rRNA methyltransferase

The aminoglycosides are a structurally diverse family of poly-cationic compounds containing a central aminocyclitol ring, most frequently 2-deoxystreptamine or streptamine, connected via glycosidic bonds to amino sugars. They can be conveniently divided into three structural classes based on the position of these bonds. First class includes 4,6-disubstituted 2-deoxystreptamines (4,6-DOS) like kanamycin, and most clinically useful aminoglycosides, such as gentamicin, tobramycin, amikacin and netilmicin. Aminoglycosides with 4,5-disubstituted 2-deoxystreptamine (4,5-DOS) as in neomycin, ribostamycin and paromomycin belong to the second class. The third class consists of those compounds that do not fit into either of the previously described groups, such as 4-monosubstituted 2-deoxystreptamines (apramycin, neamine), streptomycin, hygromycin B, and spectinomycin. Aminoglycosides are widely used in clinical practice, as the antibiotics of choice, especially for the treatment of life-threatening infections caused by Gram-negative and Gram-positive bacteria.

Aminoglycosides inhibit the translation process by causing misreading and/or hindering of the translocation step. It is believed that the fidelity of translation depends on two steps, an initial recognition

between the codon of the mRNA and the anticodon of a charged tRNA, and subsequent proofreading (52). Kanamycin, gentamicin, neomycin, and paromomycin are believed to bind in a similar fashion to the base of 16S rRNA helix 44, that together with portions of the 530 loop and helix 34 form the tRNA acceptor aminoacyl site (A-site) (*Figure 1A*). It has been shown that the aforementioned antibiotics have two binding sites in common – bases A1408 and G1494 (*Figure 1B*). On the other hand, tRNA interacts with four bases, A1408, A1492, A1943, and G1494, in the 16S rRNA A-site (53). Binding of aminoglycosides to the A-site on the 16S rRNA mimics the conformation adopted by the 16S rRNA fidelity gatewatch nucleotides A1492 and A1493 in the presence of cognate tRNA-mRNA codon association; therefore they shunt a molecular switch and cause a loss of translational fidelity (54).

Aminoglycosides producers

Many aminoglycoside-producing bacteria protect themselves from the toxic effects of antibiotics by methylating a specific nucleotide in antibiotic-binding sites of the ribosome, thus disrupting the antibiotic binding without much interference with other functions of the ribosome. Two distinct groups of 16S rRNA aminoglycoside resistance MTases, Kgm and Kam families, conferring resistance to overlapping sets of aminoglycosides, have been distinguished based upon their target nucleotides, G1405 or A1408, respectively (55, 56). Methylation of these two nucleotides affects drug binding not only by steric hindrance but also by charge repulsion.

The KgmB (kanamycin-gentamicin methyltransferase) from *Streptoalloteichus tenebrarius* (57), formerly *Streptomyces tenebrarius*, a producer of nebramycin complex (58), and the Sgm (sisomicin-gentamicin methyltransferase) from *Micromonospora zionensis*, a producer of G-52 (59), modify the N7 position of G1405, in the A-site of 16S rRNA (22, 56). Closely related GrmA (gentamicin-resistance methyltransferase), found in the gentamicin-producing strain *Micromonospora echinospora* (formerly known as *Micromonospora purpurea*) (60) also catalyze the modification of G1405 at the N7

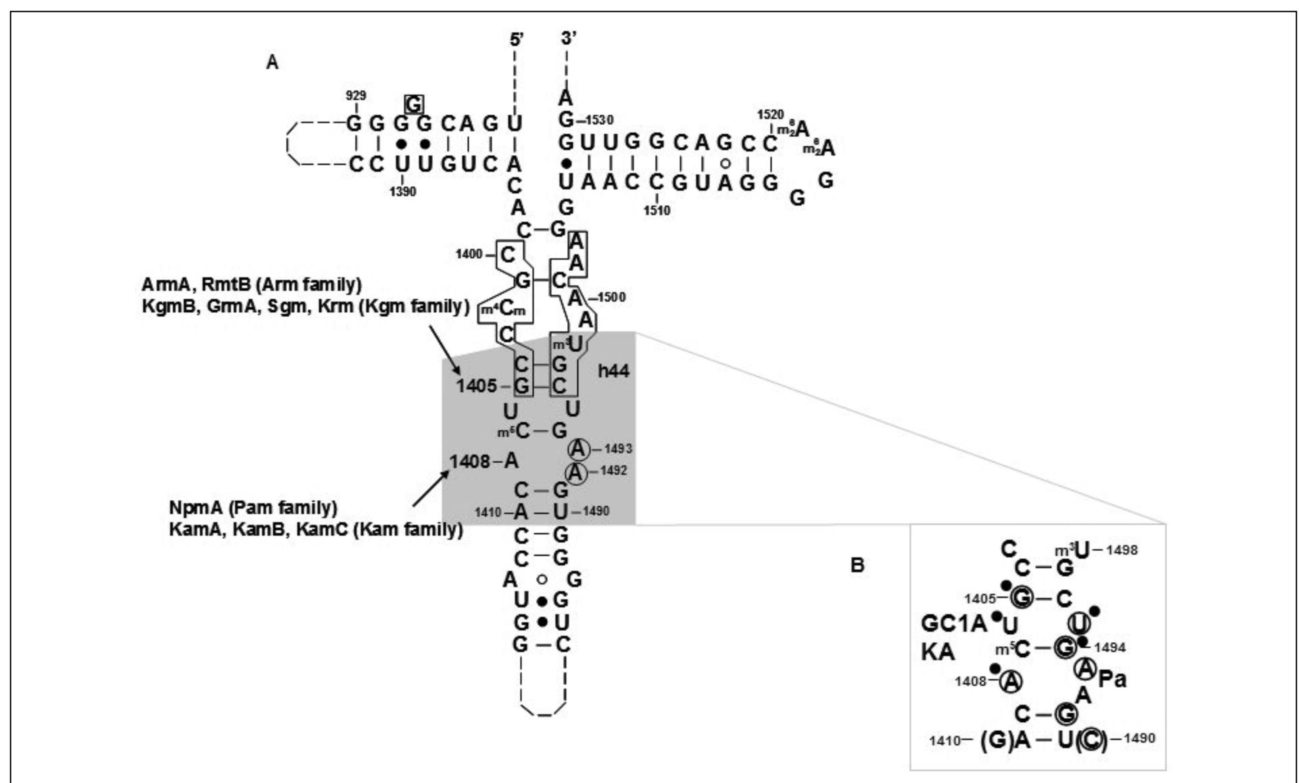

Figure 1 Secondary-structure model of the 16S rRNA A-site and aminoglycoside binding.

E. coli 16S rRNA secondary structure model is presented. A) Experimentally determined G1405 target site for Arm (ArmA and RmtB MTases) and Kgm (KgmB, GrmA and Sgm MTases) families is indicated by arrow as well as A1408 target site for Pam (NpmA MTase) and Kam (KamA-C MTases) families. Basis of helix 44 is shaded. A-site nucleotides (A1492 and A1493) important for tRNA anticodon–mRNA interaction are shown in circles. P site nucleotides, responsible for binding of peptidyl tRNA, are framed. The sites of post-transcriptional »house-keeping« modifications in this *E. coli* rRNA region are indicated.

B) Sequence and secondary structure of the bacterial decoding site is expanded. Nucleotides involved in paromomycin ('Pa') binding are shown in circles, nucleotides involved in gentamicin C1A ('GC1A') and kanamycin A ('KA') binding are marked with black dots. Paromomycin binds C1490, however used 16S rRNA *E. coli* model has A1410:U1490 pair instead of G1410:C1490, which is given in brackets.

position (56). These enzymes provide high-level resistance to 4,6-DOS. The Krm MTase (kanamycin-resistance methyltransferase) from *Frankia* sp. Ccl3 has been shown to methylate the same position in the A-site (*Figure 1A*) (56). All aforesaid MTases belong to Kgm family.

According to predicted site of action (G1405), several other MTases from the antibiotic-producing organisms are Kgm family members, such as FmrO from *Micromonospora olivasterospora*, (61), Grm from *M. rosea* (60), Srm1 from *M. inyonensis* (GenBank accession number AY661430), and *NbrB Streptomyces hindustanus* (GenBank accession number AF03808).

The KamA MTase from *Streptomyces tenjimariensis* acts at the N1 position of A1408 conferring resistance to kanamycin, tobramycin, sisomicin, and apramycin but not to gentamicin (22). The exact same site of the methylation is also confirmed for the KamB MTases from *Streptoalloteichus tenebrarius* (56) and KamC MTases from *Saccharopolyspora hirsuta* (62) (*Figure 1A*). These MTases constitute the Kam family of producers' MTases.

The Kgm MTase family members share among themselves 33.2–58.0% amino acid sequence identity, except for Sgm and GrmA, which share sequence identity of 89.8%. When compared to the KamB MTase, the identity ranges from 22.0% (Krm MTase) to 29.4% (KgmB MTase).

It is worth noting that resistance to some members of the third class of aminoglycosides, such as spectinomycin, streptomycin, and hygromycin B, is not conferred through 16S rRNA methylation but by other resistance mechanisms.

Pathogenic bacteria

Since the introduction of antibiotics into clinical practice, bacteria have struck back and collected an array of resistance mechanisms that have spread extensively, forcing physicians to reconsider their prescriptions (63). Although most of the resistance mechanisms to antibiotics among antibiotic-producing strains and pathogenic bacteria are shared, certain mechanisms were considered to be unique to either of them. Until recently, it was thought that antibiotic target site methylation is exclusively conveyed to antibiotic-producing bacteria. During the last decade, six plasmid-mediated MTases that confer high-level resistance against a broad spectrum of aminoglycosides were discovered in pathogenic bacteria isolated from human and animal specimens. Two distinct groups of resistance MTases have been distinguished, based upon their target nucleotide preferences. Five of them – ArmA, RmtA, RmtB, RmtC, and RmtD, that confer resistance to 4,6- but not to 4,5-DOS antibiotics, have been globally found in *Enterobacteriacea*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Proteus mirabilis* and *Acinetobacter* spp., and they are classified as the Arm MTase family (Aminoglycoside Resistance Methyltransferase) (64–69). It has been experimentally determined that only two members of the Arm family – ArmA and RmtB, methylate A1405 position at the ribosomal A-site (70), similarly to Kgm MTase family from producers. Unlike Arm family members, a 16S rRNA MTase identified in clinically isolated *E. coli*, NpmA, is responsible for resistance to both 4,5-DOS and 4,6-DOS aminoglycosides, and was shown to modify A1408 nucleotide (69), just like the Kam MTase family from producers. NpmA MTase is not just the sole member of the Pam (Pan-Aminoglycoside Methyltransferase) family, but also the only aminoglycoside resistance MTase that confers resistance to both 4,5- and 4,6-DOS antibiotics.

In general, it is now accepted that two 16S rRNA methylations, at G1405 and A1408 position, confer resistance to overlapping sets of aminoglycoside antibiotics regardless of the origin of the MTase (55, 71). *In silico* comparison has revealed variation of identity at the amino acid sequence level among the members of Arm resistance MTase family, as well as when they have been compared to NpmA MTase, ranging from 25.5–82.0% and 20.0–44.8%, respectively, indicating that not all of them share the same ancestor, at least not in recent past. Evaluating the identity among MTases from pathogens and antibiotic-producers, the differences in their origin have become evident. Shared sequence identity of 18.3–35.8% indicates that if they have had a common ancestor, it is most likely distant. On the other hand, phylogenetic analysis of two groups of 16S rRNA resistance MTases (G1405 and A1408) supports the possibility that these groups, regardless of the source of the enzyme, evolved from common ancestors (*Figure 2A* and *2B*).

Taken together, regardless of the origin of 16S rRNA MTase families, they constitute a unique superfamily of resistance 16S rRNA MTases, named Rma (Resistance Methyltransferases for Aminoglycosides) (55).

From fundamental research to application

Antibiotics are probably the most successful form of chemotherapeutics developed in the twentieth century and perhaps over the entire history of medicine. On the other hand, they are class of drug with built-in obsolescence, due to continued growth of resistance among microorganisms in the presence of cytotoxic concentration of antibiotics. Resistance has been a continuing problem since antibiotics were introduced into clinical practice and numerous factors threaten the future clinical use of antibiotics, including multi-antibiotic resistant bacteria (73–75).

Aminoglycosides are among the most commonly used broad-spectrum antibiotics in the battle against microorganisms. Methylation of 16S rRNA nucleotides, an effective means of conferring resistance to antibiotics targeting the bacterial ribosome, that evolved as a self-defense mechanism in aminoglycoside-producing bacteria, is on the increase in pathogenic bacteria, and it has rendered many commonly used aminoglycosides therapeutically ineffective. Combating bacterial infections that are resistant to aminoglycoside treatment includes the search for new antibiotics or modification of existing ones that would be preferentially able to bind to the ribosome despite the presence of methylated rRNA nucleotides. Resolved crystal structures of the ribosomes and the antibiotics binding sites could be a basis for improvement of existing drugs or for novel drug design. Another potential solution to the problem, though a considerably greater challenge, would be in designing novel compounds that would act against new ribosomal targets. Many chemically diverse antibiotic compounds, including aminoglycosides, interfere with the synthesis of proteins, but they target the ribosome at surprisingly few locations, which results in overlap between many of their binding sites. It is necessary to emphasize that it is not known whether there are other possible sites of inhibition on the ribosome, besides those few already identified, that remain undiscovered (14). The next approach in addressing the issue of raising resistance to aminoglycosides requires in-depth understanding of MTase expression and its mechanism of action, resolution of a tertiary structure of 16S rRNA MTase with its cofactor, and structure of 30S-MTase complex in order to develop MTase inhibitors. The novel, inhibitory drugs ought to prevent the methylation of bacterial 16S rRNA, and thus restore the therapeutic power of existing aminoglycosides. Comparing the

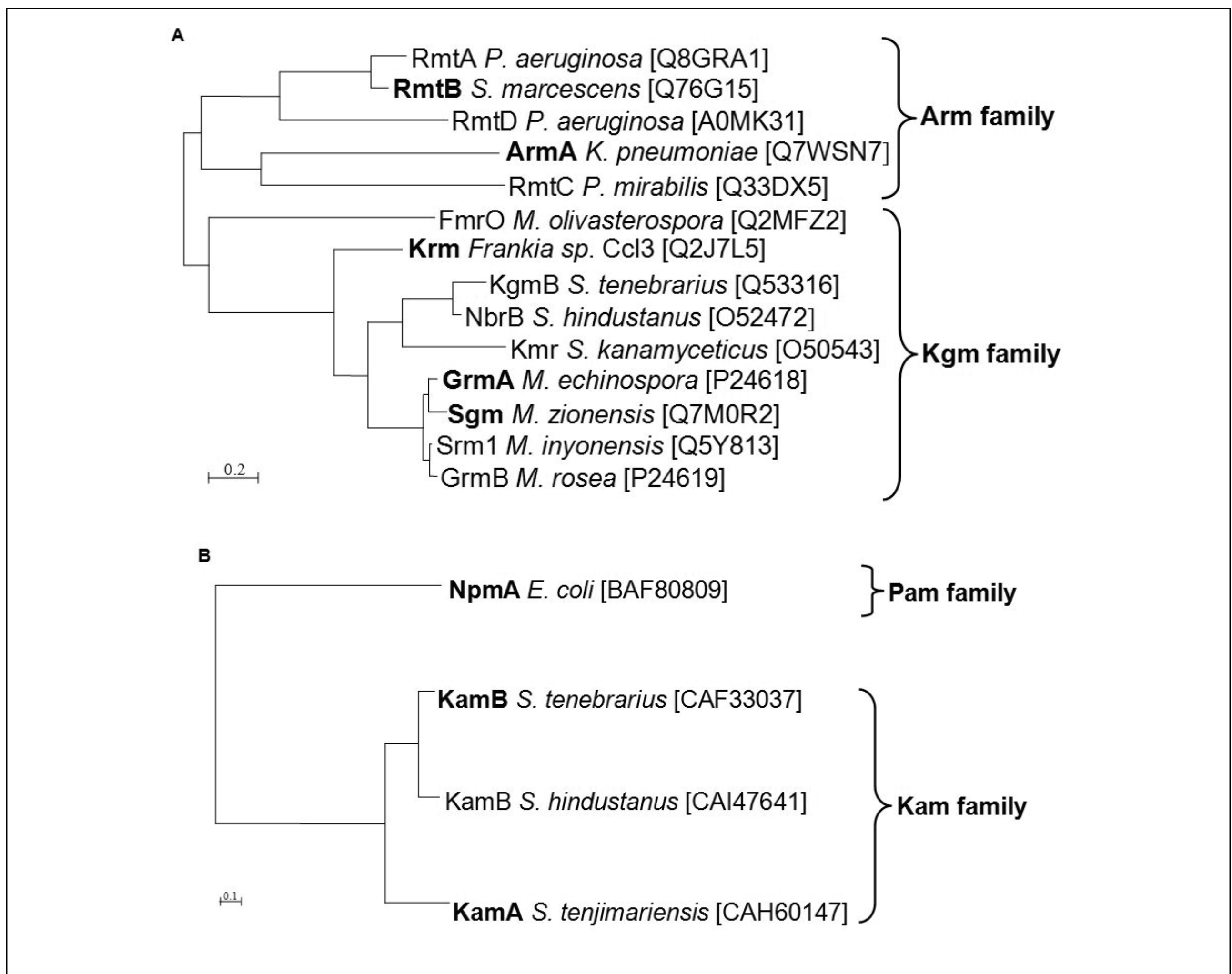

Figure 2 Phylogenetic relationship of 16S rRNA resistance MTase families.

A) G1405 MTases (Arm and Kgm families) and B) G1408 MTases (Pan and Kam families). The bar represents amino acids substitutions per position. In bold letters are indicated MTases whose target nucleotide has been experimentally determined. The numbers given in parentheses are UniProtKB/TrEMBL database protein identification numbers. Phylogenetic analysis has been performed using maximum likelihood method (PHYML) at Pasteur Institute (72; http://mobyle.pasteur.fr/cgi-bin/portal.py).

above-mentioned strategies to reinstate the power of aminoglycoside antibiotics, it seems that the most promising and most feasible approach is the development of enzyme inhibitors that confer high level resistance to aminoglycosides, as there is a significant and still growing body of knowledge related to MTases.

Although several Kgm and Kam MTase genes have been cloned in the last two decades, just a small number of the encoding enzymes have been partially characterized until recently, when a breakthrough has been made. Having in mind the emergence and worldwide spreading of 16S RNA MTases and their effect on the usefulness of aminoglycoside antibiotics, fundamental research related to MTases driven resistance mechanisms has gained new significance. Since the Sgm MTase from *Micromonospora zionensis* has been cloned in our laboratory (59), the focus of our research was not just on the in-depth understanding of Sgm gene expression regulation (76), but also on the deciphering of details of the exact mechanism of Sgm MTase action.

Very little direct experimental evidence for target site modification existed for the majority of 16S rRNA MTases for a long time. Until recently, the only target sites that have been experimentally determined were G1405 for KgmB from *Streptoalloteichus tenebrarius* (62), and A1408 for KamA and KamC from *Streptomyces tenjimariensis* and *Saccharopolyspora hirsute*, respectively (22, 62). The site of action for other MTases has been usually inferred indirectly by their inability to further methylate ribosome subunits already protected by one of these enzymes, just as we have shown it for Sgm MTase to be G1405 (77). Even more frequently, resistance profiles were used to assume the site of action for particular MTase, although systematic analysis of the resistance profiles is lacking. Soon after circuitously proving that Sgm MTase shares the same site of modification with KgmB, we have experimentally determined that G1405 is the Sgm target nucleotide, as well as for GrmA from *M. echinospora* and Krm from *Frankia* sp. CcI3 (56). Methylation sites have also been identified for functionally equivalent MTases from isolates of bacterial pathogens, as G1405 for ArmA and RmtB, and A1408 for NpmA (69, 78, 79). The importance of these data is in that they provide a more secure and systematic basis for the classification of new aminoglycoside resistance MTases from producers and pathogenic bacteria based on their sequences and resistance profiles, thus enabling prompt and accurate deduction of their sites of action.

Very limited biochemical data on actinomycetes' G1405 MTases that were recently improved by probing the Sgm MTase from *M. zionensis* paved the path for inhibitor(s) development. These studies have demonstrated the existence of two structural domains within Sgm (80) – the smaller, N-terminus is most likely involved in target site recognition and binding, while the larger, C-terminus is responsible for cofactor binding and catalysis (80, 81). Based on *in silico* sequence analysis and modeling, the key amino acids were mutated and we identified three functional classes of amino acids responsible for SAM binding, target recognition and methyl group transfer (81–83). Identification of important amino acids for reaction catalysis and rRNA binding provides essential data necessary for the design of specific inhibitors that could be used together with aminoglycosides to treat infections due to bacteria resistant to this class of antibiotics.

Although the Sgm was the first 16S resistance MTase to be analyzed in detail, crystal structures have been originally determined for ArmA and RmtB MTases, the members of Arm resistance family (70). It has been established that both of them are composed of two domains, N- and C- terminus, with the same functions as in Sgm MTase. Not long after, the Sgm crystal structure was resolved, and it has become the first determined structure of 16S rRNA MTases from antibiotic producers (84). Also, for the first time the interactions between G1405 MTase and its substrate, the 30S subunit, were characterized (84). It has been learned that conformational change of the 30S subunit upon Sgm binding involves the rearrangement of helix 44, and most likely base-flipping of G1405. The finding that 16S rRNA resistance MTases exhibit different modes of protein–ligand interaction in comparison with other physiologically important methylations of G at N7 position, suggests that specific inhibitor(s) can be developed.

Taken altogether, detailed analysis of Sgm, ArmA, and RmtB can serve as a starting point towards developing drugs that would specifically block the activity of resistance MTases and thereby make aminoglycosides useful again.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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