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Ribosome-targeting antibiotics and mechanisms of antibiotic resistance

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LIST OF PUBLICATIONS

The current dissertation is based on the following original publications, which will be referred to by the corresponding Roman numbers in the text:

I. **Allas Ü**, Tenson T. (2010). A method for selecting *cis*-acting regulatory sequences that respond to small molecule effectors. BMC Mol Biol 11: 56.

II. Li W, Atkinson GC, Thakor NS, **Allas Ü**, Lu CC, Chan KY, Tenson T, Schulten K, Wilson KS, Hauryliuk V, Frank J. (2013). Mechanism of tetracycline resistance by ribosomal protection protein Tet(O). Nat Commun 4: 1477.

III. **Allas Ü**, Toom L, Selyutina A, Mäeorg U, Medina R, Merits A, Rinken A, Hauryliuk V, Kaldalu N, Tenson T. (2016). Antibacterial activity of the nitrovinylfuran G1 (Furvina) and its conversion products. Sci Rep 6: 36844.

Author's contribution

I. Performed all experiments, analyzed results and participated in writing the manuscript.

II. Conducted mutagenesis and measured effects of mutations on Tet(O) functionality, participated in writing the manuscript.

III. Performed colorimetric measurements as well as all susceptibility tests with bacteria and yeast, participated in writing the manuscript.

LIST OF ABBREVIATIONS

aa-tRNA – aminoacyl-tRNA
ATP – adenosine triphosphate
CAMHB – cation-adjusted Mueller-Hinton broth
CAT – chloramphenicol acetyltransferase
DMSO – dimethyl sulfoxide
DOS – 2-deoxystreptamine
EF-G – elongation factor G
EF-Tu – elongation factor thermo unstable
FDA – The Food and Drug Administration
fMet – N-formylmethionine
GDP – guanosine diphosphate
GFP – green fluorescent protein
GTP – guanosine triphosphate
HPLC – high-performance liquid chromatography
HSL – N-(3-oxohexanoyl)-L-homoserine lactone
IF2 – prokaryotic initiation factor-2
IMDM – Iscove's Modified Dulbecco's Medium
MIC – minimum inhibitory concentration
NMR – nuclear magnetic resonance
ORF – open reading frame
P_i – inorganic phosphate
PrAMP – proline-rich antimicrobial peptide
PTC – peptidyl transferase center
RBS – ribosome-binding site
RPP – ribosomal protection protein
SAM – S-adenosylmethionine
SD – Shine-Dalgarno sequence

INTRODUCTION

Bacterial ribosomes represent one of the most important targets for antibiotics in the cell and several larger and smaller classes of protein synthesis inhibitors have been discovered or designed. Many years of biochemical and structural studies have revealed the molecular basis by which antibacterial compounds inhibit translation. Tetracyclines and most of aminoglycosides bind to the decoding site of the 30S ribosomal subunit and interfere with its ability to decipher the genetic information encoded in the mRNA. The 50S subunit has three major antibiotic binding sites. One of them is the peptidyl-transferase centre (PTC) where the peptide bond is being catalyzed between the growing polypeptide chain attached to the peptidyl tRNA and the amino acid on the incoming aminoacyl-tRNA. Antibiotics such as phenicols, oxazolidinones, tiamulin and streptogramin A interfere with the binding of tRNA into the PTC or inhibit peptide bond formation. The second prominent target site on the 50S subunit is the protein exit tunnel. The tunnel serves as a binding site for macrolides, lincosamides and streptogramin B antibiotics. These drugs inhibit protein synthesis by interfering with the progression of the nascent peptide. Another target for antibiotics is the GTPase-associated region that provides a docking site for elongation factors and coordinates GTP hydrolysis during protein synthesis. This region acts as a binding site for the thiopeptide antibiotics such as thiostrepton. Considering the chemically large diversity of antibiotics, they target the ribosome at surprisingly few locations.

Protein synthesis can be divided into four distinct phases (initiation, elongation, termination and recycling). Regulation of translation occurs largely at the level of initiation and is often accomplished by small proteins or RNA molecules that bind to the mRNA. However, in some cases translation is regulated via nascent polypeptide-mediated ribosome stalling. This regulation mechanism may require the presence of a small ligand molecule that specifically interacts with the nascent peptide inside the ribosomal exit tunnel. The first part of my experimental work describes a novel method for selecting peptides capable of inhibiting translation in response to different chemicals. We believe that applying our method on a larger scale could contribute to identification of the sequence rules underlying the activity of *cis*-acting regulatory peptides. In addition, this method might be applicable in the development of novel gene expression systems.

Since their discovery in the 1930s, antibiotics have saved a countless number of lives. However, recent years have seen events that are more characteristic to the pre-antibiotic area. The most well-known problem is the spread of drug-resistant “superbugs”. It is very likely that resistance to even a new class of antibiotics will be observed already within a few first years of marketing. Genes encoding components of resistance machinery are often located on mobile genetic elements and can be transferred between bacteria via horizontal gene transfer. Bacteria use a great variety of mechanisms to achieve resistance to

antibiotics. Among these, ribosomal protection represents a unique tactics for promoting tetracycline resistance. Ribosomal protection proteins are known for their ability to dislodge tetracycline from the ribosome. The second part of my experimental work explores the molecular mechanism of this important type of resistance.

As the emergence and spread of multidrug-resistant pathogens continues to be one of the major public health concerns, the development of new antimicrobials has become an important issue. While 30 new antibiotics have been launched worldwide since the year 2000 and many promising drug candidates are currently in clinical trials, development of many compounds has been discontinued due to different reasons. On the other hand, some antimicrobial agents are used clinically, although their mechanism of action is still a matter of debate. An example of such drugs is the putative translation inhibitor nitrovinylfuran G1 (or Furvina®) that is already many years medically used in Cuba. The third part of my experimental work focuses on aspects concerning antibacterial activity and stability of G1 compound.

1. REVIEW OF LITERATURE

1.1. Major classes of bacterial translation inhibitors

Antibiotics possess three main targets or pathways in bacteria: the ribosome, cell wall synthesis and DNA gyrase or DNA topoisomerase. The current thesis focuses on antibiotic classes that target the bacterial ribosome and interfere with microbial protein synthesis.

Ribosomes are macromolecular machines that convert the genetic information encoded in the messenger RNA into proteins. Considering the essential function of protein synthesis, it is not surprising that this process is a target for many antibacterials. The majority of translation inhibitors affect the translation elongation cycle. Nevertheless, almost every step of bacterial protein synthesis can be inhibited with antibiotics (Wilson, 2009). Many translation inhibitors are clinically important drugs.

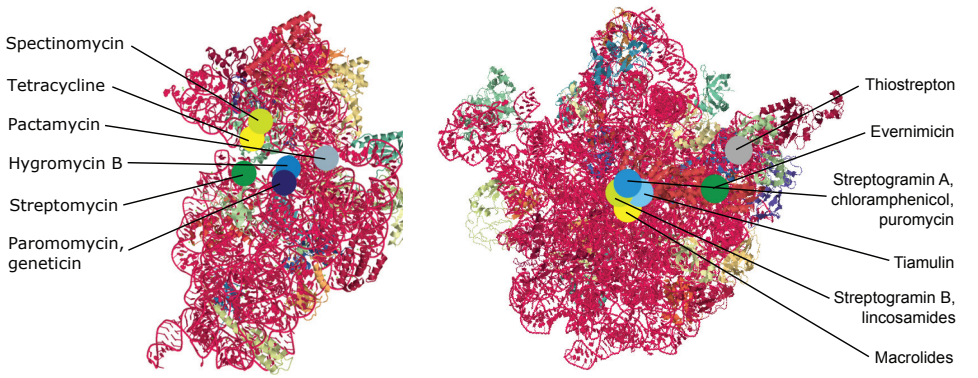


Figure 1. Antibiotic binding sites on 30S and 50S ribosomal subunits. The figure is adapted and modified from Poehlsgaard and Douthwaite (2005).

1.1.1. Tetracyclines

Tetracyclines (Figure 2) are known as historically first described group of antibiotics possessing broad-spectrum antibacterial activity. In addition to inhibiting growth of Gram-positive and Gram-negative bacteria, they are also active against mycobacteria, spirochetes, and malaria-causing protist *Plasmodium falciparum*.

First reported tetracyclines were chlortetracycline and oxytetracycline, produced by soil bacteria *Streptomyces aureofaciens* and *Streptomyces rimosus*, respectively. Chlortetracycline was discovered in 1948 by Benjamin Duggar (American Cyanamid) while oxytetracycline was isolated in the early 1950s by Alexander Finlay (Pfizer) (Duggar, 1948; Finlay et al., 1950). After the chemical structures of both antibacterials were solved in 1953, the Pfizer chemists

chemically modified chlortetracycline and generated an antibiotic tetracycline. However, tetracycline was soon found also from spent broth of both *S. aureofaciens* and *S. rimosus* (Backus et al., 1954; Perlman et al., 1960). Subsequent studies revealed that tetracycline is a precursor of chlortetracycline (McCormick et al., 1960).

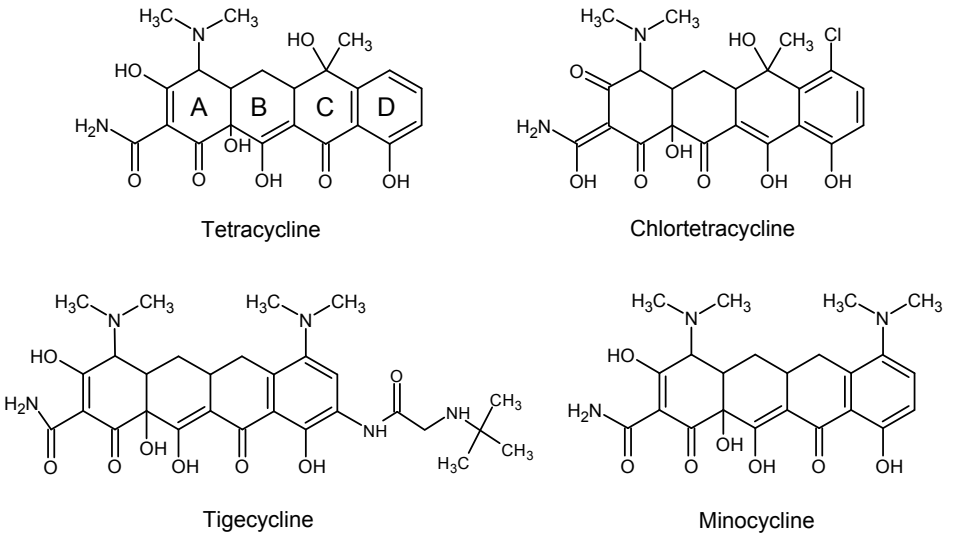


Figure 2. Chemical structures of tetracycline antibiotics.

The era of second-generation tetracyclines started with the emergence of semisynthetic tetracycline derivatives. The most well-known semisynthetic tetracyclines doxycycline and minocycline were introduced in 1967 and 1972, respectively (Chopra and Roberts, 2001). The semisynthetic analogs appeared to be more lipophilic, thus being able to penetrate bacterial cell membranes more efficiently. They also demonstrated improved antimicrobial activity, higher affinity to the ribosome and decreased toxicity (Nguyen et al., 2014). Nevertheless, the importance of tetracyclines in human medicine started to diminish mainly because of the rise of antibacterial resistance, and the appearance of more effective drugs. Still, in 2006, tigecycline was approved by FDA and became clinically available. Tigecycline is classified as a member of third-generation tetracycline family, also known as glycycyclines (Peterson, 2008). The glycycyclines bear N,N-dimethylglycylamido moiety at the C9 position of carbocyclic skeleton. They are promising drugs because they have improved ribosome binding properties and they retain activity against some bacterial strains that contain *tet* resistance genes (Bauer et al., 2004., Bergeron et al., 1996). Two additional glycycyclines, omadacycline and fully synthetic eravacycline, are currently in Phase III clinical trials (Sun et al., 2015).

Tetracyclines are structurally similar as they all contain a 19-carbon four-ring cyclic skeleton. The members of tetracycline family differ from each other

by the structural variations, which mainly affect the C5, C6 or C7 carbons of the molecule. The naphthacene core of tetracyclines is formed from a starter molecule acetyl-CoA. Ketosynthase and other enzymes are responsible for the iterative condensation of 8 molecules of malonyl-CoA to yield the polyketone backbone of tetracycline (Pickens and Tang, 2009).

Members of tetracycline family may be divided into two classes according to their mode of action: (1) "typical" tetracyclines (e.g. oxytetracycline and minocycline), which are bacteriostatic and inhibit prokaryotic protein synthesis, and (2) "atypical" tetracyclines (e.g. chelocardin and 6-thiatetracycline), which interfere with electrochemical gradient of the bacterial cell membrane and induce cell lysis, thus being bactericidal.

The crystal structures of tetracycline in complex with the *Thermus thermophilus* 70S ribosome and with the 30S ribosomal subunit revealed that the binding site of the drug is located near the A-site where it partly overlaps with the anticodon stem-loop of the tRNA (Brodersen et al., 2000; Jenner et al., 2013; Pioletti et al., 2001). The binding pocket of tetracycline is formed by the irregular minor groove of helix 34 of 16S rRNA (rRNA residues 1054–1056 and 1196–1200; *Escherichia coli* base numbering is used throughout) in combination with residues 964–967 of helix 31 of 16S rRNA. The lower peripheral region of tetracycline molecule forms hydrogen bonds with the sugar-phosphate backbone of rRNA residues that constitute the binding pocket. The contacts are therefore sequence-independent, thus explaining the broad-spectrum activity of the drug. The binding of tetracycline to the ribosome is coordinated through a magnesium ion that facilitates interaction with the phosphate backbone of helix 34 (White and Cantor, 1971; Brodersen et al., 2000). It has been proposed that another magnesium ion mediates the interaction between the phosphate backbone of G966 in helix 31 and the ring A of tetracycline (Jenner et al., 2013). Tigecycline has been shown to bind 30S subunit analogously to tetracycline (Jenner et al., 2013).

Tetracyclines are inhibitors of translation elongation cycle. In the presence of tetracycline, aminoacyl-tRNA:EF-Tu:GTP ternary complex is still able to bind the ribosome. Tetracycline does not interfere with codon-anticodon interaction and GTP hydrolysis by EF-Tu (Brodersen et al., 2000; Gordon, 1969). However, tetracycline blocks progression of aminoacyl-tRNA from the initial codon recognition state to the A-site of the ribosome (Blanchard et al., 2004). As a result, aminoacyl-tRNA is prematurely released and the elongation cycle terminates without peptide bond formation. Tetracyclines can make protein synthesis energetically expensive for cells, as GTP hydrolysis by EF-Tu occurs without peptidyl transfer (Brodersen et al., 2000).

In addition to the primary binding site, biochemical and X-ray crystallography studies have revealed five other tetracycline binding sites at different locations on the head and the body of 30S subunit (Oehler et al., 1997; Pioletti et al., 2001). According to current knowledge, these secondary binding sites are not involved in translation inhibitory mechanism as resistance to the drug has

been shown to occur through mutations of nucleotides within the primary binding site (Gerrits et al., 2002).

Resistance to tetracyclines can be conferred by the unique ribosome protection proteins, such as Tet(M) and Tet(O). Membrane-bound efflux proteins, including Tet(A), Tet(B), Tet(K) and Tet(L) can similarly be responsible for the resistance. Alternative resistance mechanisms involve mutations in 16S rRNA as reported in *Helicobacter pylori* and inactivation of the drug by hydroxyase Tet(X) (Wu et al., 2005; Yang et al., 2004).

1.1.2. Aminoglycosides

Aminoglycosides (Figure 3) are active against many aerobic Gram-negative and Gram-positive pathogens, including *Klebsiella pneumoniae*, *Yersinia pestis* and *Haemophilus influenza*. These drugs are also efficient for curing infections caused by *Pseudomonas* and *Acinetobacter* that are not effectively treatable with other antibiotics. It is a common practice to use aminoglycosides in combination with other antibiotics in order to reduce microbial resistance. They are poorly absorbed via the gastrointestinal tract and, thus, are preferably administered via the intramuscular or intravenous route (Craig 2011). Inhaled delivery of aminoglycosides has become an area of renewed interest and inhaled tobramycin is available in the European Union. Aminoglycosides are bactericidal, which is uncommon among antibiotics acting as inhibitors of protein biosynthesis.

The first member of aminoglycoside group was isolated in 1943 by Albert Schatz who was working in Selman Waksman's laboratory at Rutgers University. It was a fermentation product of *Streptomyces griseus*, hence the name streptomycin (Schatz et al., 1944). Streptomycin was the first antibiotic that was successfully used to cure tuberculosis. Intensive research leaded to the discovery of other natural aminoglycosides, such as neomycin (1949), kanamycin (1957), gentamicin (1963) and tobramycin (1967). The spread of microbial resistance to aminoglycosides and their obvious toxicity were the ground for improving pharmacological profile of aminoglycosides. These efforts led to introduction of semisynthetic derivatives in 1970s. Amikacin (1972), arbekacin (1973) and isepamicin (1975) are some examples of these second-generation aminoglycosides.

Naturally occurring aminoglycosides are products of secondary carbohydrate metabolism. They are mostly produced by the genuses *Streptomyces* and *Micromonospora* and carry suffixes "-mycin" or "-micin", respectively. An exception is butirosin, which is produced by *Bacillus circulans* (Woo et al., 1971).

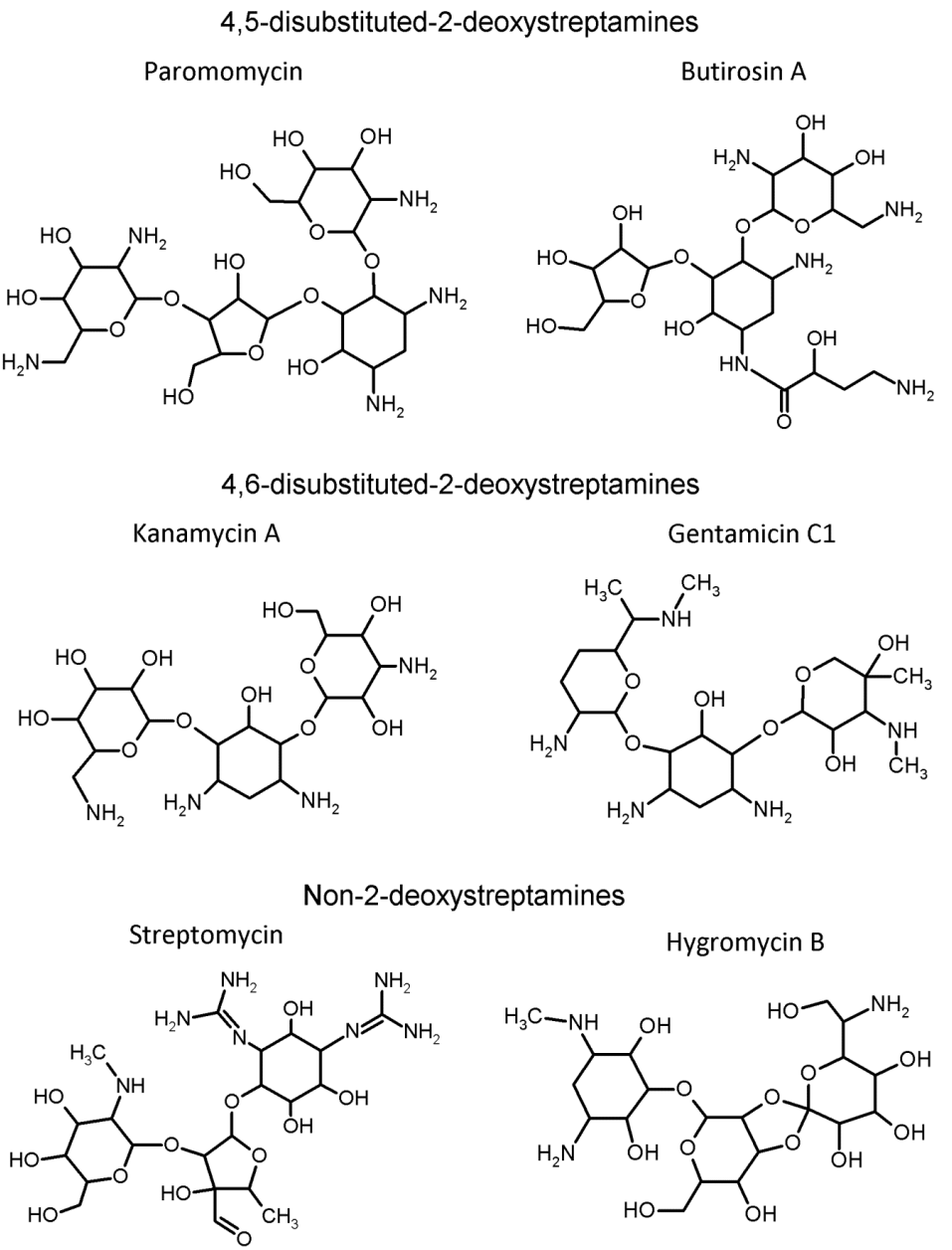


Figure 3. Structures of representative aminoglycoside antibiotics.

Aminoglycosides can be divided into 3 families according to their chemical structure.

(1) 4,5-disubstituted-2-deoxystreptamines

Their molecule contains an aminocyclitol 2-deoxystreptamine (DOS), which is disubstituted at C4 and C5 positions. Members of this family can be either pseudotrisaccharides (butirosin, ribostamycin) or pseudotetrasaccharides (neomycin, lividomycin, paromomycin).

(2) 4,6-disubstituted-2-deoxystreptamines

This aminoglycoside family is clinically most widely used. These drugs contain sugars at C4 and C6 positions of DOS. Gentamicin, kanamycin, tobramycin and amikacin are prominent members of this family. They are further divided into groups based on their sugar substituents.

(3) Non-2-deoxystreptamine aminoglycosides

These molecules contain an aminocyclitol other than 2-deoxystreptamine. Well-known members of this group are streptomycin and its derivatives. The molecule of streptomycin can be categorized as a pseudotrisaccharide. It contains a 6-carbon aminocyclitol called streptidine. Sugar derivatives pentose (L-streptose) and glucosamine are linked to aminocyclitol ring via glycosidic bonds.

Up to date, a number of aminoglycosides have been crystallized in complex with 70S ribosome, 30S ribosomal subunit or small RNA fragments mimicking helix 44 of 16S rRNA. Aminoglycosides are a large group of antibiotics whose members display diversity in binding to the target as well as in their mechanism of action. Aminoglycosides have been shown to inhibit translational fidelity (streptomycin, paromomycin, geneticin), translocation (hygromycin B) and also ribosome recycling (gentamicin, neomycin).

The most studied mechanism of aminoglycoside action is the effect on the accuracy of translation. Aminoglycosides, such as paromomycin and geneticin, interact with the 16S rRNA helix 44 that is located within the A site of 30S ribosomal subunit (Vicens and Westhof, 2001). This region comprises the decoding center of the ribosome and functions by monitoring the formation of correct interaction between mRNA codon and tRNA anticodon. Two universally conserved nucleotides of helix 44, A1492 and A1493, are crucial for this process. A cognate codon-anticodon interaction induces a conformational change accompanied by the flip-out of A1492 and A1493. This event in turn induces other rearrangements inside the ribosome that lead to tight codon-anticodon interaction (Nierhaus, 1993). When aminoglycosides bind into A-site, their amino-sugar rings interact with helix 44 and force A1492 and A1493 to flip outside (Vicens and Westhof, 2001; Vicens and Westhof, 2003). As a

result, the decoding center adopts a conformation that allows the binding of noncognate tRNAs to the A-site. Therefore, incorrect amino acids can be incorporated to growing polypeptide chain. Mistranslated proteins insert into and cause damage to the cytoplasmic membrane. This leads to rapid uptake of additional aminoglycoside molecules that in turn increase mistranslation (Davis et al., 1986). It has been estimated that normally the ribosome is responsible for one misreading for every 1000 – 10,000 correct amino acids (Zaher and Green, 2009). Aminoglycosides can decrease translation fidelity down to one misreading for 100 amino acids (Wilson, 2009).

Streptomycin binds to a distinct site near the A-site and makes contacts with 16S rRNA helices 1, 18, 27 and 44 as well as with the ribosomal protein S12 (Carter et al., 2000). The binding of the drug induces a lateral shift of decoding region of helix 44, thereby causing translational misreading by a mechanism unrelated to other aminoglycosides (Demirci et al., 2013). Streptomycin alters the rate by which GTP hydrolysis occurs during the selection of aa-tRNA in the A-site. Normally, GTP hydrolysis by EF-Tu is very fast for cognate codon-anticodon complexes and slower for near-cognate complexes. Streptomycin has been shown to reduce the rate of GTPase activation for cognate codons and increase the rate of GTPase activation for near-cognate codons, resulting in reduction of selectivity (Gromadski and Rodnina, 2004).

In contrast to paromomycin and geneticin, the binding of hygromycin B to helix 44 induces the flip-out of A1493 alone. In this position, A1493 would block the movement of tRNAs between the A and P sites (Borovinskaya et al., 2008). Hygromycin B effectively inhibits translocation in both bacteria and eukaryotes (Eustice and Wilhelm, 1984).

Studies of X-ray crystal structures of *E. coli* 70S ribosome revealed that aminoglycosides, such as gentamicin and neomycin, possess an additional binding site located at 23S rRNA helix 69. Indeed, these drugs interact with the region that overlaps the binding site of ribosome recycling factor. Therefore, binding of aminoglycosides to 50S subunit interferes with the release of ribosomes from mRNA and inhibits recycling of ribosomes (Borovinskaya et al., 2007).

The use of aminoglycosides in human medicine is complicated because of their adverse effects. Nephrotoxicity, ototoxicity and neuromuscular blockade are most serious problems to the patients. It has been shown that nucleotides A1408 and G1491 of prokaryotic 16S rRNA are essential for aminoglycoside binding (Francois et al., 2005). Human mitochondrial rRNA contains adenine and guanine at the same positions, which can provide some explanation for aminoglycoside toxic side effects (Hobbie et al., 2008).

Enzymatic modifications of drug molecule contribute the major resistance mechanism to aminoglycosides. Mutations in helix 44 and genes encoding ribosomal proteins S12 and S5 can also confer resistance. Some efflux systems are involved in moderate resistance to aminoglycosides (Moore et al., 1999). Many natural aminoglycoside producers protect themselves by having methyltransferases that methylate residues A1405 and A1408 of 16S rRNA (Cundliffe,

1989). Recently, several plasmid-borne methyltransferases have emerged in clinical isolates that show high-level resistance to many aminoglycosides (Krause et al., 2016).

1.1.3. Macrolides

Macrolides (Figure 4) represent a large and clinically important class of antibiotics. They inhibit the growth of many Gram-positive and certain Gram-negative bacteria. On the other hand, their activity against *Enterobacteriaceae* and *Pseudomonas spp* is weak. First macrolides were isolated in the early 1950s. First, pikromycin was discovered in 1950 (Brockmann and Henckel, 1951). Erythromycin was found shortly thereafter from the fermentation broth of actinomycete *Saccharopolyspora erythraea* (formerly known as *Streptomyces erythreus*) (McGuire et al., 1952).

All medically used macrolides comprise either 14-, 15- or 16-membered macrolactone ring to which certain saccharide molecules are attached. Molecules possessing smaller or larger lactone ring tend to exhibit weak antibacterial activity.

Erythromycin A is considered the prototype of 14-membered macrolides. The molecule of erythromycin consists of 14-membered lactone with desosamine and L-cladinose linked to C5 and C3 position, respectively. Other well-known naturally occurring 14-membered macrolides are oleandomycin, lankamycin and megalomicin. Semi-synthetic members of this group include clarithromycin and roxithromycin as well as ketolides telithromycin and cethromycin.

The 16-membered macrolide family can be further divided into subfamilies according to substitution patterns of their macrolactones (Kirst, 2014). Tylosin, produced by *Streptomyces fradiae*, along with its semi-synthetic derivatives constitutes the first subfamily. The molecule of tylosin contains the disaccharide D-mycaminosy-L-mycarose at C5 and D-mycinose at C14 of the macrolactone ring. The second subfamily is referred as the leucomycin-spiramycin group. Several naturally occurring members of this group, such as josamycin and spiramycin are used in human medicine.

Clinically, macrolides can be classified into three generations. The first-generation macrolides were launched as drugs in the 1950s. Erythromycin was the first macrolide developed for treatment of bacterial infections in humans. Despite of effectiveness, the use of erythromycin in medicine encountered problems such as low oral bioavailability and low stability in acidic digestive tract. Development of first-generation derivatives, such as 2'-esters and acid-addition salts, slightly helped to reduce these difficulties.

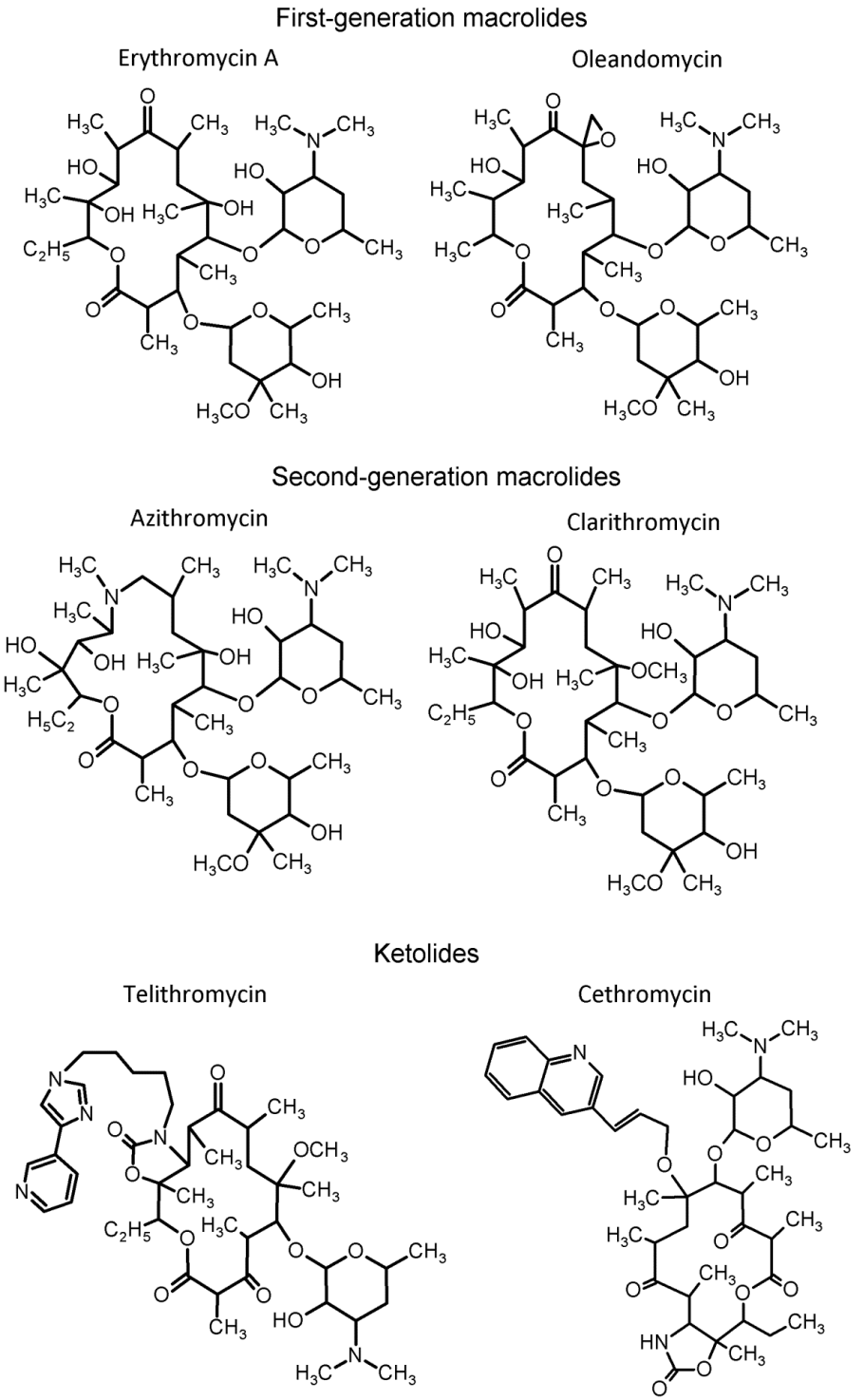


Figure 4. Chemical structures of representative macrolide antibiotics.

The search for compounds with more favorable pharmacokinetics resulted in launching of semi-synthetic second-generation macrolides in the 1980s. Clarithromycin and azithromycin are most prominent members of this group and they are still widely used in medicine. Clarithromycin differs from erythromycin only in the 6-O-methyl group (Morimoto et al., 1984). Azithromycin contains a chemically expanded 15-membered macrolactone ring and the ketone group at the C9 position has been removed (Retsema et al., 1987). Both compounds demonstrated improved oral bioavailability and diminished side effects. Nevertheless, these drugs still exhibit low stability in acidic media due to the presence of 3-L-cladinose.

Dramatic rise in bacterial resistance to macrolides was the main reason for developing novel types of macrolides. The third-generation derivatives are called ketolides, because their molecule contains a 3-ketone group instead of 3-cladinosyl moiety. Other features of ketolides include the presence of carbamate side ring and the substitution at the C6 position. Ketolides like telithromycin exhibit improved stability in acidic environment, low propensity for selection of resistant mutants and stronger antimicrobial activity (Bryskier, 2000). Unfortunately, the use of telithromycin has been associated with rare but severe health issues including hepatotoxicity and visual disturbances (Kirst, 2014). The molecule of telithromycin has a pyridinyl-imidazolyl-butyl group linked to the C6 position of the carbamate ring. Cethromycin that contains a quinolinyl-allyl side chain at the C6 position is another promising example of ketolide family.

Macrolides inhibit protein synthesis by impeding the progression of nascent peptide chain. During translation, the nascent peptide exits from the ribosome through a tunnel that begins near the PTC (Choi and Brimacombe, 1998). The tunnel wall is composed mainly of rRNA, although ribosomal proteins L4 and L22 also contribute in the narrowest part of the tunnel. Macrolides can bind to free ribosomes, but not during elongation when the exit tunnel is occupied by the nascent peptide (Contreras and Vazquez, 1977a). The binding site of macrolides is located adjacent to PTC within the tunnel where they interact with the 23S rRNA. The prevailing view suggests that the presence of macrolide molecule in the exit tunnel blocks the extension of growing polypeptide. As a result, the short peptidyl-tRNA dissociates from the ribosome (Menninger and Otto, 1982). Smaller macrolides, such as erythromycin were shown to enable the synthesis of oligopeptides consisting of 6–8 amino acids while josamycin and spiramycin that penetrate deeper into exit tunnel generate shorter oligopeptides. Telithromycin lacks C3-cladinose and enables synthesis of 9–10 amino acids before peptidyl-tRNA drop-off (Tenson et al., 2003). Recent studies have challenged the general view of macrolide action and suggested that these drugs are rather protein specific translation inhibitors. It appears that some nascent peptides can pass through the ribosome exit tunnel even in the presence of very high levels of macrolides (Kannan et al., 2012). However, macrolides can arrest translation of the majority of polypeptides at the later stages of elongation (Davis et al., 2014; Kannan et al., 2014). Such translational arrest occurs at specific stalling sites that are located throughout the entire sequence (Davis et al., 2014).

New data indicate that marolides act as inhibitors of peptide bond formation between specific combinations of PTC donor and acceptor substrates. This mode of action is possibly mediated by drug-induced conformational changes in the PTC (Kannan et al., 2014; Sothiselvam et al., 2014).

More than 20 crystal structures of macrolides in complex with 70S ribosome or 50S ribosomal subunit have been analyzed. Structure of the 50S subunit complexed with erythromycin revealed that the 2'OH group of desosamine sugar of the drug forms hydrogen bonds with nucleotides A2058 and A2059 in domain V of 23S rRNA (Schlunzen et al., 2001). A ketolide telithromycin has been crystallized in complex with ribosomes derived from eubacteria (Berisio et al., 2003; Bulkley et al., 2010; Dunkle et al., 2010) as well as in complex with 50S subunit from an archaea *Haloarcula marismortui* bearing a mutation G2058A in 23S rRNA (Tu et al., 2005). In all structures, the lactone ring of telithromycin is positioned similarly to that of erythromycin. However, variations in binding mode seem to exist between species due to differences in their 23S rRNA sequence. In the crystal structure of *E. coli* 50S subunit, the alkyl-aryl side-chain of telithromycin reaches into exit tunnel and stacks upon the A752-U2609 base pair (Dunkle et al., 2010). In contrast, the same side-chain is rotated by 120° and interacts with C2609 when bound to archaeal 50S subunit (Wilson et al., 2005). Additional interactions with the ribosome contribute to the higher antibacterial activity of ketolides.

Studies of crystal structures of azithromycin in complex with ribosomes show that the binding mechanisms of macrolides possessing 14- or 15-membered lactone ring are very similar (Bulkley et al., 2010; Hansen et al., 2002). Interestingly, analysis of *Deinococcus radiodurans* 50S subunit revealed the second binding site for azithromycin. When bound to the second site, the drug interacts with ribosomal proteins L4 and L22 as well as with domain II of 23S rRNA (Schlunzen et al., 2003).

The molecule of macrolides bearing 16-membered ring is larger and often contains disaccharides at the C5 position of the macrolide ring. Despite of larger size, the placement of the lactone ring and C5-sugar resembles that observed for erythromycin. The C14 sugar moiety of tylosin penetrates deeper into exit tunnel and forms additional interactions with nucleotides A748–A752 in domain II of 23S rRNA (Hansen et al., 2002).

A common mechanism for resistance to macrolides is modification of ribosomes by methylation. Methylation of 23S rRNA is accomplished by the Erm family of methylases. Dimethylation of A2058 in the PTC leads to steric clash with the C5-monosaccharide. Such methylation pattern is characteristic to so-called MLS_B phenotype, which is resistant to macrolides, lincosamides and streptogramin B (Weisblum, 1995a). Both A2058G and A2059G transitions lower macrolide affinity (Franceschi et al., 2004). Resistance to macrolides can also be conferred by mutations in genes encoding proteins L4 and L22, although there is no direct contact between these proteins and the drug. Instead, L4 and L22 are responsible for maintaining the 23S rRNA conformation necessary for macrolide binding (Gregory and Dahlberg, 1999).

Another mechanism of resistance is hydrolysis of macrolactone ring by esterases or modification of antibiotic by phosphotransferases (Arthur et al., 1986; O'Hara et al., 1989). Efflux systems that confer resistance to macrolides are more common in Gram-negative bacteria, but they can also be found in Gram-positive cocci (Katz and Ashley, 2005).

1.1.4. Phenicols

Chloramphenicol (Figure 5) was originally isolated in 1947 as a fermentation product of a soil bacterium *Streptomyces venezuelae* (Ehrlich et al., 1947). It is a broad-spectrum antibiotic, being active against both Gram-negative and Gram-positive bacteria as well as *Chlamydiae*, *Rickettsiae* and *Mycoplasma*. Although chloramphenicol is bacteriostatic against many bacteria, it has been shown to be bactericidal at clinically achievable concentrations against *Haemophilus influenzae, Streptococcus pneumoniae*, and *Neisseria meningitidis* (Rahal and Simberkoff, 1979). Although initially considered as a promising drug, undesirable side effects were soon linked with the use of chloramphenicol. Among those adverse effects, aplastic anemia and bone marrow suppression are most problematic (Alavi, 1983; Kucers, 1980). Increased risk of leukemia and association with the so-called Gray-baby syndrome has also been observed (Mulhall et al., 1983). Although the use of chloramphenicol in human medicine has been decreased in developed world, it is still used as a topical ointment. In addition, chloramphenicol is favored for the treatment of staphylococcal brain abscesses and meningitis for which less toxic remedies are not available. Chloramphenicol application in animals has been banned in many countries as its residues in carcasses of food animals could potentially cause adverse side effects to meat consumers (Schwarz et al., 2004).

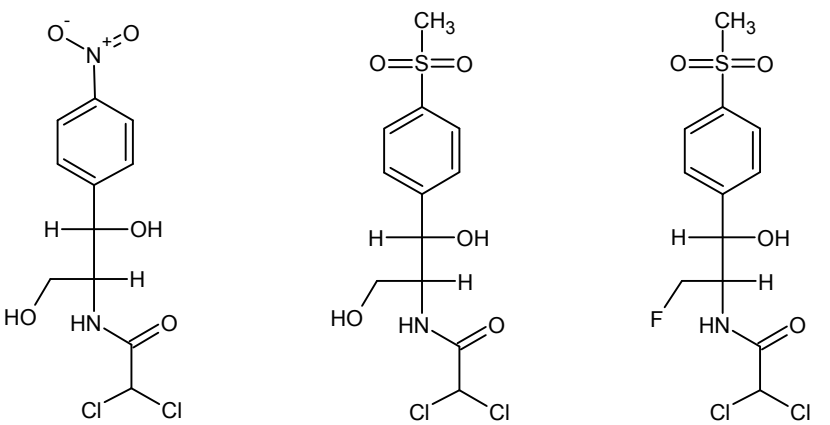


Figure 5. Chemical structures of chloramphenicol antibiotics.

The detailed pathway by which *S. venezuelae* produces chloramphenicol from chorismic acid is not yet completely clear. Since 1950, chloramphenicol is being produced synthetically as the procedure is relatively simple and inexpensive (Schwarz et al., 2004). The molecule of chloramphenicol consists of a 1,3-propanediol bearing a para-nitrophenyl ring at C1 position and a dichloroacetamido tail at C2 position. By modifying this molecule, several derivatives of chloramphenicol have been generated. Thiamphenicol has a sulfomethyl group attached to phenyl ring instead of nitro group. As the connection between thiamphenicol and aplastic anemia has never been reported, this drug is used for human treatment in several countries (Lambert, 2012). Florfenicol is similar to thiamphenicol, but contains fluorine attached to C3 of propanediol moiety instead of hydroxyl group. Florfenicol is used only in veterinary medicine.

The binding site of chloramphenicol is located within the A site of PTC on 50S ribosomal subunit. In this position, the drug overlaps with the amino acid-containing end of an aa-tRNA. By occupying the A-site of 50S subunit, chloramphenicol acts as an obstacle for the incoming aa-tRNA. Biochemical experiments show that chloramphenicol obstructs small tRNA fragments from binding to the A-site of the PTC (Celma et al., 1971). The drug interferes with the puromycin model reaction in which ribosomes containing peptidyl-tRNA in the P-site are exposed to puromycin (Cannon, 1968).

Structure of chloramphenicol in complex with *D. radiodurans* 50S subunit reveals that the drug interacts with seven conserved nucleotides, which constitute the central loop of domain V of 23S rRNA (G2061, A2451, C2452, U2500, U2504, G2505 and U2506) (Schlunzen et al., 2001).

High-resolution X-ray structures of chloramphenicol in complex with 70S ribosomes from *E. coli* and *T. thermophiles* are available. These structures show drug in different positions, rotated by 180° (Bulkley et al., 2010; Dunkle et al., 2010). Chloramphenicol contacts the ribosome through a stacking interaction between its para-nitrobenzyl ring and the base C2452. A single potassium ion identified in PTC also contributes in binding as it links methylene hydroxyl group of chloramphenicol with nucleotides G2447, G2501 and G2061 of 23S rRNA (Bulkley et al., 2010).

Archaea are less susceptible to chloramphenicol possibly due to rRNA sequence differences within the PTC. However, structure of chloramphenicol bound to the large ribosomal subunit of *H. marismortui* revealed a novel chloramphenicol binding site. This second binding site is located at the entrance to the peptide exit tunnel and it overlaps the binding site of macrolides (Hansen et al., 2003). The presence of secondary binding site was confirmed with experiments in which chloramphenicol was cross-linked to *E. coli* and *H. halobium* ribosomes (Long and Porse, 2003). The low affinity of the drug to the second binding site suggests that this additional site is not crucial for the translation inhibition. Nevertheless, the release of peptidyl-tRNA's containing short peptides *in vitro* has been observed in the presence of chloramphenicol (Rheinberger and Nierhaus, 1990).

Several studies have demonstrated that chloramphenicol can not be viewed as a universal inhibitor of peptide bond formation. Instead, the inhibitory effect of chloramphenicol depends on the nature of mRNA being translated. For instance, translation of mRNAs encoding small or charged amino acids, such as poly(A) for lysine and poly(C) for proline is more effectively inhibited by chloramphenicol than translation of mRNAs encoding larger aromatic amino acids, such as poly(U) for phenylalanine (Pestka, 1977). Recent experiments have shown that chloramphenicol blocks translation at specific locations within the mRNA in a context-specific manner. The two C-terminal nascent peptide residues as well as of the A-site acceptor strongly influence the ability of chloramphenicol to inhibit peptidyl transfer. Inhibition is most efficient when the nascent peptide in the ribosome carries an alanine residue in its penultimate position (Marks et al., 2016).

Most of the resistance to chloramphenicol and its derivatives is mediated by chloramphenicol acetyltransferases (CATs). All CATs transfer an acetyl group from a donor molecule to the hydroxyl group at C3 of the phenicol molecule (Murray and Shaw, 1997). However, CATs do not confer resistance to florfenicol, because their hydroxyl group at C3 is replaced by fluorine (Schwarz et al., 2004). Efflux systems encoded by *elm* genes constitute the second important resistance mechanism. Other mechanisms including methylation of A2503 by the methyltransferase Cfr, mutations in 23S rRNA and porin alternations have been described (Kehrenberg et al., 2005).

1.1.5. Oxazolidinones

There are three major antibiotic classes of purely synthetic origin: (1) the quinolones that target bacterial topoisomerases, (2) sulfonamides that interfere with folate biosynthesis, and, (3) the oxazolidinones that are translation inhibitors. In 1970s, an American chemical company DuPont started a screening program in order to identify novel agents for treatment of certain plant diseases. This screening leaded to discovery of first bioactive oxazolidinones (Fugitt and Luckenbaugh, 1978). A common characteristic of these compounds is the presence of a 2-oxazolidinone ring, which is a heterocyclic 5-membered ring bearing both a nitrogen and an oxygen atom. Compounds Dup 105 and Dup 721 gained special attention because they exhibited promising characteristics required for developing new medications. These features included an ability to inhibit bacterial translation via novel mechanism, activity against multidrug-resistant Gram-positive pathogens and inability to generate resistant mutants *in vitro* (Slee et al., 1987). However, the safety profile of Dup 105 and Dup 721 was deemed to be inappropriate for human use (Ranger, 2004).

Extensive synthesis and examination of oxazolidinone analogues continued at The Upjohn Company. Finally, two compounds were found to exhibit good balance between antibacterial efficacy, pharmacokinetics, water solubility and other properties. These compounds were a piperazine analog eperezolid (PNU-

100592) and a morpholine analog linezolid (PNU-100766). Both drug candidates demonstrated acceptable safety, bioavailability and clearance in animal tests (Slatter et al., 2002). Eperezolid and linezolid entered into human clinical trials in 1994 and 1995, respectively. Eventually, linezolid (Figure 6) was preferred for further studies due to more favorable pharmacokinetic profile (Zurenko et al., 1997). Linezolid successfully passed through Phase II and Phase III trials and was approved for human use in 2000. The drug is currently marketed by Pfizer under the name Zyvox®.

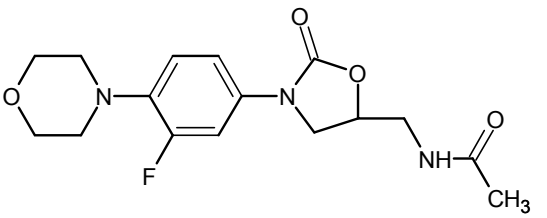


Figure 6. The structure of an oxazolidinone antibiotic linezolid.

The molecule of linezolid is composed of three aromatic rings (oxazolidinone A-ring, fluorophenyl B-ring, morpholine C-ring) and an acetamide C5-tail (Figure 6). Early experiments showed that eperezolid competes with chloramphenicol and puromycin for ribosome binding (Lin et al., 1997). Subsequent crosslinking experiments demonstrated that oxazolidinones interact with components of the PTC (Colca et al., 2003).

Ipplito and others have published a structure of linezolid bound to *H. marismortui* 50S subunit with and without the addition of CCA-N-acetylphenylalanine (CCA-Phe), an analog of the 3'-terminus of aminoacyl-tRNA (Ippolito et al., 2008). In these structures, linezolid is seen in the A site of the PTC. The structure reveals that linezolid and CCA-Phe bind the PTC simultaneously, indicating that the drug does not interfere with the binding of peptidyl-tRNA substrates to the P-site (Ippolito et al., 2008).

Another crystal structure shortly became available in which linezolid is bound to 50S ribosomal subunit from *D. radiodurans*. The binding region of linezolid was shown to overlap those of chloramphenicol and the aminoacyl moiety of an A-site bound tRNA. Morpholine ring of the molecule heads toward the intersubunit interface, whereas oxazolidinone ring is oriented in the general direction of the ribosomal tunnel (Wilson et al., 2008).

According to the proposed model for the mechanism of action, linezolid interferes with both translation initiation and elongation phases. When bound to the A-site of the 50S subunit, linezolid perturbs the orientation of nucleotide U2585 of 23S rRNA (Wilson et al., 2008). As the correct conformation of U2585 is required for the positioning of initiator-tRNA to the P-site (Schmeing et al., 2005), the drug is thought to inhibit translation initiation (Aoki et al., 2002). When bound to the A-site of a translating ribosome, linezolid does not interfere neither with the binding of aa-tRNA:EF-Tu:GTP ternary complex to the initial

A/T site of the ribosome nor the GTP hydrolysis by EF-Tu (Matassova et al., 1999). However, linezolid blocks the subsequent accommodation of an incoming tRNA into the A-site, similarly to tetracycline. As a result, aa-tRNA dissociates from the ribosome and peptidyl-tRNA becomes "locked" in the P-site (Wilson et al., 2008). Similarly to chloramphenicol, linezolid does not actively block formation of every peptide bond, but rather stalls ribosomes at specific mRNA locations. The action of linezolid is defined by the nature of the penultimate residue of the nascent peptide as well as by the amino acid residues directly participating in peptide bond formation. The presence of Ala in the penultimate position of the peptide stimulates the action of linezolid, while Gly strongly counteracts the inhibitory effect (Marks et al., 2016). However, the mechanistic principles of context specificity are not completely understood.

Linezolid is a bacteriostatic agent active against Gram-positive bacteria. It is used mainly for treatment of bacteremia, pneumonia and skin diseases, including infections caused by vancomycin-resistant *Enterococcus* and methicillin-resistant *Staphylococcus*. Oxazolidinones have been shown to cross-link to the PTC of human mitochondrial ribosomes (Leach et al., 2007). This can be the explanation for some adverse effects like diarrhea, nausea and headache, although linezolid is generally well tolerated by patients.

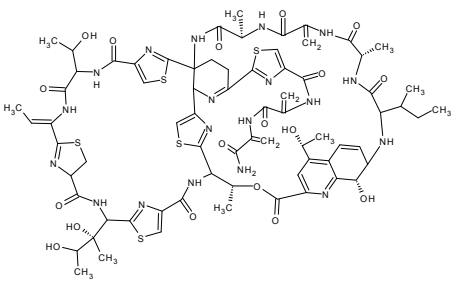
Numerous attempts have been made in order to discover new oxazolidinone analogues and to improve the potency of existing molecules. Although linezolid is so far the only commercial oxazolidinone, several novel oxazolidinones are currently under investigation. Radezolid is the first biaryloxazolidinone that has recently successfully completed Phase II clinical trials. Radezolid displays improved antibacterial properties in comparison with linezolid, as it also inhibits growth of Gram-negative bacteria and retains activity against linezolid-resistant strains (Locke et al., 2010). The molecules of second-generation oxazolidinones are generally larger as they contain an extra D-ring or side chain. Improved molecules are able to form additional interactions with the ribosome, thus explaining the greater potency of radezolid (Shaw et al., 2008). Another oxazolidinone analogue, tedizolid, has entered into Phase III trials. Tedizolid is suggested to be less toxic when compared to linezolid, as it does not bind to mitochondrial ribosomes (Das et al., 2012).

Resistance to linezolid is not widespread, but can occur through mutations in the 23S rRNA region associated with the binding site of the drug (nucleotides A2451, C2452, U2504 and G2505; reviewed in Shaw and Barbachyn, 2011 and in Wilson, 2009). Resistance can also be conferred by mutations in 23S rRNA nucleotides that do not interact with the drug. One such mutation, G2576U, acts via perturbing the position of G2505 and U2506 (Wilson et al., 2008). Furthermore, reduced susceptibility to oxazolidinones is associated with mutations in genes encoding ribosomal proteins L3, L4 and L22 (Locke et al., 2009; Wong et al., 2010). Cfr methytransferase that modifies 23S rRNA nucleotide A2503 within the PTC is responsible for the cross-resistance to oxazolidinones, macrolides, phenicols, pleuromutilins, lincosamides and streptogramin A (Long et al., 2006).

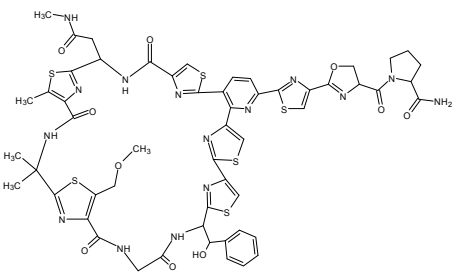
1.1.6. Thiopeptides

Thiopeptide class of antibiotics is distinctive by their highly modified, sulfur-containing macrocyclic structure and high molecular weight. Thiopeptide molecules compose largely of heteroaromatic rings such as indoles, thiazoles, oxazoles and pyridines that are linked together (Figure 7). This class comprises more than 100 compounds that can be subdivided into subfamilies according to the size of the macrocyclic ring(s) or the oxidation state and substitution pattern of the central pyridine core (Bagley et al., 2005; Malcolmson et al., 2013). Thiopeptides possess either 26-, 29- or 35-membered macrocyclic rings. The most-studied thiopeptides belong to classes containing 26-membered macrocyclic ring (thiostrepton, micrococcin, siomycin, thiocillin, nosiheptide) and 29-membered ring (GE2270A) (Figure 7).

The first discovered thiopeptide was isolated from *Micrococcus ssp* in 1948 and was named micrococcin (Su, 1948). Thiostrepton was first isolated in 1954 from an actinomycete *Streptomyces azureus* (Donovik et al., 1955) but its structure was not completely solved until 1983 (Hensens and Albers-Schonberg, 1983). It took 60 years before the details of thiopeptide biosynthesis became evident. Maturation of a thiopeptide molecule begins with the synthesis of a 50–60 amino acids long precursor protein by the ribosome. The precursor must pass through a cascade of post-translational modifications before the active compound is formed (Walsh et al., 2010; Kelly et al., 2009; McIntosh et al., 2009).



Thiostrepton



GE2270A

Figure 7. Structures of thiopeptide antibiotics thiostrepton and GE2270A.

Thiostrepton has been shown to be effective especially against Gram-positive bacteria as well as against malaria-causing protozoan parasite *Plasmodium falciparum* (McConkey et al., 1997). Gram-negative organisms are naturally resistant to thiostrepton, as the drug is unable to penetrate their outer membrane. Although thiopeptides have been applied in veterinary medicine, their use in treatment of human infections has faced several problems due to chemical and physical properties of the molecules. The toxicity of thiopeptides to eukaryotes is relatively low. On the other hand, low solubility in water complicates their parenteral administration and the large size of molecules creates problems in oral bioavailability. One opportunity for overcoming these drawbacks could be

the reduction of the size of large thiopeptide molecules without affecting their antibacterial activity (Nicolaou et al., 2005).

Thiopeptides exhibit different mechanisms of action depending on macrocycle size. The target of compounds with 35-membered rings remains uncertain, although they maintain antibacterial activity (Just-Baringo et al., 2014). Thiopeptides with 26-membered macrocycles bind to 50S ribosomal subunit while those with a 29-membered ring, such as GE2270A, bind to EF-Tu. GE2270A has been shown to inhibit the formation of EF-Tu:GTP:aa-tRNA ternary complex (Anborgh and Parmeggiani, 1991). So far, GE2270A remains the only thiopeptide that has been completed Phase I clinical trials for human treatment (Butler, 2008).

Studies of crystal structures of *D. radiodurans* 50S ribosomal subunit in complex with thiostrepton, nosiheptide and micrococcin have elucidated the binding site of thiopeptides on the ribosome. Thiostrepton was shown to bind into crevice between helices 43 and 44 of 23S rRNA and N-terminal domain of ribosomal protein L11 (Harms et al., 2008). Thiostrepton forms hydrogen bonds with nucleotides A1067 and A1095 that are situated at the tips of H43 and H44, respectively. Thiostrepton does not bind to free L11, although the absence of L11 decreases dramatically the binding affinity of the antibiotic (Porse et al., 1998).

The target site of thiostrepton as well as related compounds nosiheptide, siomycin and micrococcin is recognized as a GTPase-associated center since it operates as the binding site for translational GTPases (Margus et al., 2007; Lentzen et al., 2003). Thiostrepton mimics EF-G region V, interferes with the stable interaction between EF-G and the ribosome and prevents ribosome-dependent GTP hydrolysis by EF-G (Cameron et al., 2002; Harms et al., 2008). An EF-G mutant lacking domains IV and V is insensitive to thiostrepton (Walter et al., 2011). In addition, thiostrepton has been shown to inhibit stable ribosome binding of ribosome protection proteins Tet(O) and Tet(M) (Connell et al., 2003a; Mikolajka et al., 2011), elongation factor 4 (LepA) (Walter et al., 2011), BipA (Mikolajka et al., 2011) and IF2 (Grunberg-Manago et al., 1972).

Despite of their similar structure, thiostrepton and micrococcin are shown to have different effect on the GTPase activity of EF-G. Similarly to thiostrepton, micrococcin inhibits the GTPase activities of translational GTPases Tet(M), EF4, BipA and IF2 (Mikolajka et al., 2011). However, micrococcin does not interfere neither with the binding of EF-G:GTP to the ribosome nor with the ribosome-induced GTPase activity of EF-G and, contrary to thiostrepton, enhances the GTPase activity of EF-G (Lentzen et al., 2003). This essential difference can be explained by the fact that micrococcin binds to slightly different position and interacts with A1095 rather than with A1067 (Harms et al., 2008). By binding to the ribosome, micrococcin can stabilize the interaction between proteins L11 and L7, thereby bending L7 into position in which it can interact with EF-G and stimulate the GTP turnover (Mikolajka et al., 2011).

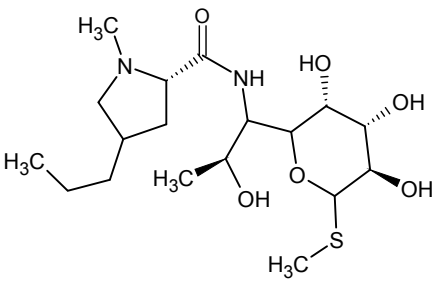
Cross-resistance between thiopeptides and other antibiotics is unlikely to happen because the binding site of thiopeptides is unique (Figure 1). Thiostrepton

producer *S. azureus* protects his own ribosomes by methylation of A1067 (Thompson et al., 1982). Although resistance development to thiopeptides has not been documented *in vivo*, several resistance-conferring spontaneous mutations have been found during selection experiments. Resistance to thiostrepton can be conferred by methylation or base changes at 23S rRNA conserved positions A1067 and A1095 (Cundliffe and Thompson, 1979; Hummel and Bock, 1987b; Rosendahl and Douthwaite, 1993). Additionally, mutations in gene encoding protein L11 can also be responsible for the resistance (Cameron et al., 2004).

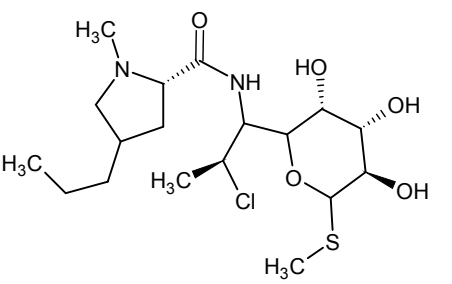
1.1.7. Lincosamides

The prototypical member of lincosamide group is lincomycin. It was discovered as a fermentation product of actinomycete *Streptomyces lincolnensis* (Mason et al., 1962). Later studies revealed that lincomycin can also be produced by other organisms, such as *Streptomyces espinosus* and *Actinomyces roseolus* (Wilson et al., 2009).

The molecule of lincomycin (Figure 8) comprises a propyl-pyrrolidinyl moiety (a proline derivative) and a lincosamine sugar ring that are connected via an amide bond. Although licensed for use in human medicine, lincomycin is rarely used nowadays. A large number of lincomycin modifications have been prepared (Magerlein, 1971) but only a few of them are used in medicine. Clindamycin is a semi-synthetic derivative of lincomycin in which the 7-hydroxyl group is replaced by chlorine (Figure 8) (Lewis, 1974). Consequently, clindamycin is 20 times more effective than lincomycin in inhibiting the growth of *E. coli* (Douthwaite, 1992). Clindamycin is most widely used in medicine due to highest potency and good oral absorption. Another clinically important derivative of lincomycin is pirlimycin, which contains six-membered cyclic amino acid amide instead of the five-membered proline ring (Birkenmeyer et al., 1984). Pirlimycin is used to treat bovine mastitis (Watts and Yancey, 1994).



Lincomycin



Clindamycin

Figure 8. Structures of lincosamide antibiotics lincomycin and clindamycin.

Lincosamides have only a limited spectrum of activity, as Gram-negative bacteria tend to be intrinsically resistant to lincosamides. Nevertheless, lincosamides are active against Gram-positive bacteria, such as the genera *Staphylococcus* and *Streptococcus* (Spizek and Rezanka, 2004). Moreover, clindamycin has been shown to be active against some protozoa and can serve as an antimalarial drug (Lell and Kremsner, 2002).

Lincosamides interact with the A- and the P-site of the 50S ribosomal subunit and directly inhibit peptide bond formation. They cause dissociation of short peptidyl-tRNAs from the ribosome in early elongation phase but lose their effect when peptides have grown beyond a critical length (Tenson et al., 2003). Currently, there are three crystallographically resolved structures of clindamycin bound to the 70S ribosome or to 50S ribosomal subunit. The propylpyrrolidinyl group of clindamycin occupies the same region as the aminoacyl-moiety of A-site bound tRNA and interferes with binding of aminoacyl-tRNA into the A-site (Tu et al., 2005; Dunkle et al., 2010). In this region, the binding site of clindamycin partially overlaps with that of chloramphenicol (Schlunzen et al., 2001). The sugar moiety of clindamycin extends into the peptide exit tunnel and overlaps with the desosamine sugar of macrolides (Tu et al., 2005). Consistently, lincosamides have been shown to compete with both chloramphenicol and erythromycin for ribosome binding (Fernandez-Munoz et al., 1971). According to the crystal structures, several hydrogen bonds can form between the hydroxyl groups of sugar ring of clindamycin and nucleotides within the PTC and the peptide exit tunnel, such as A2058, A2059, G2505, and A2503 (Schlunzen et al., 2001, Tu et al., 2005, Dunkle et al., 2010). This is consistent with earlier experiments in which the interactions between clindamycin and lincomycin with *E. coli* ribosomes were studied by chemical footprinting. Both drugs protected 23S rRNA bases A2058, A2451, G2505 and G2061. Clindamycin additionally protected A2059. Nevertheless, the affinity of the two drugs for the ribosome is approximately the same (Douthwaite, 1992). The propyl tail of lincosamides is suggested to be highly flexible and therefore less relevant in the binding to the ribosome (Wilson, 2009).

As macrolides, lincosamides and streptogramin B bind to an overlapping region, they are together often referred as MLS_B group of antibiotics despite of large differences in their molecular structure. Addition of one or two methyl groups to 23S rRNA nucleotide A2058 renders cells resistant to MLS_B group of drugs and is the most prevalent cause of resistance to lincosamides (Poehlsgaard and Douthwaite, 2003). Methylation of A2058 is carried out by rRNA methyltransferases encoded by *erm* genes (Roberts, 2011). Another methyltransferase, Cfr, confers resistance to five different classes of antibiotics (phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramins A) by adding a methyl group to nucleotide A2503 of 23S rRNA (Long et al., 2006).

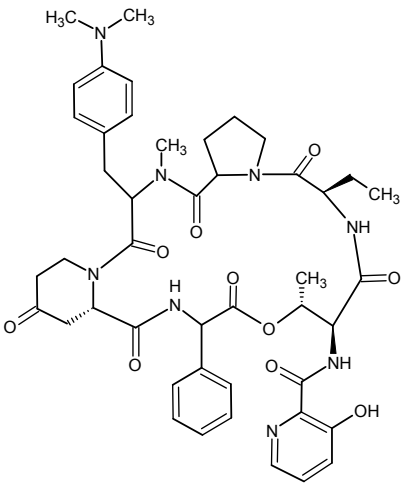
Active efflux across the cell membrane is a less common lincosamide resistance mechanism. It can be mediated by *car*, *lsa*, *lmr* and *vga* gene products (Roberts, 2011). An unusual co-resistance phenotype has been described in a *Streptococcus agalactiae* strain that exhibits resistance to lincosamides, strepto-

gramins A and pleuromutilins (co-called LS_AP phenotype). Studies have revealed that such type of resistance is caused by the presence of a *lsa(C)* gene encoding for a specific ABC transporter (Malbruny et al., 2011). Other lincosamide resistance mechanisms include mutations in 23S rRNA nucleotides A2058 and A2059 (Poehlsgaard et al., 2005) and enzymatic inactivation of drugs via adenylation of their 4- or 3-hydroxyl group (Brisson-Noel et al., 1988).

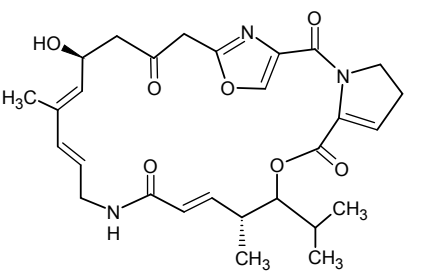
1.1.8. Streptogramins

Family of streptogramins is unique among antibiotics as it consists of a mixture of two chemically unrelated substances, types A and B (Figure 9). Both streptogramin A and B (S_A and S_B) substances are co-synthesized by the same producer organism in a 7:3 ratio (Mast and Wohlleben, 2014). S_A substances are cyclic polyunsaturated macrolactones that are synthesized by polyketide synthases and nonribosomal peptide synthetases. S_B compounds are cyclic hexadepsipeptides of nonribosomal origin (Barriere et al., 1998).

The family obtained its name from the mixture of compounds that was isolated from *Streptomyces graminofaciens* (Charney et al., 1953). During few years, several other streptogramins were discovered from strains of *Streptomyces*, *Micromonospora*, *Actinoplanes* and *Actinomadura*. However, these new antibiotics such as mikamycin, synergistin and madumycin did not become subjects for further development (Ahmed and Donaldson, 2007; Barrière et al., 1998).



Pristinamycin I



Pristinamycin II

Figure 9. Structures of pristinamycin components pristinamycin I (S_B) and pristinamycin II (S_A).

Pristinamycin was found from *Streptomyces pristinaespiralis* and consists of a mixture of streptogramine A (S_A) type pristinamycin II and streptogramin B (S_B) type pristinamycin I (Figure 9). Pristinamycin is an effective drug against

Gram-positive bacteria, including methicillin-resistant *S. aureus* as well as vancomycin-resistant *S. aureus* and *Enterococcus faecium*. Streptogramins are generally unable to penetrate the outer membrane of most Gram-negative bacteria. Nevertheless, pristinamycin is active against a few Gram-negative pathogens, *Mycoplasma* and *Legionella* (Mast and Wohlleben, 2014). The use of pristinamycin in therapy is limited due to poor solubility in water. Nevertheless, the drug is available for oral use in some countries (Eliopoulos et al., 2005). To overcome the solubility of natural streptogramins, an injectable semi-synthetic derivative Synercid was developed. Synercid is composed of a mixture of the S_A type dalfopristin and the S_B type quinupristin (Barrière et al., 1994). However, the antibacterial efficiency of Synercid is low and the use of the drug is associated with some severe side effects (Delgado et al., 2000). A novel pristinamycin-derivative NXL-103 is currently in Phase II clinical trial. NXL-103 is a mixture of S_A type flopristin and the S_B type linopristin. This streptogramin drug candidate is reported to have a 2-fold higher activity than pristinamycin and shows fewer side effects than Synercid (Politano and Sawyer, 2010). It has been suggested that NXL-103 is more effective mainly due to flopristin, as its counterpart dalfopristin exhibits significantly lower antimicrobial activity (Dupuis and Leclercq, 2006).

Another commercially used streptogramin was isolated from *Streptomyces virginiae*, and was named virginiamycin. It is composed of a mixture of virginiamycin M (type S_A) and virginiamycin S (type S_B) and has been used as an animal feed supplement for disease prevention and growth promotion (Yates and Schaible, 1962). Since 1999, the use of virginiamycin as a growth promoter is banned in the European Union, but it is still used in some countries including USA, China and Japan (Casewell et al., 2003).

To a date, crystal structures of 50S ribosomal subunits or 70S ribosomes from *H. marismortui* (Hansen et al., 2003; Tu et al., 2005), *D. radiodurans* (Harms et al., 2004) and *E. coli* (Noeske et al., 2014) in complex with streptogramins are available. Both S_A and S_B type substances bind to the 50S ribosomal subunit. S_A binds into a hydrophobic pocket within the PTC and forms hydrophobic interactions as well as hydrogen bonds with the surrounding nucleotides. The binding site of S_A molecule overlaps with the aminoacyl moieties of both A- and P-tRNAs and therefore prevents tRNAs from positioning into both A- and P-sites (Tu et al., 2005; Hansen et al., 2003; Harms et al., 2004). Consequently, peptide bond formation is hampered and elongation of the growing polypeptide chain stops. Binding of S_A to the ribosome is suppressed when the P-site or the A-site is occupied. Therefore, S_A molecules do not inhibit ribosomes that are actively engaged in protein synthesis (Chinali et al., 1988; Cocito et al., 1997).

Surprisingly, the bacteriostatic activity of S_A has been shown to persist for a prolonged period even after removal of the compound (Parfait and Cocito, 1980; Nyssen et al., 1989). As studies of the *D. radiodurans* 50S ribosomal subunit in complex with Synercid have shown, binding of dalfopristin induces a conformational change of 23S rRNA nucleotide U2585. U2585 is known to be involved in correct positioning of tRNA substrates and peptide bond formation

(Schmeing et al., 2005). In a dalfopristin-induced alternative conformation, U2585 forms hydrogen bonds with G2588 and C2606. Spontaneous reversal of such non-productive U2585 conformation occurs relatively slowly after removal of the drug, thus explaining the post-antibiotic effect (Harms et al., 2004).

The presence of S_A substance in its target site has been shown to enhance the binding of S_B substance to the ribosome (Contreras and Vazquez, 1977b). Binding of S_A type dalfopristin alters the conformation of 23S RNA nucleotide A2062 within the PTC. The base of A2062 moves toward the dalfopristin molecule, thus enabling better accommodation for S_B compound. A2062 forms interactions with both streptogramin A and B (Harms et al., 2004; Noeske et al., 2014).

The binding site of S_B substances is localized within the ribosomal exit tunnel, adjacent to S_A compound. Quinupristin has been shown to form hydrophobic interactions with nucleotides of domain II, IV and V of 23S rRNA and hydrogen bonds with A2062 and C2586 (Harms et al., 2004). The presence of S_B compounds in the exit tunnel blocks the elongation process after a few cycles of peptide bond formation. As a result, the short peptidyl-tRNA is released from the ribosome (Chinali et al., 1988). Therefore, the mode of action of S_B substances is similar to that of the macrolide antibiotics. Unlike S_A compounds, S_B molecules also interact with ribosomes engaged in protein synthesis (Vasquez, 1975).

S_A and S_B type antibiotics show moderate bacteriostatic activity when applied individually. However, the combination of both S_A and S_B substances is bactericidal as it results in a strong synergistic effect that is up to 100-fold higher than measured for both substances separately (Vazquez, 1966; Di Giambattista et al., 1989).

Resistance to S_A and S_B compounds can be different due to differences in their chemical structures and binding sites. Synergy between S_A and S_B compounds may reduce the likelihood of acquired resistance mutations at their binding sites. S_B substances are often classified into MLS_B group together with macrolides and lincosamides due to their overlapping binding sites around the peptide exit tunnel and their cross-resistance due to target modification (Canu and Leclercq, 2001; Tenson et al., 2003). S_A compounds can in turn show cross-resistance with lincosamides and pleuromutilins and are therefore grouped to the LS_AP group (Mast and Wohlleben, 2014). MLS_B resistance phenotype does not confer resistance to S_A, whereas S_A resistance is often associated with S_B resistance (Barriere et al., 1998; Cocito et al., 1997).

Resistance to MLS_B antibiotics can be conferred by rRNA methylases, which add one or two methyl groups to A2058 in 23S rRNA thus blocking antibiotics from attaching to the ribosome (Roberts, 2011). Both S_A and S_B compounds can be removed from the cell via active efflux. Resistance to streptogramins may also be caused by enzymatic inactivation of the drugs. S_A compounds are inactivated by acetyl transferases that are encoded by the *vat* and *satA* genes. S_B substances can be degraded by hydrolases that are products of the *vgb* genes (Allignet and el Solh, 1995). Mutations of A2062 can give rise to both S_A and S_B resistance (Depardieu and Courvalin, 2001).

1.1.9. Other protein synthesis inhibitors

Translation is a complex and highly regulated process that can be divided into distinct phases. Research has been identified natural, semi-synthetic or fully synthetic antibiotics that inhibit every translation phase. In addition to large classes, many smaller groups of antibiotics are known. There are also numerous antibiotics that cannot be grouped together with other compounds due to their unique structure or mechanism of action. Some of these compounds (e. g. antimicrobial peptides) are considered as potential candidates for the development of new therapeutic antimicrobial agents, while some are used in molecular biology as research tools (e. g. puromycin) or have gained interest due to their additional effects (e. g. anisomycin). There are many excellent publications on the subject (Hermann, 2005; McCoy et al., 2011; Poehlsgaard and Douthwaite, 2005; Wilson, 2009; Wilson, 2014). The following is a short review of some translation inhibitors belonging to smaller antibiotic groups.

Kirromycin

Kirromycin (Figure 10) is produced by *Streptomyces collinus.* It was the first discovered antibiotic that targets EF-Tu (Wolf et al., 1972). Other discovered members of kirromycin family include aurodox (N-methyl kirromycin) from *Streptomyces goldingiensis* (Berger et al., 1973) and mocimycin from *Streptomyces ramocissimus* (Vos, 1972). However, the latter turned out to be identical with kirromycin (Vos and Verwiel, 1973). Kirromycin is a narrow-spectrum antibiotic. It exhibits activity against *Streptococci*, some *Enterococci, Neisseria gonorrhoeae, Haemophilus influenza*, and the malaria parasite *Plasmodium falciparum* with no toxic effects on higher eukaryotic cells (Schmid et al., 1978). Natural resistance to the drug is more spread among Gram-positive bacteria as they harbor EF-Tu with less conserved kirromycin-binding site (Landini et al., 1993). The kirromycin producer *S. ramocissimus* has three EF-Tu isoforms. Two of those are sensitive to the drug whereas one is resistant and provides a self-resistance mechanism (Olsthoorn-Tieleman et al., 2007). The molecule of kirromycin is a linear polyketide containing three intramolecular ring systems: the pyridone ring, the central tetrahydrofurane moiety and a goldinonic moiety (Fabbretti et al., 2013)**.** The antibiotic is synthesized by a complex of type I polyketide synthase and nonribosomal peptide synthetases (Weber et al., 2008). A number of natural and semi-synthetic kirromycin analogs have been described that all share similar mechanism of action. Kirromycin binds at the interface of domains 1 and 3 of EF-Tu in the EF-Tu:GDP complex. Mutations on each side of the interface can render EF-Tu insensitive to kirromycin (Abdulkarim et al., 1994).

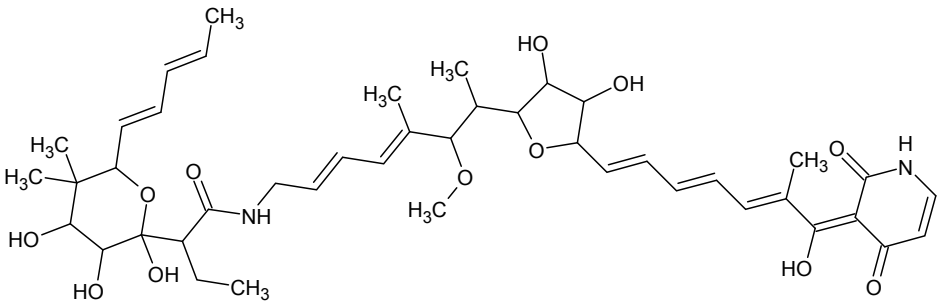


Figure 10. The structure of kirromycin.

EF-Tu:GTP:kirromycin ternary complex is still able to bind aminoacyl-tRNA and enter the ribosomal A-site. However, after GTP hydrolysis, EF-Tu does not dissociate from the ribosome. The aminoacyl end of the tRNA remains in contact with EF-Tu and is unable to enter the PTC, whereas the anticodon of the tRNA is in the decoding center of the 30S subunit (Stark et al., 1997). Kirromycin induces a conformation of EF-Tu:GDP that is similar to the GTP form, thus preventing its release from the ribosome after and despite hydrolysis of GTP to GDP and P_i (Vogeley et al., 2001). Consequently, the ribosome stalls and also blocks all other ribosomes upstream on the same mRNA. Kirromycin-like antibiotics have been effectively used for studying the structure-function relationships of EF-Tu.

Anisomycin

Anisomycin (Figure 11) was purified from the fermentation broth of *Streptomyces griseolus* (Sobin and Tanner, 1954). Anisomycin does not inhibit bacterial translation. Instead, this drug binds into the A-site of the PTC of archeal and eukaryotic ribosomes (Grollman, 1967; Barbacid and Vazquez, 1974). The molecule of anisomycin is composed of a methoxyphenyl group and a pyrrolidine ring (Beereboom et al., 1965). In the *Haloarcula marismortui* large ribosomal subunit, the methoxyphenyl group of anisomycin inserts into the crevice that normally accepts the amino acid side-chains of A-site bound aminoacyl-tRNA. The methoxyphenyl moiety stacks onto the 23S rRNA nucleotide C2452, pyrrolidine group forms a hydrogen bond with A2451 and the hydroxyl group of anisomycin interacts with U2504 (Hansen et al., 2003, Rodriguez-Fonseca et al., 1995). The binding site overlaps with those of chloramphenicol, puromycin and linezolid. In bacterial large ribosomal subunit, U2504 forms a base pair with C2452 thus obstructing the binding site of anisomycin (Wilson, 2009).

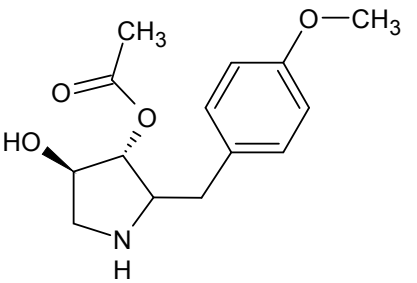


Figure 11. The structure of anisomycin.

It has been reported that anisomycin also inhibits binding of the P-site substrates (Carrasco and Vazquez, 1972). However, latter structural analysis showed that the P-site substrate is out of the reach of anisomycin (Hansen et al., 2003). It is still possible that anisomycin can inhibit P-site binding indirectly by inducing changes in the PTC structure. Resistance to anisomycin can be conferred by mutation of the nucleotide C2452, which is in direct contact with the drug and nucleotides G2447, G2576, C2499 and U2500 that are located close to the A-site crevice and cause subtle changes within the PTC (Hummel and Bock, 1987a; Blaha et al., 2008). Interestingly, anisomycin has many activities apart from being a translation inhibitor. It can act as a modulator of eukaryotic signal transduction (Torocsik and Szeberenyi, 2000), exhibits antitumor activity (You et al., 2013) and is considered as a potential psychiatric drug (Barrientos et al., 2002).

Puromycin

Puromycin was originally isolated from *Streptomyces alboniger* (Porter et al., 1952). This antibiotic inhibits translation on ribosomes of all three kingdoms of life and therefore cannot be used in therapy. The molecule of puromycin is a structural analogue of the 3' end of an aminoacyl-tRNA (Waller et al., 1953) (Figure 12). It consists of an aminonucleoside that is linked to a phenylalanine moiety via an amide bond (but not via an ester bond as aminoacyl-tRNA). Puromycin binds into the A-site region of the PTC via its 3' terminal adenine. When located in the A-site, the nascent polypeptide is covalently linked from the peptidyl-tRNA to the amino acid moiety of puromycin. When puromycin has acquired the nascent polypeptide, the drug dissociates from the ribosome due to low affinity. Peptidyl-puromycin can be transferred into the P-site but no further peptidyl transfer takes place as the ribosome is unable to cleave the amide bond (Wilson, 2009). This leads to a premature termination of translation process (Nathans, 1967). Since decades, puromycin has been an extremely important tool for studying of peptidyltransferase reaction mechanisms.

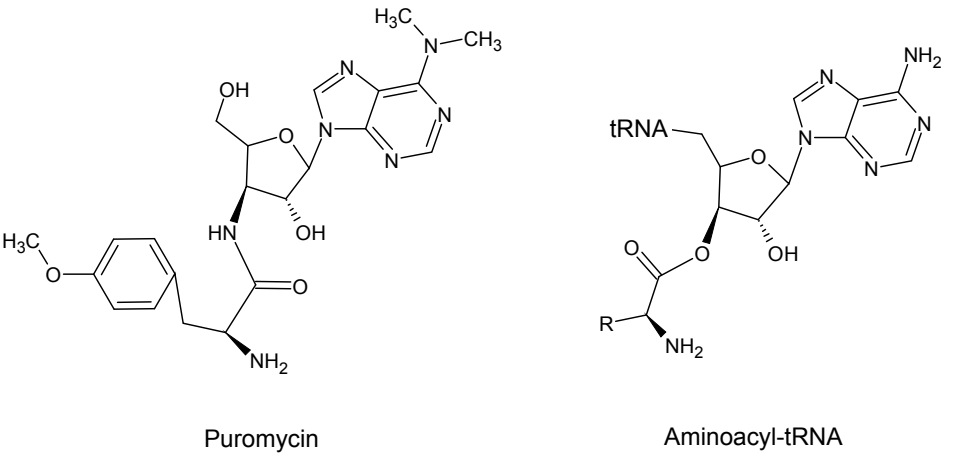


Figure 12. The structures of puromycin and 3' end of an aminoacyl-tRNA.

Sparsomycin

Sparsomycin is a universal translational inhibitor as it affects peptidyltransferase activity in bacteria, archea and eukaryotes. Therefore, this compound is not suitable for usage neither for human nor veterinary therapy, although it has been shown to possess antitumor activity against human epidermoid carcinoma cells (Owen et al., 1962). Sparsomycin is a fermentation product of *Streptomyces sparsogenes* (Argoudelis and Herr, 1962). The molecule of the compound is composed of a uracil acrylic moiety and a mono-oxodithioacetal moiety (Wiley and MacKellar, 1976). Crystal structure of *Deinococcus radiodurans* 50S ribosomal subunit in complex with sparsomycin showed that the uracil ring of sparsomycin forms stacking interactions with the highly conserved P-site base A2062 of 23S rRNA (Bashan et al., 2003). This observation is consistent with previous cross-linking experiments (Porse et al., 1999). By binding to A2062, sparsomycin significantly alters the conformation of nucleotides of the PTC (Bashan et al., 2003). In addition to contacts with A2062, sparsomycin also interacts with the P-site substrate. Indeed, ribosomes do not bind sparsomycin with high affinity unless the P-site is occupied by an N-blocked aminoacyl-tRNA molecule (Lazaro et al., 1991b). Thus, the first peptide bond formation is insensitive to the drug (Busiello and Di Girolamo, 1973). Analysis of *Haloarcula marismortui* crystal structure located sparsomycin along the backbone of nucleotides C75 and A76 of the P-site tRNA and the linked peptide (Hansen et al., 2002). By interacting with both 23S rRNA and peptidyl-tRNA, sparsomycin enhances the binding of peptidyl-tRNA to the P-site (Monro et al., 1969; Hansen et al., 2002). The tail of sparsomycin molecule occupies the position that is needed for normal accommodation of aminoacyl-tRNA 3' terminus into the A-site. Thus, the drug impedes peptide bond synthesis by interfering with the binding of tRNA to the A-site (Pestka, 1969). Other A-site attacking antibiotics, such as chloramphenicol, lincomycin and puromycin compete with

sparsomycin for binding to the ribosome (Ottenheijm et al., 1986; Lazaro et al., 1991a). It has been shown that sparsomycin promotes ribosomal translocation in the absence of EF-G and GTP (Fredrick and Noller, 2003). Resistance to sparsomycin is rare as this drug targets a very critical and conserved region of the ribosome. It has been suggested that insusceptibility of producer organism *S. sparsogenes* might be related to an alteration in the sparsomycin permeability barrier (Lazaro et al., 2002).

Pactamycin

Pactamycin was isolated from *Streptomyces pactum* (Bhuyan, 1962). Despite of numerous studies, the mode of action of pactamycin is not unambiguously understood. Structurally, pactamycin is a complex aminocyclopentitol antibiotic bearing a cyclopentane core (Wiley et al., 1970). This antibiotic gained interest as a potential anti-tumor agent (Bhuyan et al., 1961) but the interest ceased when it appeared that pactamycin inhibits both prokaryotic and eukaryotic ribosomes (Colombo et al., 1966). According to initial studies, the binding site of pactamycin was located to the P-site of the ribosome (Cohen et al., 1969). However, structural studies have specified that the binding site of the drug is actually in the E-site of 30S subunit (Carter et al., 2000; Brodersen et al., 2000). Pactamycin interacts with the 16S rRNA helices 23 and 24 with the universally conserved 16S rRNA nucleotides G693 (h23) and C795 (h24) being most crucial for binding (Brodersen et al., 2000; Woodcock et al., 1991). Pactamycin mimics an RNA dinucleotide and was thought to disrupt the path of real mRNA (Brodersen et al., 2000). Pactamycin was shown to block the formation of new polypeptide chains, while allowing the completion of chains already started on polyribosomes (Macdonald and Goldberg, 1970). When bound to bacterial 30S ribosomal subunit, pactamycin prevents dissociation of initiation factors from the 30S initiation complex, thus interfering with the formation of functional 70S ribosomes (Kappen and Goldberg, 1976). In addition, pactamycin inhibits initiation factor and GTP-dependent binding of tRNA to the P-site (Cohen et al., 1969). However, latter experiments demonstrated that pactamycin cannot be regarded as a selective inhibitor of initiation. The drug was shown to inhibit both chain elongation and initiation in overlapping concentration ranges, whereas effect on elongation was more apparent at higher pactamycin concentrations (Tai et al., 1973). Surprisingly, a comprehensive study of effects of pactamycin on initiation and elongation revealed that the drug interferes with the first translocation reaction whereas no inhibitory effect on the initiation phase was noted (Dinos et al., 2004). Nevertheless, a study by Dinos and co-workers did not explicitly rule out subtle possible inhibitory effects on initiation and pactamycin is therefore classified as both an initiation and elongation inhibitor (Wilson, 2009). Resistance to pactamycin has been studied in an archaea *Halobacterium halobium*. Resistance was caused by mutations A694G, C795U and C796U in the single 16S rRNA operon of this organism (Mankin, 1997).

Fusidic acid

Fusidic acid is an antibiotic that was isolated from the fungus *Fusidium conccineum* (Godtfredsen et al., 1962). Fusidic acid is a steroidal compound with a tetracyclic triterpenoid structure (Godtfredsen et al., 1965). It affects Gram-positive bacteria and is licensed for treatment of staphylococcal infections in Europe and Australia since the 1960s (Farrell et al., 2011). The drug has been noted for its good oral absorption, low toxicity and low probability for development of cross-resistance with other antibiotics (Kirst, 2014). Fusidic acid inhibits translocation phase of prokaryotic translation via interfering with the function of EF-G. The drug binds to EF-G:GTP in complex with the ribosome (Bodley et al., 1969). Free EF-G has a low affinity for fusidic acid, indicating that a specific EF-G conformation is needed for binding (Wilson, 2009). Cryo-electron microscopy studies of *T. thermophilus* ribosome revealed that fusidic acid binds into a pocket between domains I, II and III of EF-G (Gao et al., 2009). EF-G-induced GTP hydrolysis is not prevented in the presence of fusidic acid. Instead, the drug stabilizes the complex after GTP hydrolysis and translocation event (Seo et al., 2006). Fusidic acid traps EF-G on the ribosome in a conformation intermediate between the GTP and GDP forms (Gao et al., 2009). As an outcome, the elongation cycle discontinues and translation process is hampered. The main mechanism of resistance to fusidic acid is due to point mutations in *fusA* gene that encodes EF-G (O'Neill et al., 2004). Some alternations in ribosomal protein L6 are also associated with resistance to the drug (Norstrom et al., 2007). In addition, several protective proteins such as FusB and FusC have been identified that bind EF-G and protect translational apparatus from inhibition by fusidic acid (O'Neill and Chopra, 2006; O'Neill et al., 2007).

Edeines

Edeines are a class of universal translation inhibitors produced by *Bacillus brevis* strain Vm4 (Kurylo-Borowska, 1959). The producer strain generates different edeine variants during biosynthesis, including edeines A, B, D and F. Each of those compounds exists as two isomers – an active α isomer and an inactive β isomer (Borowski et al., 1966; Czajgucki et al., 2006). Edeines are broad-spectrum antibiotics and affect the growth of both Gram-positive and Gram-negative bacteria as well as yeasts, molds and mycoplasma (Gale et al., 1981). However, they are unsuitable for the use as clinically relevant antimicrobial agents due to high toxicity to eukaryotic systems. Edeines are closely related pentapeptide amides composed of glycine, polyamine and four non-protein amino acids (Czajgucki et al., 2006). Edeines are inhibitors of translation initiation phase. Early data indicated that edeines inhibit poly(U)-directed binding of Phe-tRNA to ribosomes, while no inhibiting effect was seen on extension of peptides (Hierowski and Kurylo-Borowska, 1965; Obrig et al., 1971). Further experiments confirmed that edeine inhibits mRNA-directed binding of aminoacyl-tRNA to the 30S subunits and 70S ribosomes but does not

affect binding of aminoacyl-tRNA to the 70S ribosomes in the absence of mRNA (Dinos et al., 2004). The biding site of edeine is located on the 30S subunit, near the E-site where the drug interacts with 16S rRNA helices 24, 28, 44 and 45 (Pioletti et al., 2001). Edeine blocks interaction between the anticodon of initiator-tRNA and the start codon of mRNA (Wilson, 2009). Edeines are also known as effective inhibitors of DNA biosynthesis (Kuryło-Borowska and Szer, 1972). The edeine producer strain protects itself by rapidly releasing active edeine isoform to the growth medium and by only maintaining low concentrations of inactive edeine in the cells (Kurylo-Borowska, 1975). A mutant of *S. cerevisiae* has been isolated in which edeine effect is abolished due to increased affinity of 30S subunit for mRNA (Herrera et al., 1984).

Cycloheximide

Cycloheximide is a eukaryotic-specific translation inhibitor. It was originally isolated from *Streptomyces griseus* (Whiffen, 1947) and reported to inhibit protein synthesis in yeast (Kerridge, 1958). Cycloheximide molecule contains a glutarimide moiety. Studies of cycloheximide biosynthesis revealed that the molecule is formed by condensation of five acetate units and one malonamide unit and the methyl groups of a dimethylcyclohexanone ring are formed by transmethylation reactions (Vanek and Vondracek, 1965).

Cycloheximide is one of the most common laboratory reagents used to block protein synthesis. It is rutinely used to avoid new synthesis of proteins in cell signaling and degradation studies. It also has an instrumental role in ribosome profiling experiments (Ingolia et al., 2009) and can be used as an experimental tool to determine the half-life of proteins. Moreover, it is used as a plant growth regulator and a fungicide (Milenkovski et al., 2010).

The precise position of cycloheximide binding site remained unclear for a long time due to lack of structural information. Footprinting experiments revealed protection of an rRNA nucleotide C3993 in the E-site of 60S ribosomal subunit (Schneider-Poetsch et al., 2010). A structure of the 60S ribosomal subunit from the unicellular ciliate *Tetrahymena thermophila* cocrystallized with cycloheximide did not unambiguously assign the orientation of the drug molecule. Nevertheless, the structure analysis confirmed that cycloheximide binds in a tight pocket on the 60S subunit that was previously identified as the binding site for nucleotides C75 and A76 of E-site tRNA (Klinge et al., 2011). An X-ray study of high-resolution structure of *Saccharomyces cerevisiae* 80S ribosome positioned cycloheximide to the E-site, into a pocket formed by the 25S rRNA and a stretch of the protein L42 (Garreau de Loubresse et al., 2014).

Cycloheximide inhibits the eEF2-mediated translocation of eukaryotic translation by skewing the binding of deacylated tRNA to the E-site. Translocation stalls only if the E-site is occupied by both cycloheximide and deacylated tRNA and binding of cycloheximide alone to the E-site does not affect translocation. Cycloheximide shares the same binding site with lactimidomycin which is another glutarimide-containing antibiotic. Although both compete with the

binding of the tRNA CCA-end in the E-site, they affect translation in a different way. Lactimidomycin arrests ribosomes at the first peptide bond, while cycloheximide stalls ribosomes during ongoing translation (Schneider-Poetsch et al., 2010).

Although most eukaryotic cells are sensitive to cycloheximide, naturally occurring resistance is widespread amongst yeast species because they possess a cycloheximide-resistant ribosome. Resistance has been shown to arise from the single proline substitution in a gene encoding for ribosomal protein L42 (formerly L41) or from amino acid replacement in ribosomal protein L29 (Dehoux et al., 1993; Stocklein et al., 1981). In addition, monomethylation at a single lysine in the gene encoding for L42 is associated with decreased susceptibility to cycloheximide (Shirai et al., 2010).

New and interesting translation inhibitors

With bacterial resistance becoming a threat to public health, there is a demand for new chemical scaffolds that interact with new ribosomal sites and inhibit translation via novel mechanisms of action. Recent years have faced the gaining interest towards antimicrobial peptides (AMPs) as potential therapeutics against antibiotic-resistant bacteria (Li et al., 2014). AMPs represent a diverse group of molecules that form part of the innate immune response of a variety of invertebrate, plant and animal species (Brogden, 2005). Proline-rich antimicrobial peptides (PrAMPs) represent a group of AMPs that were identified in the late 1980s independently in honeybees (Casteels et al., 1989) and cattle (Gennaro et al., 1989). Many AMPs kill bacteria by disrupting their cell membrane, whereas some are transported inside the bacterial cell where they bind and inactivate specific targets, including the bacterial ribosome. A study of the crystal structures of *Thermus thermophilus* 70S ribosome in complex with the PrAMPs $Bac7_{1–35}$, pyrrhocoricin, metalnikowin and two oncocin derivatives revealed that these peptides share a common mechanism of action. The N-terminal domain of ribosome-bound PrAMPs interferes with the simultaneous binding of the CCA-end of aminoacyl-tRNA in the A-site. Therefore, binding of PrAMPs allows 70S initiation complex formation, but prevents subsequent rounds of translation elongation (Seefeldt et al., 2015). The variable C-terminal regions extend to the ribosome exit tunnel and overlap with the binding site of macrolide and streptogramin B antibiotics. The conserved middle part of each of the studied PrAMPs forms interactions with the elements of the ribosome that are known to be the binding site for several antibiotics, including chloramphenicol (Gagnon et al., 2016). PrAMPs are synthesized on ribosomes as inactive precursors, which undergo proteolysis to release the active peptide. The mammalian-derived Bac7 peptide inhibits also eukaryotic translation. To avoid self-toxifcation, the Bac7 precursor is activated by a protease upon fusion with the phagosome or during exocytosis and release into the extracellular matrix where it can attack the bacteria (Scocchi et al., 1992; Seefeldt et al., 2016).

Negamycin is a natural peptide-like antibiotic. It was originally isolated from *Streptomyces* strains (Hamada et al., 1970). Negamycin exhibits inhibitory activity against Gram-negative and Gram-positive bacteria but its binding site and mode of action remained unknown for more than four decades. The structure of the *Thermus thermophilus* ribosome in complex with negamycin revealed that the drug binds to both small and large ribosomal subunits at nine independent sites. The primary site of antibiotic action was identified in the vicinity of the 16S rRNA helix 34 where it overlaps with the binding site of tetracycline. Negamycin contacts simultaneously with the 16S rRNA as well as the anticodon loop of the A-site-bound aminoacyl-tRNA. This antibiotic stimulates tRNA binding and, thus, inhibits translocation and induces miscoding (Polikanov et al., 2014b).

Amicoumacin A is produced by several bacterial species isolated from both soil and marine environments and the antibacterial properties of this compond were discovered already decades ago (Itoh et al., 1981). However, the mechanism of action of amicoumacin A remained unknown until the year 2014 when the crystal structure of bacterial ribosome in complex with the drug was solved. It was discovered that the antibiotic makes contacts with helix 24 of 16S rRNA in the E site. In addition, amicoumacin A interacts also with the mRNA backbone suggesting that the drug interferes with translocation by stabilizing mRNA interaction with the small ribosomal subunit (Polikanov et al., 2014a). The binding site of amicoumacin A overlaps with the binding sites of three chemically unrelated antibiotics pactamycin, kasugamycin and edeine B. This feature could be used for designing new antibiotic hybrids with potentially superior antibacterial properties.

1.2. Emerging antibiotics

Active use of antibiotics has evoked a wide distribution of drug-resistant bacteria. One plausible way to overcome this concern is the development and launching of new antibiotics. However, this approach is decelerated by the high cost of necessary research. Since the year 2000, 30 new antibiotics have been launched worldwide. It is self-evident that development of novel antibiotics can be obstructed by various problems. A number of drug candidates have been discontinued from clinical development even after completing Phase I or Phase II trials (Butler et al., 2016). On the other hand, there are some cases where continuous research has solved unexpected problems in the course of time. One fitting example is the development of oxazolidinones as it took more than 20 years until the first member of this class – linezolid – was approved for clinical use (Shaw and Barbachyn, 2011). The following chapters focus on three emerging antimicrobials and describe some difficulties that have impeded their launching. These antimicrobials are: (1) evernimicin, a member of orthosomycine class of antibiotics, (2) tiamulin, a representative of the pleuromutilin class, and (3) a nitrovinylfuran derivative Furvina®, also known as G1 compound.

1.2.1. Evernimicin

Evernimicin is a prominent example of everninomicin group belonging to orthosomycine class of antibiotics. Other members of orthosomycine class include avilamycin, curamycin and olivamycin. Evernimicin is a highly modified octasaccharide produced by actinomycete *Micromonospora carbonacea* (Weinstein et al., 1964). The unique structural features of evernimicin molecule are the presence of a nitrosugar L-evernitrose and two acid sensitive orthoester linkages between carbohydrate residues (Ganguly et al., 1997). Investigations identified evernimicin to be active against a number of Gram-positive bacteria, including methicillin-resistant staphylococci and vancomycin-resistant enterococci (Jones and Barrett, 1995). Evernimicin also inhibits *in vitro* protein synthesis with ribosomes derived from *E. coli*, although wild-type *E. coli* cells are naturally resistant to the compound (McNicholas et al., 2000).

Orthosomycins are the class of antibiotics that interact with the unique region of 50S ribosomal subunit. The binding site of evernimicin is located in the vicinity of L16, 50Å away from the PTC (Wilson, 2009). Other known translation inhibiting antibiotics do not compete with evernimicin for ribosome binding (McNicholas et al., 2000). Evernimicin protected a set of adenine residues in the helix 89 (A2468, A2469, A2476, A2478, and A2482) as well as A2534 in the helix 91 of 23S rRNA in chemical footprinting experiments (Belova et al., 2001). The loops of helices 89 and 91 of 23S rRNA are conserved between Bacteria and Archaea. The cryo-EM structure of evernimicin in complex with the *E. coli* 70S ribosome revealed that the heptasaccharide core of the drug spans across the minor grooves of H89 and H91, whereas the terminal dichloro-ring interacts with the arginine residues of ribosomal protein L16. The binding position overlaps with the elbow region of a tRNA bound in the A-site (Arenz et al., 2016).

Early studies indicated that evernimicin interferes with the translation initiation. 23S rRNA helices 89 and 91 that interact with evernimicin are known to be essential for the function of IF2 (La Teana et al., 2001; Burakowskii et al., 2007). Observations suggest that evernimicin interferes with the binding of IF2 on the 50S ribosomal subunit therefore perturbing the formation of 70S pre-initiation complex (Belova et al., 2001). Later studies indicated that evernimicin can also inhibit translation elongation (Orelle et al., 2013). According to a model, evernimicin allows initial binding of aa-tRNA at the A-site, but prevents complete accommodation of the incoming aa-tRNA, thus inhibiting translation elongation (Arenz et al., 2016). However, this model has not yet been conclusively demonstrated. Evernimicin does not interfere with the puromycin reaction (Belova et al., 2001; Arenz et al., 2016).

Though the initial screenings did not reveal any bacterial isolates displaying resistance to evernimicin (Jones and Barrett, 1995), several resistance cases were later reported. Evernimicin-resistant *Streptococcus pneumonia* strains were found to bear mutations in *rplP* gene encoding the ribosomal protein L16 or in domain V of 23S rRNA (positions 2456–2485) (Adrian et al., 2000a;

Adrian et al., 2000b). Resistance to the drug is achievable also via enzymatic modification. An rRNA methyltransferase EmtA causes reduction in evernimicin binding by methylating G2470 in the helix 89 of 23S rRNA (Mann et al., 2001). 23S rRNA methylation and *rplP* mutants can also provide resistance to avilamycin, whose molecule differs from evernimycin by the absence of nitro-sugar moiety. The extensive use of avilamycin as an animal growth promotant might explain the fast appearance of evernimicin resistance (Aarestrup and Jensen, 2000; Mann et al., 2001).

Evernimicin was initially considered a promising therapeutic agent as it was active against Gram-positive pathogens resistant to clinically used antibiotics and cross-resistance to other drugs was not observed. Therefore, a pharmaceutical company Schering-Plough started development of evernimicin under the name Ziracin. However, the development of evernimicin was terminated during Phase III clinical trials in year 2000. According to press release by Schering-Plough, the balance between efficacy and safety did not justify further development. Later studies have associated administration of the drug with undesirable side effects. As observed in studies with rats, Ziracin caused anomalies of the external genitalia in F1 females along with the decreased reproductive performance (Poulet et al., 2005). Nevertheless, the lack of cross-resistance between evernimicin and other ribosome-targeting antibiotics leaves the orthosomycins attractive for further studies.

1.2.2. Tiamulin

Tiamulin (Figure 13) is a member of pleuromutilin class of antibiotics. The parent compound of this group was discovered in 1951 and was named pleuromutilin because it was isolated from an edible mushroom *Pleurotis mutilus* (later renamed *Clitopilus scyphoides*). A related basidomycete *Pleurotis passeckerianus* (now classified as *Clitopilus passeckerianus*) is another producer of pleuromutilin (Kavanagh et al., 1951, Knauseder and Brandl, 1976).

Early studies of pleuromutilin demonstrated its antimicrobial activity against Gram-positive cocci and modest activity against a number of Gram-negative pathogens (Anchel, 1952). Subsequently, the compound was shown to inhibit the growth of penicillin- and streptomycin-resistant staphylococci as well as *Mycoplasma* (Knauseder and Brandl, 1976). Therefore, pleuromutilin gained the attention of Switzerland pharmaceutical company Sandoz whose researchers attempted to improve its antibacterial properties. Experimentations leaded to the discovery of semi-synthetic derivate tiamulin in 1974. Tiamulin is active against anaerobic bacteria, intestinal spirochetes and *Mycoplasma*. Still, some Gram-negative bacteria like *Pseudomonas aeruginosa* and *Proteus* are naturally resistant to tiamulin (Drews et al., 1975).

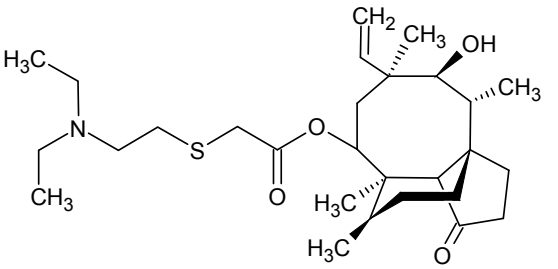


Figure 13. The structure of tiamulin.

Molecules of pleuromutilin class display an unusual structure among antibiotics. They possess a fused 5-6-8-membered tricyclidic diterpenoid structure with a C14 glycolic acid side chain (Figure 13). Chemical modification of the C14 side chain can alter the properties of the drug, such as the antibacterial activity, solubility in water and metabolism (Egger and Reinshagen, 1976; Lolk et al., 2008). Therefore, this side chain has been an object for a numerous modifications.

Tiamulin is a strong inhibitor of bacterial peptidyl transferase reaction, but it is unable to bind to mammalian ribosomes (Hogenauer, 1975). The compound prevents the correct positioning of CCA end of tRNA to the PTC and interferes with peptide bond formation. Chemical footprinting experiments showed that tiamulin interacts with the domain V of 23S rRNA (Poulsen et al., 2001). Crystal structure of tiamulin in complex with 50S subunit from *D. radiodurans* revealed that tiamulin binds to the PTC, overlapping both A- and P-site tRNA substrates (Schlunzen et al., 2004). The binding site of tiamulin overlaps those of chloramphenicol and clindamycin. Tricyclic mutilin core is located inside a pocket confined by residues of the A-site (G2061, A2451, C2452, A2503, U2504, G2505 and U2506). C14 side chain points toward the P-site and hydrogen bonds with G2061. Binding of pleuromutilins triggers an induced fit that leads to conformational change concerning nucleotides U2506 and U2585. As a result, the movement of tRNA 3' end from the A-site to the P-site is hampered due to nonproductive conformation of U2585 (Davidovich et al., 2007; Novak and Shlaes, 2010). Pleuromutilins partially destabilize fMet-tRNA binding during formation of the initiation complex (Yan et al., 2006). Based on this data, it has been suggested that tiamulin inhibits primarily translation initiation rather than elongation (Novak, 2011).

Resistance to tiamulin is shown to develop relatively slowly *in vitro* (Karlsson et al., 2001). Nevertheless, several cases of reduced susceptibility have been reported (Gentry et al., 2007). Reduced binding of tiamulin to the ribosome has been associated with point mutations in ribosomal protein L3 (*E. coli* positions 148 and 149) in combination with mutations in 23S rRNA (nucleotides G2032, G2055, G2447, C2499, U2504 or A2572) (Pringle et al., 2004). There is a chance for the emergence of cross-resistance between pleuromutilins and other antibiotics attacking the PTC. This phenomenon can be mediated by Cfr methyltransferase that methylates 23S rRNA nucleotide A2503

located within the PTC. In addition to pleuromutilins, Cfr activity confers resistance also to phenicols, lincosamides, oxazolidinones, 16-membered macrolides and streptogramin B (Long et al., 2006).

Tiamulin was approved in 1979 as a prophylactic and therapeutic agent for swine dysentery. However, initial efforts of developing pleuromutilin antibiotics suitable for human use encountered several problems. For instance, oral bioavailability of tiamulin is limited because it is rapidly metabolized by cytochrome P450 metabolism (Phillips and Sharaf, 2007). Azamulin is another derivative that was designed for human use and demonstrated good activity against many clinical isolates (Hildebrandt et al., 1982). Azamulin entered into Phase I clinical trials on volunteers, but further studies were terminated due to unsatisfactory pharmacokinetics.

In 2007, retapamulin was approved for the treatment of certain human skin infections (Novak and Shlaes, 2010). Thus, it took almost six decades until the first pleuromutilin antibiotic became available for human treatment. Some promising pleuromutilin derivatives are currently investigated at Nabriva Therapeutics (Paukner and Riedl, 2016). These new derivatives exhibit potent antibacterial activity, good pharmacokinetics and excellent safety profile. One such compound, lefamulin, was tested successfully in a Phase II clinical trial in 2011 (Prince et al., 2013).

1.2.3. G1 (Furvina®)

G1, also known by the trade name Furvina®, is a synthetic nitrovinylfuran (2-bromo-5-(2-bromo-2-nitrovinyl)-furan). G1 is a furylethylenic derivative in which the nitro group is not attached to the furan ring. The compound was developed in Cuba by researchers at the University of Las Villas. Early experiments demonstrated that nitrovinylfuran derivatives inhibit algal and yeast growth (Drobnica and Sturdik, 1980). Microbroth dilution tests confirmed antibacterial activity of G1 against Gram-positive and Gram-negative bacteria, yeasts, dermatophytes and filamentous fungi (Blondeau et al., 1999). The compound was approved in Cuba as an ointment for the treatment of dermatological infections despite of uncertain mode of action.

The exact mechanism of action of the drug is a subject of disputation. Early experiments demonstrated that vinylfurans inhibit various enzymes by chemically modifying functional thiol groups in their structures (Drobnica et al., 1981). Since then, two independent attempts have been made to elucidate the targets of G1. Scholz and colleagues showed that G1 inhibits the activity of *E. coli* MurA, an enzyme that catalyzes the assembly of peptidoglycan, and is also a potent inhibitor of bacterial methionine aminopeptidase (Scholz et al., 2012). Both enzymes contain exposed cysteine residues at their activity sites. G1 did not affect the activity of MurA in which the exposed cysteine was replaced by aspartic acid. It was concluded that G1 is reactive towards cysteine residues in proteins and participates in formation of non-native disulfide bonds,

thereby impairing stability and catalytic properties of proteins. The authors emphasized that the cytotoxic activity of G1 against human cell lines is significant and does not encourage further development of the compound as an antibacterial drug (Scholz et al., 2012).

In contrast, Fabbretti and coworkers studied the effects of G1 on prokaryotic translation (Fabbretti et al., 2012). *In vitro* translation test demonstrated that G1 effectively inhibits translation of natural-like mRNAs in a cell-free system. However, G1 did not inhibit poly(U)-dependent poly(Phe) synthesis and did not cause mRNA misreading, suggesting that G1 is not an inhibitor of translation elongation phase. Further experiments showed that G1 binds to 30S ribosomal subunits and competes with fMet-tRNA for the same binding site. IF2:GTP-dependent binding was not affected by G1. Hydroxyl radical cleavage and primer extension analysis revealed that G1 induces conformational changes at 16S rRNA site affecting the P-decoding region of the 30S subunit. Based on this data, it was reasonable to conclude that G1 is a translation inhibitor that binds to the 30S ribosomal subunit and inhibits P-site decoding thereby blocking formation of 30S initiation complex (Fabbretti et al., 2012).

1.3. General mechanisms of antibiotic resistance

1.3.1. Efflux

Bacteria are using efflux systems in order to export certain chemical substances out of their cells. Efflux is mediated by membrane-associated proteinaceous transporters that function as pumps. Efflux systems in Gram-positive bacteria compose of a single transmembrane polypeptide. On the other hand, certain pumps found in Gram-negative organisms exist as a tripartite system, spanning both the inner and outer membrane (Li and Nikaido, 2008).

First descriptions of efflux mechanisms derive from the studies of tetracycline-resistant *E. coli* (Ball et al., 1980; McMurry et al., 1980). Other types of efflux systems were discovered and characterized during next years (Poole et al., 1993; Paulsen et al., 1996). Some efflux systems are specific to a certain drug while others are able to export structurally different antimicrobials and are therefore called multidrug efflux systems (Saier et al., 1998). Genes encoding components of efflux systems can be located on the chromosome or on mobile genetic elements. Chromosomally encoded pumps can explain the inherent resistance of some bacterial species to a particular antibiotic (Munita and Arias, 2016). When present on plasmids or transposons, these genes facilitate the spread of antibiotic resistance. Today, efflux is considered to be one of the major determinants associated with the multidrug resistance (Lewis, 1994; Sun et al., 2014).

Efflux systems are thought to be widespread in bacteria already before the beginning of the antibiotic era (Saier et al., 1998). The pumps respond to a variety of stimuli and may perform different physiological functions in addition

to conferring drug resistance. For instance, efflux systems have been associated with the export of various toxic substances that are present in the environment (Piddock, 2006a; Poole, 2008; Thanassi et al., 1997). Efflux has also been shown to be involved in the secretion of quorum-sensing signals (Amaral and Molnar, 2012).

Efflux process is energy-dependent. Most of bacterial efflux systems are antiporters that use energy generated by difference of electrochemical potentials across the membrane. In such case, efflux process is coupled to the influx of protons or sodium ions (Figure 14). However, pumps belonging to the ABC family use the energy derived from ATP hydrolysis (Shlykov et al., 2013).

Five classes of efflux systems are implicated in the resistance to antibiotics (Figure 14). Classification of pumps is based on their structural characteristics as well as their energy source (Piddock, 2006b).

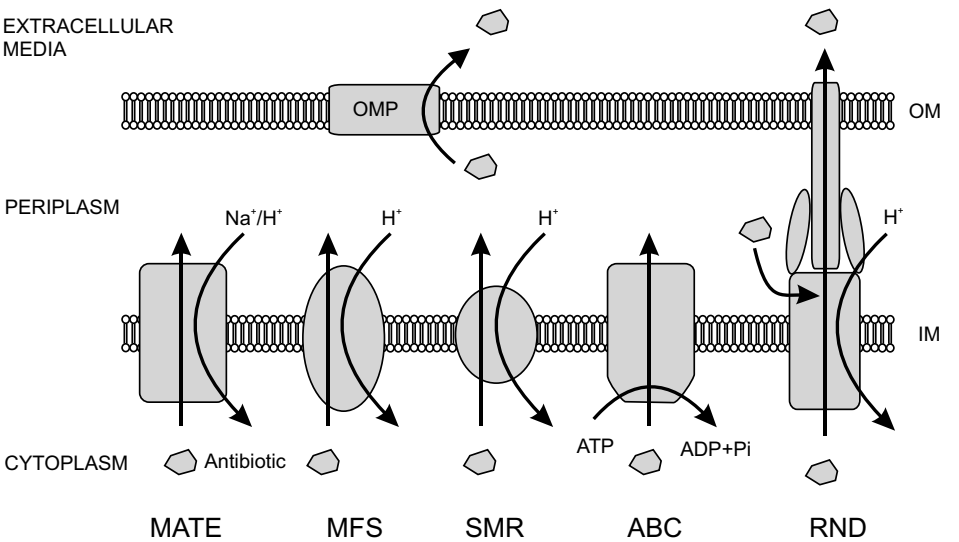


Figure 14. Schematic illustration of the main classes of bacterial efflux systems: the multidrug and toxic compound extrusion (MATE) family, the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily and the resistance-nodulation-division (RND) family. IM: inner membrane. OM: outer membrane. OMP: outer membrane protein.

Major facilitator superfamily (MFS)

MFS transporters constitute an ancient family and they are widespread in both Gram-negative and Gram-positive organisms. Members of this family are proton-driven antiporters. Their structure is composed of either 12 or 14 membrane-spanning helices (Saidijam et al., 2006). Substrates of MFS family include various biocides and dyes as well as antibiotics tetracycline, chlor-

amphenicol, macrolides and fluoroquinolones. Examples of this group include tetracycline-specific pumps Tet(K) and Tet(L). Nearly 30 *tet* genes are known that are related to efflux and most of them are found in mobile genetic elements. These pumps often decrease tetracycline and doxycycline susceptibility but do not expel the third generation tetracycline tigecycline (Roberts, 2005).

Small multidrug resistance (SMR) family

SMR transporters were first discovered in Gram-positive *S. aureus* (Tennent et al., 1989). Subsequently they were also identified in Gram-negative bacteria and archaea (Bay et al., 2008). This group comprises small transport proteins that contain 4 transmembrane segments and often form tetramers in the cytoplasmic membrane. SMR transporters are drug-proton antiporters. SMR transporters were found to be implicated in reduced susceptibility to biocides (Littlejohn et al., 1992). AbeS from *Acinetobacter baumannii* is one of few SMR pumps related to resistance to fluoroquinolones, chloramphenicol and erythromycin (Srinivasan et al., 2009).

Resistance-nodulation-division (RND) family

RND pumps usually exist as a complex composed of three components: (1) an inner membrane pump that consists of 12 transmembrane helices and uses the proton motive force for extruding the substrates, (2) a membrane fusion protein located in the periplasmic space, and (3) an outer membrane protein that forms a channel in the outer membrane and acts as an efflux duct (Zgurskaya and Nikaido, 1999). Each of these three components is essential for drug efflux and the absence of even one component makes the entire complex nonfunctional (Ma et al., 1995). One of the best studied tripartite drug effux complexes is the AcrA-AcrB-TolC transporter from *E. coli* (Du et al., 2014). TolC can also function with other types of efflux machinery (Hinchliffe et al., 2013). Due to construction of RND pumps, the drugs are transported directly into the external medium (Nikaido, 1996). Substrate specificity of RND pumps can be remarkably wide.

Many Gram-negative bacteria are resistant to β-lactams, macrolides and linezolid due to RND efflux systems (Blair and Piddock, 2009). In addition, RND pumps are responsible for removing other toxic substances (bile salts, dyes, disinfectants) from the cell. These pumps are not exclusive to Gram-negative bacteria, as some Gram-positive organisms are found to possess genes encoding proteins with structural characteristics of RND pump monomers (Schindler et al., 2015).

Multidrug and toxic compound extrusion (MATE) family

These pumps were the last discovered and are found in both Gram-negative and Gram-positive bacteria (Kuroda and Tsuchiya, 2009). MATE transporters are composed of 12 transmembrane regions and they share structural similarities

with MFS transporters. However, amino acid sequences of the two families are quite different. Moreover, MATE transporters often use Na^+ gradient as an energy source instead of proton gradient (Morita et al., 2000). VcmB and VcmD are examples of this class that reduce susceptibility to aminoglycosides, fluoroquinolones and cationic compounds in *Vibrio cholerae* (Begum et al., 2005).

ATP-binding cassette (ABC) superfamily

Transporters belonging to this family are rare and they offer rather modest level of resistance. However, they can transport various substrates including sugars, amino acids, polysaccharides and drugs like macrolides, fluoroquinolones and tetracyclines (Fernandez and Hancock, 2012). ABC transporters are either homodimers or heterodimers and they utilize the energy derived from the ATP hydrolysis (Moussatova et al., 2008). These efflux systems consist of two units: an intracellular nucleotide-binding domain dimer, which binds and hydrolyses ATP, and a membrane-domain dimer, which is embedded in the membrane and acts as a trans-membrane pathway for substrates (Du et al., 2015; Lubelski et al., 2007). The first described ABC transporter was a macrolide-specific tripartite MacABC-TolC in *E. coli* (Kobayashi et al., 2001). Based on the sequence similarity, the ABC protein family has been divided into eight subfamilies, denoted by the letters A to H. Interestingly, members of the ABC-F subfamily lack any identifiable transmembrane domain and instead of being involved in transport, these proteins mediate resistance to many antibiotics that bind to the 50S subunit of the ribosome (Sharkey et al., 2016).

Expression of efflux genes is often very complex, involving a variety of transcriptional regulators and other modulators (Grkovic et al., 2002). Some pumps can be constitutively expressed, thus providing intrinsic resistance (Poole, 2005). However, expression of many efflux systems is controlled by the presence of at least one of their substrates in the environment. For instance, expression of MFS pump QacA in *Staphylococcus aureus* is negatively regulated by transcriptional repressor QacR. When QacA substrates (e.g. certain dyes) are present, they bind to QacR thus inactivating the repressor protein and enabling the expression of QacA (Grkovic et al., 1998).

Efflux pump inhibitors (EPIs) are compounds that interfere with the function of efflux systems, thus increasing the drug concentration inside a cell (Zechini and Versace, 2009). EPIs can affect efflux systems by various ways, including blocking of energy required for the activity of pumps, repressing a gene encoding efflux pumps or interfering with the assembly of pumps (Bhardwaj and Mohanty, 2012). EPIs are considered as promising tools for restoring the activity of antibiotics that are substrates for efflux pumps. Still, none of EPIs has been approved for clinical use so far.

1.3.2. Target modifications

Resistance to antibiotics can be conferred via alternations in their target site. There are two basic ways for altering the drug binding site: (1) by mutations in genes encoding ribosomal RNA or ribosomal proteins, and (2) by enzymatic modifications of rRNA nucleotides that comprise the drug binding site. Another possibility for avoiding the antimicrobial action is to "bypass" the metabolic pathway they inhibit by overproducing the antimicrobial target (Munita and Arias, 2016).

The binding sites of most of ribosome-targeting antibiotics are composed exclusively or primarily of rRNA. Mutations in rRNA sequence may alter the conformation of antibiotic binding site and result in resistance to these antibiotics. The presence of low antibiotic levels in the environment contributes to the appearance of mutations conferring antibiotic resistance (Hughes and Andersson, 2012). The binding sites of many clinically used antibiotics are located at the decoding site of the ribosome or at the PTC (Figure 1), which are critical regions for ribosome function. Thus, mutations in rRNA may interfere with ribosome function and mutations conferring resistance to antibiotics are often accompanied by fitness cost to the cell. In several cases, fitness cost can be overcome by compensatory mutations (Bjorkman and Andersson, 2000; Schrag and Perrot, 1996). For instance, fitness cost of G2576T transversion in 23S rRNA conferring resistance to linezolid can be relieved by mutations in ribosomal proteins L3 and L16 (Billal et al., 2011). Moreover, resistant cells possessing compensatory mutations may be more fit than susceptible cells carrying the same compensatory mutants (Schrag et al., 1997). This might explain why some mutational resistance can be maintained stably in the absence of antibiotic use (Woodford and Ellington, 2007).

Direct interactions between antibiotics and ribosomal proteins are rare. Nevertheless, resistance to antibiotics can be caused by mutations in ribosomal proteins. Alternations in proteins cause resistance indirectly by perturbing the conformation of rRNA. Well-known examples are mutations in protein L3 that can render cells resistant to pleuromutilins despite of the lack of direct interaction with the drug (Pringle et al., 2004). Alternations in ribosomal protein L22 can render ribosomes resistant to erythromycin, in some cases even without reducing their affinity to the drug (Chittum and Champney, 1994; Tu et al., 2005). Sometimes the susceptibility to antibiotics is reduced by the combination of both ribosomal protein and rRNA mutations (LaMarre et al., 2013).

In most bacteria, rRNA is encoded by multiple operons. A mutation has to be present in all or in a majority of rRNA operons to achieve high-level resistance. Therefore, enzymatic modification of rRNA is a more common resistance mechanism. rRNA methyltransferase Cfr methylates the C8 position of the conserved nucleotide A2503 of the 23S rRNA (Kehrenberg et al., 2005). Methylation of A2503 occurs during ribosomal assembly (Yan et al., 2010). As A2503 is located in the cavity of the peptidyl transferase site of the ribosome, its methylation perturbs the positioning of antibiotics that target the PTC. Cfr-

mediated modification of A2503 gives rise to $PhLOPS_A$ phenotype that is resistant to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics (Long et al., 2006). Furthermore, Cfr also provides resistance to 16-member-ring macrolides (Smith and Mankin, 2008). The respective *cfr* gene was originally discovered in *Staphylococcus sciuri* isolates of animal origin (Schwarz et al., 2000). Experiments with the laboratory *S. aureus* strains revealed that only a slight fitness cost can be associated with *cfr* acquisition (LaMarre et al., 2011). This observation can explain the apparent spread of the *cfr* gene among pathogens.

Erm methyltransferases add one or two methyl groups to the N6 position of A2058 of 23S rRNA (Skinner and Cundliffe, 1982). A2058 is located inside of the nascent peptide exit tunnel that concurrently serves as a macrolide binding site (Hansen et al., 2002). Monomethylation disturbs the conformation of A2058 and confers high resistance to licosamides and moderate resistance to macrolides and streptogramin B antibiotics. Dimethylation of A2058 results in MLS_B phenotype, which demonstrates high resistance to lincosamides, steptogramin B antibiotics and macrolides including ketolide telithromycin (Weisblum, 1995a).

Currently, there are 40 known *erm* genes (http://faculty.washington.edu/marilynr). The *erm(B)* and *erm(F)* genes have the widest host range as they have been found in more than 20 different genera. Expression of *erm* genes is often regulated posttranscriptionally by translation attenuation and induced only when antibiotic is present (Weisblum, 1995b; Ramu et al., 2009). Nevertheless, several clinical isolates with mutated attenuator have been reported in which *erm* genes are expressed constitutively (Depardieu et al., 2007).

In contrast to the Cfr methyltransferases, expression of the ErmC methyltransferases causes reduction in cell fitness. Alternation of A2058 structure results in abnormal interactions between the nascent peptide and ribosomal exit tunnel. This observation explains why *erm* genes have evolved to be inducible (Gupta et al., 2013).

Methyltransferases conferring resistance to aminoglycosides form the RMA (Resistance Methyltransferases for Aminoglycosides) superfamily. RMAs provide intrinsic or acquired resistance to aminoglycosides by modifying either nucleotide G1405 or A1408 in the A-site of the 30S ribosomal subunit. RMAs found in natural aminoglycoside producers can be divided into two groups. Members of Kgm subfamily modify the N7 position of G1405 of 16S rRNA and confer high-level resistance to 4,6-DOS (Savic et al., 2009). Members of Kam subfamily act at the N1 position of 16S rRNA nucleotide A1408 and confer resistance to kanamycin and tobramycin but not to gentamicin (Beauclerk and Cundliffe, 1987). In addition to natural aminoglycosides-producers, several Gram-negative human pathogens have been found to harbor 16S rRNA methylase genes that methylate either G1405 or A1408 and confer high-level resistance to clinically useful aminoglycosides (Doi and Arakawa, 2007).

In some cases, defective methylation can also confer resistance to antibiotics. The methyltransferase KsgA methylates universally conserved nucleotides

A1518 and A1519 in helix 45 of 16S ribosomal RNA. The exact biological function of this rRNA modification is unknown. Inactivation of the *ksgA* gene and loss of the dimethylations confers resistance to the aminoglycoside antibiotic kasugamycin (Helser et al., 1972; O'Farrell et al., 2008).

1.3.3. Antibiotic modifications

Enzymatic modification or degradation of antibiotics is a widespread biochemical defense mechanism among bacteria. Enzymatic strategies of antibiotic inactivation involve: (1) inactivation by hydrolysis, (2) inactivation by group transfer and (3) inactivation by redox processes.

Antibiotic inactivation by hydrolysis

Several antibiotics contain hydrolytically susceptible chemical bonds in their structure. Hydrolysis is the most common resistance mechanism against β-lactam antibiotics. These drugs contain a β-lactam ring in their structure that can be cleaved by enzymes called β-lactamases. However, hydrolysis also provides resistance to other antibiotics including marcolides and chloramphenicol.

Macrolide esterases have been found in members of the family *Enterobacteriaceae* as well as in staphylococci (van Hoek et al., 2011). These enzymes catalyze the hydrolysis of macrolide lactone ring. The first gene encoding erythromycin esterase was discovered from *E. coli* and named *ere(A)* (Ounissi and Courvalin, 1985). Subsequently, *ere(B)* gene was isolated from another *E. coli* isolate (Arthur et al., 1986). EreB esterase can hydrolyze erythromycin and oleandomycin (Arthur and Courvalin, 1986). The amino acid sequences of EreA and EreB do not display statistically significant homology. Another erythromycin resistance gene designated *ere(*C) has been found in *Klebsiella* (Yong et al., 2009).

Antibiotic inactivation by group transfer

Bacteria are neutralizing some antibiotics by adding various functional groups to their molecules. These modifications include O- and N-acetylation, O-phosphorylation, O-nucleotidylation, O-ribosylation, O-glycosylation and thiol transfer (Wright, 2005). Modifications can dramatically reduce the binding affinity of antibiotics to their target. Group transfer reactions are catalyzed by a diverse family of intracellular enzymes called group transferases. These modifying enzymes require a co-substrate for their activity (acetyl-CoA, ATP, NAD^+, etc). An impressive number of transferases are implicated in conferring resistance to aminoglycosides (Jana and Deb, 2006; Ramirez and Tolmasky, 2010). Aminoglycoside modifying enzymes are divided into three classes according to the type of modification: O-phosphotransferases (APHs), O-nucleotidyltransferases (ANTs) and N-acetyltransferases (AACs). As each transferase targets a specific region within an aminoglycoside molecule, these enzymes are further

divided into subclasses. APHs are widely distributed among bacterial pathogens. According to their regiospecificity, seven subclasses of APHs are distinguished. They add the γ-phosphate group derived from ATP to the hydroxyl moiety which is located at the position 4, 6, 9, 3', 2'', 3'' or 7'' of an aminoglycoside molecule. ANTs adenylate hydroxyl groups of aminoglycosides at the 6, 9, 4', 2'' or 3'' positions. The ANT class has an impact on medicine as ANT(2'') can modify clinically important drugs tobramycin and gentamicin (Miller et al., 1997). AACs use acetyl-CoA as a donor substrate and catalyze the acetylation of $-NH_2$ groups at the 1, 3, 2' or 6' positions. A bifunctional enzyme AAC(6')-APH(2'') has been identified that is able to sequentially acetylate and phosphorylate its substrates (Shaw et al., 1993).

Group transfer is also a prevalent mechanism of chloramphenicol resistance. Chloramphenicol acetyltransferases (CATs) are widespread in Gram-positive and Gram-negative bacteria. They are catalyzing the transfer of an acetyl group to the 3-hydroxyl group of chloramphenicol, yielding 3-*O*-acetyl-chloramphenicol (Shaw, 1967). Acetyl-coenzyme A is utilized as a donor for acetyl group. The acetyl group can be transferred non-enzymatically from the 3-hydroxyl group to the 1-hydroxyl group of chloramphenicol. As a result, 3-hydroxyl group becomes available for another CAT-catalyzed acetylation (Thibault et al., 1980). Both di- and mono-acetylated chloramphenicol molecules are unable to bind tightly to the 50S ribosomal subunit (Bulkley et al., 2010; Shaw and Unowsky, 1968). In addition to chloramphenicol, CATs are able to inactivate thiamphenicol and azidamfenicol. Florfenicol, on the other hand, remains resistant to CAT because its molecule contains a fluor residue instead of the 3-hydroxyl group (see Figure 5).

Expression of genes encoding CATs can be constitutive or regulated by translational attenuation, with chloramphenicol acting as an inducer (Lovett, 1990; Schwarz et al., 2004). Native CAT variants are usually homotrimers. Despite of their overall similar structure, CAT proteins can be divided into three classes (Foster and Shaw, 1973). Members of CAT_I class demonstrate high similarity in their amino acid sequences. However, CAT_I proteins share only modest similarity with proteins belonging to classes CAT_{II} (46 %) and CAT_{III} (32–47%) (Biswas et al., 2012). CAT_{II} class differs from CAT_{III} class through its extreme susceptibility to thiol-modifying agents (Murray et al., 1990). Some bacteria have been found to harbor so-called xenobiotic acetyltransferases that can also acetylate chloramphenicol, but are structurally unrelated to three CAT classes (Murray and Shaw, 1997).

Chloramphenicol producer *S. venezuelae* lacks CAT activity (Shaw and Hopwood, 1976). Instead, this organism protects itself against its own toxic compound by harboring chloramphenicol phosphotransferase (CPT). CPT uses ATP as phosphoryl donor to transfer the γ-phosphate to the 3-hydroxyl group of chloramphenicol. Phosphoryl group is later removed by an extracellular phosphatase, resulting in an active antibiotic (Mosher et al., 1995). CTP does not show significant sequence similarity to aminoglycoside phosphotransferases.

Macrolide 2′-phosphotransferases [MPH(2′)] phosphorylate the 2′-hydroxyl group of an amino sugar in the macrolide molecule. The phosphoryl group is obtained from the γ-phosphate of ATP. Macrolide phosphotransferases have been found mainly in Gram-negative bacilli and they are divided into two groups. Members of MPH(2′)-I are encoded by the *mph(A)* and *mph(D)* genes and inactivate preferbly 14-membered ring macrolides (see Figure 4). MPH(2′)-II enzymes, encoded by the *mph(B)* and *mph(D)* genes are able to inactivate 14- as well as 16-membered ring macrolides (O'Hara et al., 1989; Chesneau et al., 2007).

Streptomyces lividans expresses a glycosyltransferase for self-protection. The enzyme catalyses glycosylation of macrolides at the 2' position and uses UDP-glucose as a co-factor (Jenkins and Cundliffe, 1991). However, glycosyltransfer is not considered a widely distributed resistance mechanism (Wright, 2005).

Redox processes

A few examples of resistance provided by oxidation or reduction of antibiotics can be found. The most prominent example of redox enzymes is Tet(X) that was discovered on a conjugative transposon in *Bacteoides fragilis* (Speer and Salyers, 1989). Tet(X) is a monooxygenase capable of inactivating all clinically relevant tetracycline antibiotics, including the third generation tetracycline tigecycline (Moore et al., 2005). The enzyme utilizes NADPH and O_2 to add a hydroxyl group to the C11a position located between B and C ring of tetracycline molecule. The antibiotic properties of hydroxylated tetracyclines are significantly reduced due to their fast degradation (Yang et al., 2004). Moreover, modified tetracyclines can only weakly bind Mg^{2+}-ions, which are essential for their interaction with the ribosome (Brodersen et al., 2000; Moore et al., 2005).

1.4. Target cleaning

1.4.1. Macrolide resistance peptides

Peptide-mediated resistance is a coincidentally discovered mechanism that confers resistance to macrolide antibiotics including ketolides. First evidence of a novel resistance mechanism was obtained in experiments in which *E. coli* cells expressing random fragments of ribosomal RNA operon *rrnB* were screened for the presence of erythromycin-resistant clones (Tenson et al., 1996). All resistant clones were noticed to express rRNA fragments encompassing the region 1233–1348 of 23S rRNA. It was found that expression of a 34 nucleotides long rRNA fragment (E-RNA34) corresponding to residues 1235–1268 in 23S rRNA is sufficient to provide a low-level resistance to erythromycin. E-RNA34 contained the Shine-Dalgarno sequence, initiator and terminator codons and an ORF coding for a pentapeptide MRMLT (Tenson et al., 1996).

Extensive selection experiments revealed certain pentapeptide sequences (so-called E-peptides) conferring resistance to erythromycin and other macrolides. Analysis of more than 70 E-peptide sequences helped to identify a consensus sequence fMet-(bulky/hydrophobic)-(Leu/Ile)-(hydrophobic)-Val (Tenson et al., 1997). The E-peptide MRLFV rendered cells resistant to high concentrations of erythromycin and low concentrations of oleandomycin and spiramycin, but not to ketolides. On the other hand, the peptide MRFFV providing resistance to ketolide HMR 3004 did not protect cells from high concentrations of erythromycin (Tripathi et al., 1998). As a fact, peptides with significantly different amino acid sequence are needed to confer resistance to chemically different macrolides and only a little cross-resistance was observed (Vimberg et al., 2004).

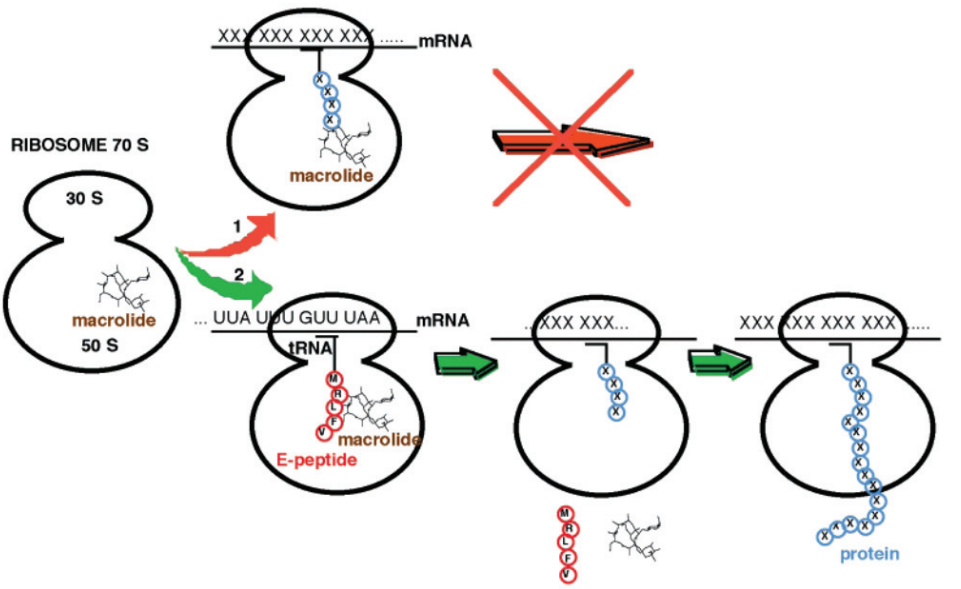


Figure 15. Model of action of macrolide resistance peptides. (1) Binding of a macrolide to the 50S ribosomal subunit hinders growth of the nascent peptide chain in the early rounds of protein synthesis and then exerts antibiotic activity in inhibiting protein biosynthesis in the elongation step. (2) Translation of a macrolide resistance peptide "cleans" the ribosome from antibiotic. If an antibiotic-free ribosome initiates synthesis of a cellular protein and polymerizes the first two to five amino acids, it will become "immune" to an antibiotic until the completion of polypeptide synthesis because macrolides cannot bind to ribosomes containing long nascent peptides. The figure is adapted from Verdier et al., 2002.

Pentapeptides confer resistance only to a ribosome on which the peptide is translated. Addition of synthetic E-peptides did not influence erythromycin sensitivity in the cell-free translation system (Tenson et al., 1996). Based on experimental data, Tenson and Mankin proposed a model for explaining the

action of macrolide resistance peptides (Figure 15). Macrolide antibiotics do not inhibit polymerization of first few amino acids. Thus, the ribosome can translate resistance peptides also in the presence of macrolides. When synthesis of the resistance peptide is almost complete, the nascent peptide forms contacts with the drug and acts as a "bottle brush" that "cleans" the ribosome from the bound antibiotic. The macrolide-free ribosome can now initiate translation from a cellular mRNA. After polymerization of a peptide 4–6 amino acids in length, the ribosome becomes "immune" to macrolides until the completion of a polypeptide synthesis (Tenson and Mankin, 2001).

The following experiments by Lovmar and colleagues demonstrated that resistance peptide can remove erythromycin from the ribosome with close to 100% probability (Lovmar et al., 2006). Biochemical data together with computational modeling suggested that the side chain of leucine in the E-peptide MRLFV binds to the hydrophobic cleft between the cladinose and desosamine moieties of erythromycin. Interaction between leucine and erythromycin in combination with extension of the resistance peptide weakens the affinity of the drug to the ribosome. However, final dissociation of erythromycin occurs not until class 1 release factor induces termination of the full-length resistance polypeptide. The determined length of resistance peptide is crucial for its activity as synthesis of a hexapeptide with the same five N-terminal amino acids leaded neither to drug ejection nor to resistance to erythromycin (Lovmar et al., 2006).

Expression of an rRNA-encoded pentapeptide in wild-type *E. coli* cells is unlikely to occur as its Shine-Dalgarno sequence is hidden into the 23S rRNA secondary structure. However, expression could be activated by site-specific fragmentation of rRNA or by mutations that enable the accessibility to the Shine-Dalgarno region (Dam et al., 1996). Interestingly, a spontaneous deletion of positions 1219–1230 in 23S rRNA was shown to render cells resistant to erythromycin (Douthwaite et al., 1985). Activation of the rRNA-encoded pentapeptide expression due to accessible ribosome-binding site can provide an explanation for this observation. Although further evidences are missing, similar mechanisms of antibiotic resistance may occur in nature. It is not clear, whether the presence of a functional gene in rRNA is a result of evolutionary selection or just a coincidence.

1.4.2. Ribosomal protection proteins

Various mechanisms may be responsible for the resistance to tetracyclines, including enzymatic inactivation of the drug, mutations in rRNA and those mediated by efflux pumps. Ribosomal protection proteins (RPPs) confer resistance by a unique mechanism that was originally described in tetracycline-resistant *Streptococcus* (Burdett, 1986). RPPs bind the ribosome and catalyze the release of tetracycline in a GTP-dependent manner. However, the release of tetracycline occurs also in the presence of non-hydrolyzable GTP analogues.

Therefore, the GTPase activity is required for the multi-turnover of RPPs rather than for the release of the drug (Burdett, 1996; Trieber et al., 1998).

RPPs belong to the translation factor superfamily of GTPases (Leipe et al., 2002) and they share 50% of sequence similarity with EF-G (Donhofer et al., 2012). RPPs are spread among both Gram-negative and Gram-positive bacteria inhabiting various environments and ecosystems (van Hoek et al., 2011). So far, 12 different classes of RPPs have been reported (http://faculty.washington.edu/marilynr/). These proteins confer resistance to tetracycline, doxycycline and minocycline, while the third generation tetracyclines generally retain antibacterial activities in the presence of RPPs (Bergeron et al., 1996; Draper et al., 2013). Genes encoding RPPs are often located on transposons or other mobile genetic elements, which facilitates their spread via lateral gene transfer.

Tet(O) and Tet(M) are the best characterized RPPs. They are soluble cytoplasmic proteins possessing 75% of sequence identity (Taylor and Chau, 1996). Tet(O) was discovered in *Camphylobacter jejuni* and became the first cloned RPP (Taylor, 1986). Tet(M) has the broadest host range among RPPs. RPPs are thought to be originated from *otrA* gene which is harbored by oxytetracycline producer *Streptomyces rimosus* (Doyle et al., 1991). Several mosaic genes have been identified that contain regions from 2 or 3 different *tet* genes. Mosaic genes, such as *tet(O/W)* are thought to arise via recombination between different *tet* genes (Stanton et al., 2005).

Early experiments demonstrated that Tet(M) and EF-G compete for binding to the ribosome (Dantley et al., 1998). Subsequent studies of cryo-EM structures showed that the RPPs bind into an intersubunit space similarly to EF-G (Spahn et al., 2001; Donhofer et al., 2012). When bound to a ribosome, Tet(M) overlaps with the anticodon stem-loop of the A-tRNA and interacts with the helix 34 of 16S rRNA, which is a component of tetracycline binding site (Donhofer et al., 2012). The presence of an antibiotic thiostrepton blocks the ability of Tet(M) to form a stable complex with ribosomes and also inhibits GTP-dependent tetracycline release from ribosomes (Dantley et al., 1998).

Donhofer and colleagues studied the cryo-EM structure of the Tet(M):70S complex at 7.2-Å resolution and suggested that binding of Tet(M) alters the conformation of nucleotides C1054 and U1196 of the 16S rRNA (Donhofer et al., 2012). Tet(M) was thought to remove tetracycline molecule from the A-site by disrupting interaction between the aromatic D ring and C1054. According to the proposed model, immediate rebinding of the drug is inhibited as the conformational changes in 16S rRNA persist after dissociation of RPP from the ribosome (Donhofer et al., 2012). However, recent work in which Tet(M):70S complex was studied at 3.9 Å resolution indicated that Tet(M) does not alter the conformation of C1054 (Arenz et al., 2015). In addition, RPPs were shown to induce a flipped-out conformation of 16S rRNA nucleotides A1492 and A1493. Such conformation persists upon dissociation of RPP from the ribosome and promotes rapid binding of the aa-tRNA:EF-Tu:GTP ternary complex (Connell et al., 2003a; Donhofer et al., 2012).

Expression of Tet(M) is controlled by translational attenuation and induced by tetracycline molecule. The gene encoding *tet(M)* is preceded by a leader ORF. In the absence of tetracycline, the ribosome binding site of *tet(M)* ORF is hidden by mRNA secondary structure, but translation of leader ORF is enabled. Tetracycline is proposed to inhibit translation of *tet(M)* leader ORF. As an outcome, mRNA folds into conformation, which enables *tet(M)* translation (Lodato et al., 2006).

The resistance mechanism provided by proteins such as Tet(O) and Tet(M) may, however, be not completely unique. Studies have suggested that proteins belonging to the ABC-F subfamily of ATP-binding cassette transporters may function in a similar manner. The family of ATP-binding transporters is divided into eight subgroups on the basis of their sequence similarity (Dean et al., 2001). Despite of sharing homology with other members of the family, ABC-F proteins are not directly involved in transport (see chapter *ATP-binding cassette (ABC) superfamily*). They lack transmembrane domanis and instead they comprise a single polypeptide that contains two ATP-binding cassette domains (Kerr, 2004). ABC-F proteins are found in Gram-positive bacteria where they can confer resistance to many antibiotic classes that bind to the 50S ribosomal subunit, including the macrolides, lincosamides, phenicols, oxazolidinones and streptogramins. However, no single protein confers resistance to every listed class. Resistance to lincosamides and streptogramins A is mediated by ABC-F proteins encoded by *vga*, *lsa* and *sal* genes, while *msr* gene products can confer resistance to macrolides and streptogramins B. Another ABC transporter gene, *optrA*, confers combined resistance to oxazolidinones and phenicols (Wilson, 2016).

Results obtained so far suggest that ABC-F proteins mediate resistance to antibiotics through direct interaction with the ribosome (Sharkey et al., 2016). The process seems to require ATP hydrolysis, since mutations in the nucleotide-binding domain of ABC-F protein Vga(A) rendered cells susceptible to antibiotics (Jacquet et al., 2008). The molecular details of mechanism by which ABC-F proteins confer resistance are not completely understood. One possibility is that the binding of ABC-F proteins physically overlaps the drug-binding sites on the ribosome. Alternatively, the binding of proteins can induce conformational changes in the ribosome that promote displacement of drug molecule.

2. AIMS OF THE STUDY

I

Several nascent polypeptide chains are able to induce translational stalling in response to specific ligand molecules (Weisblum, 1995b; Ramu et al., 2009; Gong and Yanofsky, 2002). The aim of our study was to develop a universal method for finding such *cis*-acting regulatory sequences. If the approach would turn out to be successful, our further goal was to characterize the identified sequences and shed light on the possible sequence rules.

II

Ribosomal protection proteins confer resistance to tetracycline by dislodging the drug from the ribosome (Burdett, 1996; Connell et al., 2003a). An atomic model of the ribosome-Tet(O) complex suggests that the three loops of domain IV of ribosomal protection protein Tet(O) play a crucial role in Tet(O)-mediated tetracycline resistance. The goal of current study was to validate the structural results by mutational analysis. For this purpose, I introduced deletions or substitution mutations into the three loops of Tet(O) domain IV and measured the functional activity of mutant Tet(O) variants *in vivo*.

III

G1 (or Furvina®) is an antimicrobial of 2-vinylfuran family. It has been shown to target the 30S ribosomal subunit and inhibit translation initiation (Fabbretti et al., 2012). On the other hand, G1 has a direct reactivity against thiol groups (Balaz et al., 1982). By reacting with thiol groups it causes direct damage to proteins but, as a result, is very short-living and interconverts into an array of reaction products (Scholz et al., 2013). Our aim was to characterize thiol reactivity of G1 and identify its decomposition products. We also wanted to clarify how much of antimicrobial and cytotoxic effects are due to the primary activity of the compound and how much can be attributed to its breakdown products.

3. METHODS

Peptide expression libraries

A peptide expression library is a collection of plasmid expression vectors encoding a systematic combination of different peptides. Such libraries are usually generated by PCR using primers containing random nucleotides. Plasmid libraries expressing random peptides provide a useful tool for isolating peptides with various properties. For instance, a 21-codon mini-gene library together with a pentapeptide library was used to isolate peptides conferring resistance to erythromycin (Tenson et al., 1997). Similarly, a random pentapeptide mini-gene library was successfully applied to identify sequences conferring resistance to several other macrolide and ketolide antibiotics (Tripathi et al., 1998; Vimberg et al., 2004). A random five-codon gene library was also used to isolate mini-genes whose expression causes cell growth arrest (Tenson et al., 1999). Moreover, it has been demonstrated that peptides with antimicrobial activity, 20 amino acids long, can be screened effectively from a random genetic library by *in vivo* expression (Choi et al., 2002).

We generated a plasmid library in order to find *cis*-acting regulatory sequences that suppress β-lactamase expression in response to chemically diverse compounds. Our library contained an SD element, initiation codon and 21 random nucleotides in front of the β-lactamase ORF. In theory, the number of all possible RNA sequences coded by a random sequence of 21 nucleotides is 4^{21} (more than 10^{12}) and the number of possible heptapeptide sequences is 20^7 (1.28×10^9). Our initial library contained 10^6 clones which represents only a small fraction of all possible sequence variants. Nevertheless, we expected that if strong sequence preference patterns are involved, they would be revealed.

Site-directed mutagenesis

To investigate the functional importance of ribosomal protection protein Tet(O) domain 4 loops, a series of Tet(O) mutant variants were generated by site-directed mutagenesis. This method is a fundamental tool in molecular biology that is often used to elucidate the function of a gene or protein. Several techniques have been developed to introduce substitutions, deletions or insertions into DNA. Older techniques that are based on extension of a single mutation-containing primer are now rarely used as they comprise multiple steps and require selection of the mutant products (reviwed in Braman, 2002). Site-directed mutagenesis can also be performed using PCR and primers containing the desired change. During PCR, the primer sequence replaces the original sequence, but the changes should be small enough to allow the primer to anneal to the target (Mullis and Faloona, 1987).

Site-directed mutagenesis by overlap extension employs two rounds of PCR and nested primers (Ho et al., 1989; Lee et al., 2010). Figure 16 demonstrates the strategy for generating a deletion into *tet(O)* gene sequence. Primers B and

C were designed to position at either side of the region to be deleted. The first round of PCR used primers AB and CD to create two products. The ends of these products, created by primers B and C, were complementary to each other and enabled hybridization of the two fragments during the second round of PCR. As a result, the final RCR product containing the desired mutation was created using primers A and D. Substitution mutations were generated by employing internal primers B and C which contained the desired mutation in the overhang regions. The complementary ends of the first round PCR products were hybridized and the next PCR created the final product bearing the desired insertion.

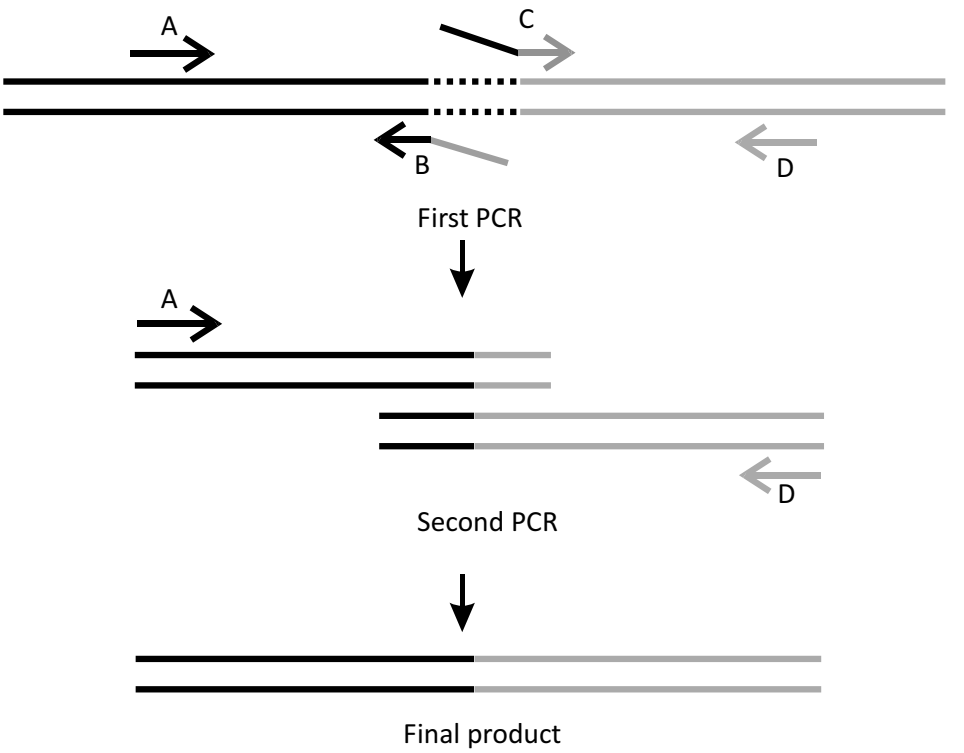


Figure 16. Deletion mutagenesis by overlap extension PCR (explanation in text).

Antimicrobial susceptibility testing

Antimicrobial activity of antibiotics is usually assessed by determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). MIC is defined as the lowest concentration of an antimicrobial that, under defined test conditions, will inhibit the visible growth of a microorganism (Wiegand et al., 2008). Most commonly used techniques to determine the MICs are agar dilution and broth dilution. Agar dilution involves the incorporation of different concentrations of an antimicrobial into a nutrient agar medium followed by the application of a standardized number of cells to the surface of

the agar plate. Broth dilution is performed using a series of dilutions of the antimicrobial in liquid culture medium, to produce a range of concentrations in test tubes (macrodilution) or in a microtiter plate (microdilution). MIC is usually read after overnight incubation, but the time period can be extended for organisms which require prolonged incubation for growth (Andrews, 2001). The recommended bacterial inoculum size for broth dilution is 10^5 colony forming units per millilitre, whereas the appropriate cell number in agar dilution experiments is set at 10^4 colony forming units per spot (Wiegand et al., 2008).

The MBC test can be viewed as an extension of an MIC test. MBC is the lowest concentration of an antimicrobial required to kill a particular microorganism. The simplest method for determining the MBC can be done by plating a subculture from antibiotic concentrations with no visible growth in the MIC test on to drug-free agar. This determines whether the bacteria have been inhibited from growing but are still viable, or whether they have been killed. An antibacterial is usually regarded as bactericidal if the MBC is not more than fourfold higher than the MIC (Levison, 2004).

MBC tests are less clinically relevant. MICs, on the other hand, are routinely determined in diagnostic laboratories to confirm resistance. MIC measurement has also become a standard procedure to evaluate the *in vitro* activity of new antimicrobials. Therefore, I determined MIC values of *E. coli* and yeast to G1 compound by broth dilution method. The results characterize the antimicrobial potency of G1.

4. RESULTS AND DISCUSSION

4.1. Selection of ligand-dependent *cis*-acting regulatory sequences (I)

Protein synthesis in living cells is a complex and highly regulated process. Most of translation regulation occurs at the level of translation initiation and is mediated by small *trans*-acting proteins or RNA-molecules (reviewed in Babitzke et al., 2009; Jackson et al., 2010). However, several studies have identified naturally existing nascent peptides that act in *cis* via inhibiting their own translation and can induce programmed translational arrest (Lovett and Rogers, 1996). In some cases, ribosome stalling occurs during translation of short upstream ORFs, so-called leader peptides. Translational arrest represents one possibility for regulating expression of downstream genes (Wilson et al., 2016).

Ribosome stalling on the mRNA is either dependent or independent from the binding of an effector molecule (an antibiotic, an amino acid, etc) to the ribosome (Ramu et al., 2009). Previous studies have identified peptide sequences that induce ribosome stalling in response to erythromycin (Weisblum, 1995b), chloramphenicol (Lovett and Rogers, 1996), tryptophan (Gong et al., 2001), arginine (Delbecq et al., 2000) and polyamides (Law et al., 2001, Raney et al., 2002). A well-studied example of effector-independent ribosome pausing is regulation of expression of *sec* operon in *E. coli* (Nakatogawa and Ito, 2002).

Many naturally occurring *cis*-acting sequences are involved in regulation of gene expression. In some cases, they control synthesis of enzymes that confer resistance to antibiotics, such as macrolides or chloramphenicol. A prominent example of ribosome stalling-mediated gene expression is the regulation of *ermC* gene expression. First described in *Staphylococcus aureus* (Weisblum et al., 1971), *ermC* was later found also in other bacteria including *Bacillus* (Monod et al., 1986) and *Neisseria* (Roberts et al., 1999). *ermC* encodes a methyltransferase which mono- or di-methylates A2058 residue in 23S rRNA. Methylation of A2058 confers so-called MLS_B resistance that interferes with binding of macrolide, lincosamide and streptogramin B antibiotics to the ribosome (Weisblum, 1995a). Expression of *ermC* is controlled by *ermCL*, a 19-codon ORF that is located 60 bp upstream of *ermC* (Gryczan et al., 1980). Both *ermCL* and *ermC* are transcribed as a single mRNA. Under normal conditions, translation of *ermCL* is enabled, while the ribosome binding site of *ermC* is sequestered in the secondary structure of mRNA. Subinhibitory concentrations of an inducing drug (erythromycin or similar macrolides) cause ribosome stalling on *ermCL* ORF, allowing mRNA to fold into alternative secondary structure. As a result, the RBS of *ermC* becomes exposed for the ribosomes (Horinouchi and Weisblum, 1980).

Results obtained so far suggest that ribosome stalling can be caused by a greater number of sequences and is more common event than initially believed.

However, the lack of a general selection method is a notable obstacle in finding new *cis*-acting regulatory sequences. Development and applying of an effective selection method would significantly accelerate the research by providing more sequences for analysis. Such selection method should be applicable for finding sequences that cause ribosome stalling in response to various compounds as most of described nascent peptide-mediated regulation mechanisms require the presence of a co-effector molecule (Tenson and Ehrenberg, 2002).

Plasmid libraries provide a useful tool for selecting *cis*-acting peptide sequences that cause ribosome stalling in the presence or in the absence of a co-effector. For instance, plasmid libraries have been successfully used for identifying pentapeptides that modulate ribosome activity in response to various macrolide antibiotics (Tenson et al., 1997; Vimberg et al., 2004).

Tanner and colleagues (2009) have selected stalling peptides from a plasmid library by using a modified tmRNA molecule. Their library was based on a gene encoding an inactive KanR protein that lacks 15 amino acids at its C-terminus. The C-terminal part of the gene was replaced by 18 random nucleotides (6 random codons). When the ribosome reaches the end of truncated mRNA, it becomes arrested. Normally, tmRNA rescues stalled ribosome by entering the empty A-site and serving a new template for interrupted protein synthesis. tmRNA encodes a peptide tag that acts as a signal for cellular proteases. Tagged and released protein becomes a subject for degradation (Barends et al., 2011). However, the modified tmRNA used by Tanner's group provides the missing C-terminal end of KanR instead of signal for proteolysis. Therefore, modified tmRNA helps to render cells resistant to kanamycin when ribosome stalling occurs during translation of six random C-terminal codons (Tanner et al., 2009). The described method enabled to identify some novel nascent peptides that induce ligand-independent ribosome stalling.

In order to identify novel *cis*-acting sequences, we created a plasmid library in which β-lactamase gene was under the control of *tac* promoter. A region between the SD element and β-lactamase ORF contained 21 random nucleotides that were inserted by PCR. In the first step of selection, *E. coli* cells transformed with the plasmid library were grown in the presence of low concentration of ampicillin. This was done in order to exclude the clones exhibiting deficient β-lactamase activity from the further selection stages. Then, cells were washed and resuspended in fresh medium containing one of the compounds used for selection. These compounds of interest included macrolides erythromycin and troleandomycin, the PTC inhibitor chloramphenicol, a non-biological compound meta-toluate and a bacterial signal molecule homoserine lactone. After the cells had been grown in the presence of the chemical, ampicillin was added to the medium. We assumed that cells, in which the ribosomes become stalled in front of β-lactamase ORF, are sensitive to ampicillin and, thus, can be lysed with the antibiotic. The released plasmids were isolated from the medium by precipitation and used to transform new bacteria. The same selection cycle was repeated for two more rounds to enrich for sequences responding to the

chemical. Three rounds of selection were found to be optimal as additional rounds tended to limit the number of emerging sequences.

Our selection method enabled to identify sequences in response to all tested compounds. An apparent conclusion is that cells became sensitive to ampicillin due to the presence of ribosome stalling sequence in front of β-lactamase ORF. However, an alternative explanation suggests that seven additional codons could disrupt the N-terminal region of β-lactamase thereby interfering with the stability or functional activity of the enzyme. To distinguish between these possibilities, we tested the functional activity of selected sequences in different reporter systems. We fused the sequences to β-galactosidase and GFP ORFs. Next, we measured the effect of sequences to the functionality of β-galactosidase and GFP reporters in the presence of their corresponding compounds. The activity of β-galactosidase was determined by β-galactosidase assay (Miller, 1992) and GFP expression was estimated by flow cytometry. We found that the sequences selected on erythromycin and chloramphenicol responded to their corresponding compounds in all of three tested expression systems (Reference I, Table I). Some troleandomycin peptides responded to troleandomycin in β-galactosidase context. However, none of troleandomycin sequences worked when inserted in front of GFP gene (Reference I, Figure 4), indicating that the reporter context may influence the specificity of the response. Sequences selected on meta-toluate and HSL displayed only weak activity in β-galactosidase or GFP context. We speculate that the activity of stalling peptides can be affected by properties of corresponding mRNA. Variations in structures of translation initiation region together with mRNA secondary structure elements are known to play role in efficiency of translation (Geissmann et al., 2009; Kozak, 2005). Therefore, a disadvantage of our method is that many sequences, potentially able to cause ribosome stalling, may remain undiscovered, because they are not active in β-lactamase context. Similarly, as we observed, sequences that are active in front of β-lactamase may not function in other reporter systems. However, *cis*-acting sequences can retain their regulatory activity when inserted in the leader sequence of another gene. For instance, arginine-sensitive leader peptide sequence of the yeast gene CPA1 can place another gene (namely, GCN4) under arginine repression (Delbecq et al., 1994).

As mentioned, *ermC* leader peptide is one of the best-characterized *cis*-acting regulators that respond to erythromycin (Dubnau, 1985; Mayford and Weisblum, 1990). We fused *ermCL* sequence to β-galactosidase ORF and compared the activities of sequences selected on erythromycin with the activity of *ermCL* using the β-galactosidase assay. We noticed that the activity of *ermCL* peptide was only a little higher than the activities of peptides selected from our library (Reference I, Figure 3). This result is somewhat surprising, as previous studies have reported stronger *ermCL* effects (Mayford and Weisblum, 1990). One may assume that stalling sequences need a specific context for showing their maximum activity.

Comparison of selected sequences did not reveal any recognizable consensus motif neither at the mRNA nor at the peptide level. This result is not surprising

as previous studies have also failed in finding common sequence motifs (reviewed in Ramu et al., 2009). Moreover, mutation of a single amino acid of the nascent chain could be sufficient to not only broaden or narrow the spectrum of molecules, which can elicit the functional response of the ribosome, but also switch the specificity of recognition from one chemical to another (Gupta et al., 2016). It is possible that certain sequence rules exist, but their discovery is hampered due to only limited number of sequences available. Thus, it is likely that increasing the number of clones in the initial library could lead to identification of more sequences thus facilitating the identification of consensus motifs.

Despite of the lack of a common consensus motif, peptides selected on different chemicals demonstrated some trends in their amino acid composition. Peptides that were selected in the presence of erythromycin often contained leucine and serine (Reference I, Table I). Sequences selected on macrolide troleandomycin, a synthetic derivative of oleandomycin, were enriched in arginine. Chloramphenicol-dependent *cis*-acting sequences tended to contain glycine and sequences selected on HSL were rich in valine. Nevertheless, none of amino acids was prevalent in peptides selected on meta-toluate.

The stalling sequences are thought to be active as peptides, i.e. they cause ribosome pausing while being translated. However, an alternative explanation to our results is that selected sequences are active as mRNA molecules. Selected mRNA sequences may interact with the compound that we use in selection and interfere with the translation process. In such manner, our selected sequences might act similarly to riboswitches. To examine the possibility that selected *cis*-acting sequences inhibit protein synthesis on the level of mRNA, we substituted some codons within the sequences selected on erythromycin or chloramphenicol with synonymous ones. Thus, mRNA sequence was changed while peptide sequence remained invariant. Mutations in mRNA are likely to disrupt the possible ligand binding site. The activity of mutated peptides was measured in β-galactosidase assay. We did not observe significant difference between the activities of selected sequences and their mutated versions (Reference I, Figure 1). We therefore conclude that at least the tested sequences (and most likely all sequences) are active as peptides.

4.2. Mechanisms of ligand-dependent translational stalling (I)

Formation of peptide bond between amino acids takes place in the peptidyl transferase center (PTC) of the ribosome. In order to exit the ribosome, the growing polypeptide chain must move through the tunnel that is located between the PTC and cytosol. The exit tunnel is 90–100 Å in length and approximately 15 Å in width, depending on the type of the ribosome (Bogdanov et al., 2010). Although the walls of tunnel are predominantly composed of 23S rRNA, ribosomal proteins L4 and L22 (L17 in eukaryotes) contribute in the

middle part where they render the tunnel as narrow as 10 Å in diameter (Nissen et al., 2000).

Accumulating evidence suggests that the walls of exit tunnel are able to monitor amino acid sequence of the nascent polypeptide. The ribosomal tunnel plays a role in early protein folding events as well as in the regulation of translation (Wilson et al., 2016). Majority of rRNA nucleotide residues within the tunnel space are exposed to the growing polypeptide by their heterocyclic bases (Nissen et al., 2000). Thus, hydrophobic contacts as well as hydrogen bonds between the tunnel wall and nascent polypeptide are possible. Strong interactions between the polypeptide and the tunnel wall may result in translation arrest (Lovett and Rogers, 1996; Tenson and Ehrenberg, 2002). There are no large hydrophilic or hydrophobic regions within the tunnel. Nevertheless, movement of the growing polypeptide chain through the tunnel can be difficult if it contains blocks of basic arginine or lysine residues (Lu and Deutsch, 2008).

We have isolated *cis*-acting peptides that cause ribosome stalling in response to three antibiotics, meta-toluate and HSL. Peptides selected on erythromycin tended to contain hydrophobic amino acids, such as leucine, glycine, valine and isoleucine. This is consistent with the fact that the critical C-terminal segments of many macrolide-regulated ORFs (ErmAL1, Erm39L, ErmCL) are hydrophobic (Mayford and Weisblum, 1990; Ramu et al., 2011). On the other hand, hydrophilic amino acids such as aspartic acid and glutamic acid were very rarely components of selected peptides. This observation suggests that ribosome stalling in the presence of erythromycin preferably requires hydrophobic nascent peptides. To test this idea, we mutated the amino acid sequence of a peptide that exhibited the highest activity in β-galactosidase expression assay. Indeed, when the peptide was made more hydrophobic, its activity increased (Reference I, Figure 2). Consistently, insertion of hydrophilic amino acids into peptide decreased its activity.

Previous studies have described a coincidentally discovered resistance mechanism to macrolides that is based on the activity of nascent peptides. These so-called macrolide resistance peptides are 4–6 amino acids in length and confer resistance to a ribosome on which the peptide is translated. During translation, the nascent peptide acts as a “bottle brush” that removes the bound macrolide molecule from the ribosome (Tenson and Mankin, 2001). It has been shown that the pentapeptide MRLFV can remove erythromycin from the ribosome. However, successful dissociation of erythromycin is dependent on the class 1 release factor-mediated translation termination. Resistance pentapeptides do not work when termination does not take place and an additional amino acid is added to the peptide. In such case, further protein synthesis is inhibited due to erythromycin and the ribosome stalls (Lovmar et al., 2006). Selection experiments have revealed peptide sequences that provide resistance to structurally different macrolides (Tenson et al., 1997; Vimberg et al., 2004). Interestingly, some similarities can be seen between macrolide resistance peptides and stalling peptides selected on macrolide antibiotics. For instance, minipeptides conferring resistance to erythromycin were found to be hydrophobic and enriched in leucine.

The same is also true for the *cis*-acting peptides causing ribosome stalling in response to erythromycin. These data suggest that the mechanism of peptide-mediated macrolide resistance may be related to nascent peptide-mediated regulation of gene expression.

4.3. Applications of *cis*-acting sequences (I)

Control over gene activity is one of the most important mainstays in synthetic biology. Many attempts have been made to generate various gene expression systems as they provide a powerful tool for applied biotechnology (transgenic disease models, drug discovery, etc) as well as for basic gene function research (May et al., 2006). In most cases, essential components of successful eukaryotic expression platforms (Cre/lox, Flp/FRT, Tet-systems) are derived from prokaryotes (Sauer and Henderson, 1989; O'Gorman et al., 1991; Gossen and Bujard, 1992). Gene activity in different expression systems is often regulated by co-effector molecules, such as antibiotics (Fussenegger et al., 2000; Weber et al., 2002), hormones (Braselmann et al., 1993), quorum sensing molecules (Neddermann et al., 2003) or vitamins (Weber et al., 2007). In addition, temperature-sensitive DNA expression systems have been generated (Boorsma et al., 2000). The systems are designed to be either inducible (ON-type) or repressible (OFF-type), i.e. gene expression occurs either in the presence or in the absence of an inducer, respectively.

In all of mentioned expression systems, gene activity is regulated at the level of transcription. However, translationally regulated systems can have advantages under circumstances where other expression platforms cannot be used. For instance, stalling sequences could be applied as *cis*-regulatory elements for gene therapy vectors that are based on RNA viruses (reviewed in Gould and Favorov, 2003; Yamanaka, 2004). Another restriction of transcriptionally regulated systems is the fact that they often contain elements of great size, which makes them inappropriate for small viral vectors.

We have shown that our selection method is suitable for finding peptides that cause ribosome stalling in the presence of certain ligands. Our findings may have important biotechnological implications. In theory, *cis*-acting nascent peptides can be used for designing novel gene expression systems that are regulated at the level of translation. Control of gene activity in such systems would be based on interaction between the co-effector molecule and a nascent peptide in the ribosome. In ligand-dependent translational systems, expression of a gene of interest can be either repressed or induced. Translation of a gene can be repressed when this gene is preceded by a regulatory upstream ORF containing a stalling sequence. Alternatively, for inducible regulation, an ORF containing a stalling sequence can be inserted upstream of an ORF encoding a specific repressor protein. In the presence of a co-effector, expression of a repressor is inhibited thereby enabling translation of a desired gene.

Translational arrest can occur in response to chemically miscellaneous compounds, but not every compound is appropriate for the use in living systems. Antibiotics are harmful to bacteria and the use of *cis*-acting sequences that response to erythromycin, troleandomycin and chloramphenicol in prokaryotic systems is therefore complicated. Nevertheless, we have successfully selected *cis*-acting sequences that respond on chemically diverse molecules and by applying our method, sequences that act in the presence of non-toxic compounds could be identified. The suitable ligands should be cell permeable molecules that are not subjects for cellular metabolism.

Recognition of a stalling sequence can evidently happen in a number of modes. Despite of generally conserved nature of the ribosome, there are species-specific significant variations in tunnel wall properties (Vazquez-Laslop and Mankin, 2011). It must be pointed out that our stalling peptides were isolated using the prokaryotic selection system. Therefore, before applying in eukaryotic system, it is critical to test their ability to stall a eukaryotic ribosome.

The *cis*-acting regulatory sequences that respond to diverse chemicals are already extensively used in molecular biology studies. For instance, ErmCL peptides were successfully used in cryo-electron microscopy reconstruction of the ribosome protection protein Tet(M) in complex with a translating ribosome (Arenz et al., 2015). Our method can be considered as a promising tool for finding new *cis*-acting regulatory sequences. Applying the method on a larger scale, by selecting and comparing an extended set of sequences, might lead to identification of yet unknown sequence rules and better understanding of mechanisms of translational arrest. This in turn could facilitate the development of novel biological sensing and expression systems or synthetic riboswitches. In these expression systems, gene expression would be controlled by interaction between the ribosome, *cis*-acting sequences and small ligand molecules.

4.4. The role of domain IV of ribosomal protection protein Tet(O) in tetracycline resistance (II)

Tetracyclines are broad-spectrum antibiotics that inhibit protein synthesis by interfering with delivery of aa-tRNAs to the A-site (Wilson, 2014). Although tetracycline has been shown to bind to multiple sites on the 30S ribosomal subunit, only one binding site has been directly associated with its antimicrobial effect. This primary binding site is located in close vicinity of the A-site codon, in a crevice between the head and platform of the small ribosomal subunit (Brodersen et al., 2000; Pioletti et al., 2001).

Resistance to tetracycline can be achieved via active efflux (Chopra and Roberts, 2001), enzymatic inactivation of the drug (Volkers et al., 2011), mutations in 16S rRNA (Ross et al., 1998) or by ribosomal protection (Connell et al., 2003a). Ribosomal protection proteins (RPPs) represent a unique resistance mechanism that was first identified in *Streptococcus faecalis* (Burdett, 1986). To date, 12 RPPs have been reported and they are widespread among both

Gram-negative and Gram-positive bacteria. RPPs can confer resistance to tetracycline and many of its derivatives by removing the drug from the drug-stalled ribosome in a GTP-dependent manner (Burdett, 1996; Trieber et al., 1998). However, the molecular details of RPP-mediated resistance started to become evident only recently with the appearance of high-resolution cryo-EM structures.

In 2001, a cryo-EM structure of Tet(O) was published with a density map at a resolution of 7.2 Å. It revealed that Tet(O) has a similar shape as elongation factor EF-G (Spahn et al., 2001). RPPs and EF-G are known to share a high degree of sequence homology (Thakor et al., 2008; Sanchez-Pescador et al., 1988). Based on the X-ray structure of EF-G, our workgroup generated a cryo-EM density map at 9.6 Å resolution for the complex of Tet(O) from *Campylobacter jejuni* bound with the *E. coli* 70S ribosome in the presence of non-hydrolysable GTP analogue GDPNP. Guided by the density map, an atomic model of Tet(O) was built and interactions between Tet(O) and the ribosome were identified (Reference II, Figure 3).

Our molecular model is consistent with the previously obtained cryo-EM structure of Tet(M):70S ribosome at 7.2 Å resolution (Donhofer et al., 2012). The binding site of RPP is located within the intersubunit cavity of the ribosome. In this position, RPP forms interactions with both small and large ribosomal subunit. The overall localization of RPP on the ribosome is similar to that observed for EF-Tu:tRNA complex and overlaps with the anticodon stem-loop of A-tRNA (Donhofer et al., 2012; Spahn et al., 2001).

RPPs are composed of five structural domains. Domain IV consists of a four-stranded β-sheet and two α-helices (Donhofer et al., 2012). These moieties are connected by three characteristic loops (the 465-, 507- and the 438 loop) that are located at the tip of domain IV (Figure 17) and extend into the head-platform crevice of 30S subunit (Reference II, Figure 3). The location and remarkable expansion of the three loops suggests that they might be critical for removal of tetracycline molecule from the ribosome. Previous work established that domain IV interacts with helix 34 of 16S rRNA adjacent to the tetracycline binding site and protects residues C1214 and C1054 within helix 34 from chemical modification (Connell et al., 2002). It was suggested that Tet(O) removes tetracycline from the ribosome indirectly via inducing conformational changes within helix 34 (Spahn et al., 2001; Connell et al., 2002; Connell et al., 2003b). However, a recent study demonstrated that Tet(M), a paralogue of Tet(O), removes tetracycline from the ribosome via direct mechanism of action. Domain IV of Tet(M) was shown to interact with the tetracycline binding site, indicating that Tet(M) can disrupt the stacking interaction between the aromatic ring D of tetracycline and C1054 (Donhofer et al., 2012).

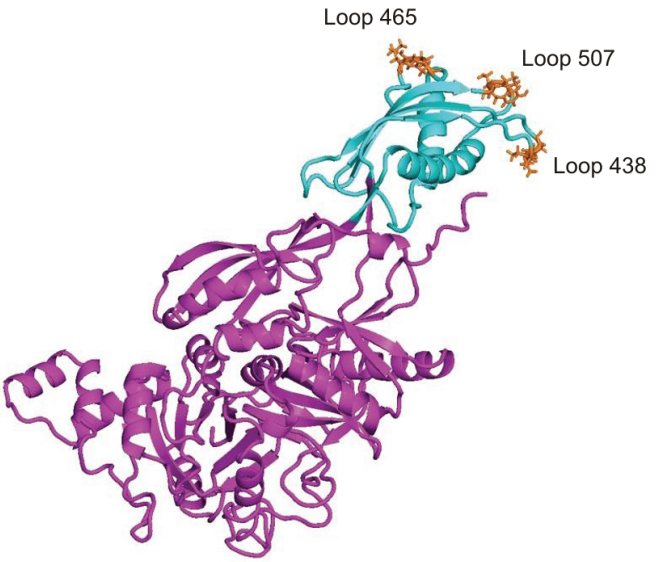


Figure 17. The model structure of Tet(O) with domain IV rendered in teal. Loops 438, 465 and 507 are shown in orange.

We performed a mutational analysis in order to evaluate the importance of the three loops of Tet(O) domain IV in expelling the antibiotic from the ribosome. We introduced a series of substitution and deletion mutations into regions that are either directly or closely involved in the interactions in the tetracycline-binding site. Plasmids bearing either *C. jejuni* wild-type Tet(O) or one of mutant variants were transformed into *E. coli* strain BW 25993 and the change in tetracycline susceptibility was measured by minimum inhibitory concentration (MIC) assay (Reference II, Figure 4c and Table 1).

The atomic model suggests that tetracycline and Tet(O) cannot co-exist on the same ribosomal complex as there would be a clash between the 507-loop of Tet(O) and the drug. Therefore, it was expected that mutation of 507-loop might have a crucial influence on the release of tetracycline from the ribosome. Indeed, substitution mutation Y507A resulted in 83% loss of Tet(O) activity when compared to the wild-type (Reference II, Table 1). Moreover, replacement YSP507-509G completely abolished Tet(O) activity (Reference II, Table 1). Members of Daniel Wilson's lab have performed a similar alanine-scanning mutagenesis to investigate the role of Tet(M) in tetracycline release (Donhofer et al., 2012). Consistent with our result, they observed that double and triple mutations within the corresponding loop rendered Tet(M) inactive. Surprisingly, no single alanine substitution within the loop exhibited a significant effect of Tet(M) to confer tetracycline resistance (Donhofer et al., 2012). Our results show that the 507-loop is absolutely necessary for RPP-mediated tetracycline resistance. Interestingly enough, sequence alignment of translational GTPases belonging to tetracycline-family revealed that the three loops are not very strongly conserved within the Tet family (Reference II, Figure 4a). This finding

along with experimental data suggested that the loops of domain IV can maintain their functionality in spite of a certain amount of sequence variability.

The 465-loop of Tet(O) extends into pocket near nucleotides 1051 and 1209 of 16S rRNA helix 34 (Reference II, Figure 3). The primary binding site of tetracycline is located in the immediate vicinity of this region and the drug forms hydrogen-bond interactions with the nucleotides in helix 34 (Brodersen et al., 2000; Jenner et al., 2013). Our atomic model suggested that the 465-loop can reorient the nucleotides 1051–1054 in helix 34 thus ruining interactions between tetracycline and 16S rRNA. In accordance with that, replacement LGY466-468G in the 465-loop resulted in complete loss of Tet(O) activity (Reference II, Table 1). Point mutations L466A and S472A resulted in 33% and 76% loss in Tet(O) activity, respectively. These observations emphasized the importance of 465-loop to the functionality of Tet(O) and were consistent with our structural data. Contrary to our conclusion, experiments with Tet(M) mutant variants have suggested that the 507-loop rather than the 465-loop alters the conformation of helix 34 to chase tetracycline from the ribosome (Donhofer et al., 2012). This indicates that Tet(O)- and Tet(M)-mediated mechanisms possess small differences at the molecular level.

Finally we examined the influence of the 438-loop on Tet(O) functionality. Substitution VPP436-438G abolished Tet(O) activity and a single substitution P438A resulted in 83% reduction of Tet(O) activity (Reference II, Table 1). These strong effects were rather surprising as according to the atomic model, the 438-loop lacks a direct contact with tetracycline molecule. We proposed that the 438-loop together with 16S rRNA nucleotides 966 and 1196 forms a structural corridor, which acts as the pathway for the release of tetracycline from the ribosome.

In combination with the structural data, our mutational analysis enabled a first insight into molecular mechanism of Tet(O)-mediated tetracycline resistance. According to our model, the 465-loop distorts the backbone shape of the 16S rRNA at the tetracycline-binding site. This in turn weakens interaction between the tetracycline molecule and 16S rRNA and enables the 507-loop to directly dislodge tetracycline from the ribosome. The 438-loop along with nucleotides 966 and 1196 should form a corridor allowing tetracycline to exit.

Recently, Daniel Wilson' group published a paper in which they presented a Tet(M):70S ribosome complex at a resolution of 3.9 Å (Arenz et al., 2015). They demonstrated that Pro509 at the tip of 507-loop, rather than previously reported Y506 and Y507, overlaps with ring D of ribosome-bound tetracycline. Therefore, Pro509 is directly involved in dislodging tetracycline from the ribosome. Pro509 is identical in all available RPP sequences. New model suggests that the role of Y506 and Y507 is to stabilize conformation of the 507-loop in Tet(M) (Arenz et al., 2015). It is very probable that the details and mechanism of action are conserved for other RPPs. Our results support this idea as mutations in positions 507 and 507–509 of domain IV strongly inhibited Tet(O) activity.

4.5. Stability and reactivity of G1 in biological culture-media (III)

2-Bromo-5-(2-bromo-2-nitrovinyl)furan, also known as G1 or Furvina®, is a synthetic nitrovinylfuran (Drobnica and Sturdik, 1980). This compound is unstable in aqueous media and has been shown to degrade into 5-bromo-2-furaldehyde and 2-bromo-5-(2-nitrovinyl)furan (Castro-Hermida et al., 2004; Scholz et al., 2013). The presence of thiol groups in medium stimulates degradation as G1 is a thiolreactive compound like other nitrovinylfurans (Balaz et al., 1982; Scholz et al., 2013). Our interest to decomposition of G1 arose with the random observation that the yellow colour of G1 solution vanishes rapidly when some drops of cysteine hydrochloride are added to the mixture. This discovery gave an idea that decomposition of G1 can be characterized using colorimetric methods.

G1 has the absorption maximum at 390 nm. We dissolved G1 in DMSO and measured its half-life in media that are often used in biological assays. Among these selected media, M9 minimal medium does not contain thiol groups. On the other hand, bacterial growth medium CAMHB and mammalian cell culture medium IMDM contain thiols at unknown concentrations. As expected, G1 was more stable in M9 medium (half-life 58 minutes), while decomposition occurred faster in thiole-containing CAMHB and IMDM (half-lives 6 and 11 minutes, respectively; Reference III, Figure S1 in the Supplemental material).

In order to elucidate G1 decomposition in the presence of thiols, we dissolved G1 in M9 minimal medium at the concentration of 16 μg/ml (0.054 mM). To this solution we added cysteine hydrochloride monohydrate to the final concentration of 20 μg/ml (0.114 mM), 50 μg/ml (0.285 mM), 100 μg/ml (0.569 mM) or 500 μg/ml (2.85 mM). We observed that in the presence of cysteine hydrochloride, the adsorption maximum peak shifted from 390 nm to 372 nm (Reference III, Figure 2a). We hypothesized that the change in spectrum is caused by conversion of G1 into new yet unknown compound. Increasing of cysteine concentration reduced adsorbance at 372 nm but a large molar excess of cysteine was needed for disappearance of adsorbance maximum between 350–400 nm. In addition, we found that G1 decomposition in the presence of thiols depends on the pH of the medium. The reaction rate increased at higher pH values (Reference III, Figure 2c). This is consistent with a previous finding (Sturdik et al., 1979).

The details of G1 decomposition along with the structures of breakdown products were characterized by NMR and HPLC analysis. We found that in the presence of cysteine hydrochloride, G1 is converted to 2-bromo-5-(2-nitrovinyl)-furan (Reference III, Figure 3a). Conversion occurs over an unstable intermediate compound that cannot be detected in our colorimetric measurements as its maximum absorbance is in ultraviolet area. On the other hand, 2-bromo-5-(2-nitrovinyl)furan has a maximum absorbance at 372 nm and is visible in colorimetric measurements. By increasing the concentration of cysteine hydrochloride, 2-bromo-5-(2-nitrovinyl)furan converts further into covalently bonded

cysteine conjugate. In the presence of remaining cysteine this conjugate transforms back to 2-bromo-5-(2-nitrovinyl)furan. In the course of hours 2-bromo-5-(2-nitrovinyl)furan was finally found to decompose irreversibly to 5-bromo-2-furaldehyde. NMR spectrocopy also revealed that reduced cysteine molecules that form during G1 decomposition react with each other to produce cystine. This explains why disappearance of absorbance maximum between 350–400 nm requires large amount of cysteine.

The exact mechanism of action of G1 has been a matter of debate. One study demonstrated that G1 targets the P-site of small ribosomal subunit and inhibits protein synthesis *in vitro* by interfering with the binding of fMet-tRNA during 30S initiation complex formation (Fabbretti et al., 2012). Another work indicated that G1 is reactive towards cysteine residues in proteins, thereby inhibiting their functional activity (Scholz et al., 2013). Our current experimental finding demonstrates that activity of G1 is rather based on non-specific modification of thiol groups. Our data together with other reports indicate that the same mode of action is shared by all vinylfurans (Scholz et al., 2013; Sturdik et al., 1979).

4.6. Antimicrobial properties of nitrovinylfuran derivative G1 (III)

G1 possesses antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts, dermatophytes and filamentous fungi (Blondeau et al., 1999). As presented above, G1 is unstable in biological culture media and interconverts into an array of reaction products. It has been shown that at least some of these decomposition products exhibit antibacterial or cytotoxic effects (Scholz et al., 2013). Standard tests for measuring antimicrobial activity, such as minimum inhibitory concentration, are endpoint measurements and score the compound's effect usually 24–72 h after its application. Such tests do not explain, how much of the antibacterial effects are attributed to the compound itself and how much are due to its breakdown products. To make a distinction between the antimicrobial properties of G1 and its conversion products, we added G1 to different growth media and scored MIC after preincubation of these mixtures for 5, 60 or 120 minutes prior to inoculation. The tested microorganisms included two *Escherichia coli* strains and a clinically relevant yeast *Candida albicans*. We found that G1 has much lower MIC in cysteine-free M9 minimal medium when compared to CAMHB medium (Reference III, Table 1). The antimicrobial potency of G1 was considerably reduced in both M9 and CAMHB when cysteine hydrochloride (final concentration of 100 μg/ml) was added to media. However, the antimicrobial properties of the compound were not lost even after 120 minutes of preincubation with cysteine indicating that the reaction products possess antimicrobial activity.

Previous studies have tested *in vitro* activity of G1 against different organisms using microbroth dilution (Blondeau et al., 1999). As mentioned before, this

simple test is not a suitable method for studying unstable compounds such as G1. Our aim was to delineate the bacteriostatic and bactericidal effects of G1. For this, we performed a time-dependent killing experiment with different drug concentrations in CAMHB medium. We added G1 to log-phase bacteria at the concentrations corresponding to 1-, 2-, 4-, and 10-fold MIC value (8, 16, 32, and 80 μg/ml respectively). Samples were collected before addition of G1 and during a 5-hour growth period. Samples were serially diluted on microwell plate and drop-plated on LB agar. After 20 hours of incubation we determined the number of viable bacteria. We found that at the G1 concentration of 8 μg/ml, bacterial growth decreased but did not stop completely (Reference III, Figure 4a and 4b). At 16 μg/ml, the drug was bacteriostatic. Higher G1 concentrations were bactericidal to proliferating *E. coli* culture.

Most antibiotics are ineffective against non-growing bacteria. In addition, the antimicrobial efficiency of antibiotics depends on the culture density of the targeted microbe (Udekwu et al., 2009). The reactivity of G1 against thiol groups is rather nonselective. Therefore we tested the activity of G1 on non-growing cells. Stationary phase *E. coli* cells were collected by centrifugation and the growth medium was filter-sterilized. Bacteria were resuspended in the conditioned medium. Simultaneously, 10-, 100-, and 1000-fold dilutions of bacteria were prepared. All cultures were grown in the presence of different G1 concentrations. Samples were collected before adding G1 and after five hours of incubation with the compound. All samples were washed, serially diluted on microwell plate and drop-plated on LB agar. We found that the effect of G1 on stationary phase cells depends on the concentration of the drug and also on the concentration of bacteria (Reference III, Figure 5). Efficient killing of stationary phase cells was achieved only with the highest tested G1 concentration (80 μg/ml). Moreover, at least 100-fold dilution of bacteria (corresponding to 10^7 CFU/ml) was required for killing. This result shows that killing of the non-growing *E. coli* is considerably less efficient than killing of proliferating bacteria. Thus, G1 cannot be regarded as a powerful drug against non-growing pathogens. Despite of nonselective reactivity against thiols, the antimicrobial activity of G1 depends on bacterial growth stage.

5. CONCLUSIONS

Reference I

We have developed a method for identifying *cis*-acting sequences that suppress expression of a marker gene in response to diverse chemicals. At least the sequences selected on erythromycin act at the peptide level. We could not identify any consensus motifs. Activity of *cis*-acting sequences may depend on the genetic context.

Reference II

The three characteristic loops (465-, 507- and 438 loop) in domain IV of ribosomal protection protein Tet(O) are essential for Tet(O)-mediated tetracycline resistance. Two-amino-acid-long deletions in the tips of either loop 438, 465 or 507 abolished Tet(O) activity. Single substitutions in the loops reduced Tet(O) activity in less extent, suggesting that a certain amount of sequence variability is tolerated in the loops.

Reference III

A nitrovinylfuran derivative G1 (or Furvina®) reacts rapidly in aqueous media with thiols such as cysteine and interconverts into an array of reaction products. The antimicrobial effect of G1 is a sum of its immediate reactivity and effects of its breakdown products. G1 is bacteriostatic for *E. coli* at the concentration of 16 μg/ml, bactericidal at 32 μg/ml and inefficient against non-growing *E. coli*.

REFERENCES

Aarestrup, F.M., and L.B. Jensen. 2000. Presence of variations in ribosomal protein L16 corresponding to susceptibility of enterococci to oligosaccharides (Avilamycin and evernimicin). *Antimicrob Agents Chemother*. 44:3425–3427.

Abdulkarim, F., L. Liljas, and D. Hughes. 1994. Mutations to kirromycin resistance occur in the interface of domains I and III of EF-Tu.GTP. *FEBS Lett*. 352:118–122.

Adrian, P.V., C. Mendrick, D. Loebenberg, P. McNicholas, K.J. Shaw, K.P. Klugman, R.S. Hare, and T.A. Black. 2000a. Evernimicin (SCH27899) inhibits a novel ribosome target site: analysis of 23S ribosomal DNA mutants. *Antimicrob Agents Chemother*. 44:3101–3106.

Adrian, P.V., W. Zhao, T.A. Black, K.J. Shaw, R.S. Hare, and K.P. Klugman. 2000b. Mutations in ribosomal protein L16 conferring reduced susceptibility to evernimicin (SCH27899): implications for mechanism of action. *Antimicrob Agents Chemother*. 44:732–738.

Ahmed, F., Donaldson W. A. 2007. Chemistry and biology of the streptogramin a antibiotics. *Mini-Rev. Org. Chem.* 4:159–181.

Alavi, J.B. 1983. Aplastic anemia associated with intravenous chloramphenicol. *Am J Hematol*. 15:375–379.

Allignet, J., and N. el Solh. 1995. Diversity among the gram-positive acetyltransferases inactivating streptogramin A and structurally related compounds and characterization of a new staphylococcal determinant, vatB. *Antimicrob Agents Chemother*. 39:2027–2036.

Amaral, L., and J. Molnar. 2012. Inhibitors of efflux pumps of Gram-negative bacteria inhibit Quorum Sensing. *Open J. Pharmacol*. 2.

Anborgh, P.H., and A. Parmeggiani. 1991. New antibiotic that acts specifically on the GTP-bound form of elongation factor Tu. *Embo J*. 10:779–784.

Anchel, M. 1952. Chemical studies with pleuromutilin. *J Biol Chem*. 199:133–139.

Andrews, J.M. 2001. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*. 48 Suppl 1:5–16.

Aoki, H., L. Ke, S.M. Poppe, T.J. Poel, E.A. Weaver, R.C. Gadwood, R.C. Thomas, D.L. Shinabarger, and M.C. Ganoza. 2002. Oxazolidinone antibiotics target the P site on Escherichia coli ribosomes. *Antimicrob Agents Chemother*. 46:1080–1085.

Arenz, S., M.F. Juette, M. Graf, F. Nguyen, P. Huter, Y.S. Polikanov, S.C. Blanchard, and D.N. Wilson. 2016. Structures of the orthosomycin antibiotics avilamycin and evernimicin in complex with the bacterial 70S ribosome. *Proc Natl Acad Sci U S A*. 113:7527–7532.

Arenz, S., F. Nguyen, R. Beckmann, and D.N. Wilson. 2015. Cryo-EM structure of the tetracycline resistance protein TetM in complex with a translating ribosome at 3.9-A resolution. *Proc Natl Acad Sci U S A*. 112:5401–5406.

Argoudelis, A.D., and R.R. Herr. 1962. Sparsomycin, a new antitumor antibiotic. II. Isolation and characterization. *Antimicrobial Agents and Chemotherapy*:780–786.

Arthur, M., D. Autissier, and P. Courvalin. 1986. Analysis of the nucleotide sequence of the ereB gene encoding the erythromycin esterase type II. *Nucleic Acids Res*. 14:4987–4999.

Arthur, M., and P. Courvalin. 1986. Contribution of two different mechanisms to erythromycin resistance in Escherichia coli. *Antimicrob Agents Chemother*. 30:694–700.

Babitzke, P., C.S. Baker, and T. Romeo. 2009. Regulation of translation initiation by RNA binding proteins. *Annu Rev Microbiol*. 63:27–44.

Backus, E.J., B.M. Duggar, and T.H. Campbell. 1954. Variation in Streptomyces aureofaciens. *Ann N Y Acad Sci*. 60:86–101.

Bagley, M.C., J.W. Dale, E.A. Merritt, and X. Xiong. 2005. Thiopeptide antibiotics. *Chem Rev*. 105:685–714.

Balaz, S., E. Sturdik, and L. Drobnica. 1982. Biochemically Important Reactions of 2-Furylethylenes, Characterization of the Reactivity Towards Thiols. *Coll Czech Chem Comm.* 47:1659–1666.

Ball, P.R., S.W. Shales, and I. Chopra. 1980. Plasmid-mediated tetracycline resistance in Escherichia coli involves increased efflux of the antibiotic. *Biochem Biophys Res Commun*. 93:74–81.

Barbacid, M., and D. Vazquez. 1974. (3H)anisomycin binding to eukaryotic ribosomes. *J Mol Biol*. 84:603–623.

Barends, S., B. Kraal, and G.P. van Wezel. 2011. The tmRNA-tagging mechanism and the control of gene expression: a review. *Wiley Interdiscip Rev RNA*. 2:233–246.

Barrientos, R.M., R.C. O'Reilly, and J.W. Rudy. 2002. Memory for context is impaired by injecting anisomycin into dorsal hippocampus following context exploration. *Behav Brain Res*. 134:299–306.

Barriere, J.C., N. Berthaud, D. Beyer, S. Dutka-Malen, J.M. Paris, and J.F. Desnottes. 1998. Recent developments in streptogramin research. *Curr Pharm Des*. 4:155–180.

Barrière, J.C., D.H. Bouanchaud, J.F. Desnottes, and J.M. Paris. 1994. Streptogramin analogues. *Expert Opin Investig Drugs.* 3:115–131.

Bashan, A., I. Agmon, R. Zarivach, F. Schluenzen, J. Harms, R. Berisio, H. Bartels, F. Franceschi, T. Auerbach, H.A. Hansen, E. Kossoy, M. Kessler, and A. Yonath. 2003. Structural basis of the ribosomal machinery for peptide bond formation, translocation, and nascent chain progression. *Mol Cell*. 11:91–102.

Bauer, G., C. Berens, S.J. Projan, and W. Hillen. 2004. Comparison of tetracycline and tigecycline binding to ribosomes mapped by dimethylsulphate and drug-directed Fe2+ cleavage of 16S rRNA. *J Antimicrob Chemother*. 53:592–599.

Bay, D.C., K.L. Rommens, and R.J. Turner. 2008. Small multidrug resistance proteins: a multidrug transporter family that continues to grow. *Biochim Biophys Acta*. 1778:1814–1838.

Beauclerk, A.A., and E. Cundliffe. 1987. Sites of action of two ribosomal RNA methylases responsible for resistance to aminoglycosides. *J Mol Biol*. 193:661–671.

Beereboom, J.J., K. Butler, F.C. Pennington, and I.A. Solomons. 1965. Anisomycin. I. Determination of the structure and stereochemistry of anisomycin. *J Org Chem*. 30:2334–2342.

Begum, A., M.M. Rahman, W. Ogawa, T. Mizushima, T. Kuroda, and T. Tsuchiya. 2005. Gene cloning and characterization of four MATE family multidrug efflux pumps from Vibrio cholerae non-O1. *Microbiol Immunol*. 49:949–957.

Belova, L., T. Tenson, L. Xiong, P.M. McNicholas, and A.S. Mankin. 2001. A novel site of antibiotic action in the ribosome: interaction of evernimicin with the large ribosomal subunit. *Proc Natl Acad Sci U S A*. 98:3726–3731.

Bennett, A.D., and W.V. Shaw. 1983. Resistance to fusidic acid in Escherichia coli mediated by the type I variant of chloramphenicol acetyltransferase. A plasmid-encoded mechanism involving antibiotic binding. *Biochem J*. 215:29–38.

Berger, J., H. Lehr, S. Teitel, H. Maehr, and E. Grunberg. 1973. A new antibiotic X-5108 OF Streptomyces origin. I. Production, isolation and properties. *J Antibiot (Tokyo)*. 26:15–22.

Bergeron, J., M. Ammirati, D. Danley, L. James, M. Norcia, J. Retsema, C.A. Strick, W.G. Su, J. Sutcliffe, and L. Wondrack. 1996. Glycylcyclines bind to the high-affinity tetracycline ribosomal binding site and evade Tet(M)- and Tet(O)-mediated ribosomal protection. *Antimicrob Agents Chemother*. 40:2226–2228.

Berisio, R., J. Harms, F. Schluenzen, R. Zarivach, H.A. Hansen, P. Fucini, and A. Yonath. 2003. Structural insight into the antibiotic action of telithromycin against resistant mutants. *J Bacteriol*. 185:4276–4279.

Bhardwaj, A.K., and P. Mohanty. 2012. Bacterial efflux pumps involved in multidrug resistance and their inhibitors: rejuvinating the antimicrobial chemotherapy. *Recent Pat Antiinfect Drug Discov*. 7:73–89.

Bhuyan, B.K. 1962. Pactamycin production by Streptomyces pactum. *Appl Microbiol*. 10:302–304.

Bhuyan, B.K., A. Dietz, and C.G. Smith. 1961. Pactamycin, a new antitumor antibiotic. I. Discovery and biological properties. *Antimicrobial Agents Chemotherapy*:184–190.

Billal, D.S., J. Feng, P. Leprohon, D. Legare, and M. Ouellette. 2011. Whole genome analysis of linezolid resistance in Streptococcus pneumoniae reveals resistance and compensatory mutations. *BMC Genomics*. 12:512.

Birkenmeyer, R.D., S.J. Kroll, C. Lewis, K.F. Stern, and G.E. Zurenko. 1984. Synthesis and antimicrobial activity of clindamycin analogues: pirlimycin, 1,2 a potent antibacterial agent. *J Med Chem*. 27:216–223.

Biswas, T., J.L. Houghton, S. Garneau-Tsodikova, and O.V. Tsodikov. 2012. The structural basis for substrate versatility of chloramphenicol acetyltransferase CATI. *Protein Sci*. 21:520–530.

Bjorkman, J., and D.I. Andersson. 2000. The cost of antibiotic resistance from a bacterial perspective. *Drug Resist Updat*. 3:237–245.

Blaha, G., G. Gurel, S.J. Schroeder, P.B. Moore, and T.A. Steitz. 2008. Mutations outside the anisomycin-binding site can make ribosomes drug-resistant. *J Mol Biol*. 379:505–519.

Blair, J.M., and L.J. Piddock. 2009. Structure, function and inhibition of RND efflux pumps in Gram-negative bacteria: an update. *Curr Opin Microbiol*. 12:512–519.

Blair, J.M., G.E. Richmond, and L.J. Piddock. 2014. Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. *Future Microbiol*. 9:1165–1177.

Blanchard, S.C., R.L. Gonzalez, H.D. Kim, S. Chu, and J.D. Puglisi. 2004. tRNA selection and kinetic proofreading in translation. *Nat Struct Mol Biol*. 11:1008–1014.

Blondeau, J.M., N. Castanedo, O. Gonzalez, R. Mendina, and E. Silveira. 1999. In vitro evaluation of G1: a novel antimicrobial compound. *Int J Antimicrob Agents*. 11:163–166.

Bodley, J.W., F.J. Zieve, L. Lin, and S.T. Zieve. 1969. Formation of the ribosome-G factor-GDP complex in the presence of fusidic acid. *Biochem Biophys Res Commun*. 37:437–443.

Bogdanov, A.A., N.V. Sumbatyan, A.V. Shishkina, V.V. Karpenko, and G.A. Korshunova. 2010. Ribosomal tunnel and translation regulation. *Biochemistry (Mosc)*. 75:1501–1516.

Boorsma, M., L. Nieba, D. Koller, M.F. Bachmann, J.E. Bailey, and W.A. Renner. 2000. A temperature-regulated replicon-based DNA expression system. *Nat Biotechnol*. 18:429–432.

Borovinskaya, M.A., R.D. Pai, W. Zhang, B.S. Schuwirth, J.M. Holton, G. Hirokawa, H. Kaji, A. Kaji, and J.H. Cate. 2007. Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nat Struct Mol Biol*. 14:727–732.

Borovinskaya, M.A., S. Shoji, K. Fredrick, and J.H. Cate. 2008. Structural basis for hygromycin B inhibition of protein biosynthesis. *RNA*. 14:1590–1599.

Borowski, E., H. Chmara, and E. Jareczek-Morawska. 1966. The antibiotic edeine. VI. Paper and thin-layer chromatography of components of the edeine complex. *Biochim Biophys Acta*. 130:560–563.

Braman, J.E. 2002. In Vitro Mutagenesis Protocols.

Braselmann, S., P. Graninger, and M. Busslinger. 1993. A selective transcriptional induction system for mammalian cells based on Gal4-estrogen receptor fusion proteins. *Proc Natl Acad Sci U S A*. 90:1657–1661.

Brisson-Noel, A., P. Delrieu, D. Samain, and P. Courvalin. 1988. Inactivation of lincosaminide antibiotics in Staphylococcus. Identification of lincosaminide O-nucleotidyltransferases and comparison of the corresponding resistance genes. *J Biol Chem*. 263:15880–15887.

Brockmann, H., and W. Henkel. 1951. Pikromycin, ein bitter schmeckendes Antibioticum aus Actinomyceten (Antibiotica aus Actinomyceten, VI. Mitteil. *W. Chem. Ber*. 84:284–288.

Brodersen, D.E., W.M. Clemons, Jr., A.P. Carter, R.J. Morgan-Warren, B.T. Wimberly, and V. Ramakrishnan. 2000. The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. *Cell*. 103:1143–1154.

Brogden, K.A. 2005. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol*. 3:238–250.

Bryskier, A. 2000. Ketolides-telithromycin, an example of a new class of antibacterial agents. *Clin Microbiol Infect*. 6:661–669.

Bulkley, D., C.A. Innis, G. Blaha, and T.A. Steitz. 2010. Revisiting the structures of several antibiotics bound to the bacterial ribosome. *Proc Natl Acad Sci U S A*. 107:17158–17163.

Burakovskii, D.E., A.S. Smirnova, D.V. Lesniak, S.V. Kiparisov, A.A. Leonov, P.V. Sergiev, A.A. Bogdanov, and O.A. Dontsova. 2007. [Interaction of 23S ribosomal RNA helices 89 and 91 of Escherichia coli contributes to the activity of IF2 but is insignificant for elongation factors functioning]. *Mol Biol (Mosk)*. 41:1031–1041.

Burdett, V. 1986. Streptococcal tetracycline resistance mediated at the level of protein synthesis. *J Bacteriol*. 165:564–569.

Burdett, V. 1996. Tet(M)-promoted release of tetracycline from ribosomes is GTP dependent. *J Bacteriol*. 178:3246–3251.

Busiello, E., and M. Di Girolamo. 1973. Sparsomycin requirement for inhibiting peptide-bond formation. *Biochim Biophys Acta*. 312:581–590.

Butler, M.S. 2008. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat Prod Rep*. 25:475–516.

Butler, M.S., M.A. Blaskovich, and M.A. Cooper. 2016. Antibiotics in the clinical pipeline at the end of 2015. *J Antibiot (Tokyo)*.

Butler, M.S., M.A. Blaskovich, and M.A. Cooper. 2017. Antibiotics in the clinical pipeline at the end of 2015. *J Antibiot (Tokyo)*. 70:3–24.

Cameron, D.M., J. Thompson, S.T. Gregory, P.E. March, and A.E. Dahlberg. 2004. Thiostrepton-resistant mutants of Thermus thermophilus. *Nucleic Acids Res*. 32:3220–3227.

Cameron, D.M., J. Thompson, P.E. March, and A.E. Dahlberg. 2002. Initiation factor IF2, thiostrepton and micrococcin prevent the binding of elongation factor G to the Escherichia coli ribosome. *J Mol Biol*. 319:27–35.

Cannon, M. 1968. The puromycin reaction and its inhibition by chloramphenicol. *Eur J Biochem*. 7:137–145.

Canu, A., and R. Leclercq. 2001. Overcoming bacterial resistance by dual target inhibition: the case of streptogramins. *Curr Drug Targets Infect Disord*. 1:215–225.

Carrasco, L., and D. Vazquez. 1972. Survey of inhibitors in different steps of protein synthesis by mammalian ribosomes. *J Antibiot (Tokyo)*. 25:732–737.

Carter, A.P., W.M. Clemons, D.E. Brodersen, R.J. Morgan-Warren, B.T. Wimberly, and V. Ramakrishnan. 2000. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature*. 407:340–348.

Casewell, M., C. Friis, E. Marco, P. McMullin, and I. Phillips. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother*. 52:159–161.

Casteels, P., C. Ampe, F. Jacobs, M. Vaeck, and P. Tempst. 1989. Apidaecins: antibacterial peptides from honeybees. *EMBO J*. 8:2387–2391.

Castro-Hermida, J.A., H. Gomez-Couso, M.E. Ares-Mazas, M.M. Gonzalez-Bedia, N. Castaneda-Cancio, F.J. Otero-Espinar, and J. Blanco-Mendez. 2004. Anticryptosporidial activity of furan derivative G1 and its inclusion complex with beta-cyclodextrin. *J Pharm Sci*. 93:197–206.

Celma, M.L., R.E. Monro, and D. Vazquez. 1971. Substrate and antibiotic binding sites at the peptidyl transferase centre of E. coli ribosomes: Binding of UACCA-Leu to 50 S subunits. *FEBS Lett*. 13:247–251.

Charney, J., W.P. Fisher, C. Curran, R.A. Machlowitz, and A.A. Tytell. 1953. Streptogramin, a new antibiotic. *Antibiot Chemother (Northfield Ill)*. 3:1283–1286.

Chesneau, O., K. Tsvetkova, and P. Courvalin. 2007. Resistance phenotypes conferred by macrolide phosphotransferases. *FEMS Microbiol Lett*. 269:317–322.

Chinali, G., E. Nyssen, M. Di Giambattista, and C. Cocito. 1988. Inhibition of polypeptide synthesis in cell-free systems by virginiamycin S and erythromycin. Evidence for a common mode of action of type B synergimycins and 14-membered macrolides. *Biochim Biophys Acta*. 949:71–78.

Chittum, H.S., and W.S. Champney. 1994. Ribosomal protein gene sequence changes in erythromycin-resistant mutants of Escherichia coli. *J Bacteriol*. 176:6192–6198.

Choi, K.C., H.R. Kim, Y.S. Park, S.M. Park, and J.H. Kim. 2002. Design and screening of in vivo expressed antimicrobial peptide library. *Biotechnology Letters*. 24:251.

Choi, K.M., and R. Brimacombe. 1998. The path of the growing peptide chain through the 23S rRNA in the 50S ribosomal subunit; a comparative cross-linking study with three different peptide families. *Nucleic Acids Res*. 26:887–895.

Chopra, I., and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev*. 65:232–260.

Cocito, C., M. Di Giambattista, E. Nyssen, and P. Vannuffel. 1997. Inhibition of protein synthesis by streptogramins and related antibiotics. *J Antimicrob Chemother*. 39 Suppl A:7–13.

Cohen, L.B., I.H. Goldberg, and A.E. Herner. 1969. Inhibition by pactamycin of the initiation of protein synthesis. Effect on the 30S ribosomal subunit. *Biochemistry*. 8:1327–1335.

Colca, J.R., W.G. McDonald, D.J. Waldon, L.M. Thomasco, R.C. Gadwood, E.T. Lund, G.S. Cavey, W.R. Mathews, L.D. Adams, E.T. Cecil, J.D. Pearson, J.H. Bock, J.E. Mott, D.L. Shinabarger, L. Xiong, and A.S. Mankin. 2003. Cross-linking in the living cell locates the site of action of oxazolidinone antibiotics. *J Biol Chem*. 278:21972–21979.

Colombo, B., L. Felicetti, and C. Baglioni. 1966. Inhibition of protein synthesis in reticulocytes by antibiotics. I. Effects on polysomes. *Biochim. Biophys. Acta*. 119:109–119.

Connell, S.R., D.M. Tracz, K.H. Nierhaus, and D.E. Taylor. 2003a. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother*. 47:3675–3681.

Connell, S.R., C.A. Trieber, G.P. Dinos, E. Einfeldt, D.E. Taylor, and K.H. Nierhaus. 2003b. Mechanism of Tet(O)-mediated tetracycline resistance. *Embo J*. 22:945–953.

Connell, S.R., C.A. Trieber, U. Stelzl, E. Einfeldt, D.E. Taylor, and K.H. Nierhaus. 2002. The tetracycline resistance protein Tet(O) perturbs the conformation of the ribosomal decoding centre. *Mol Microbiol*. 45:1463–1472.

Contreras, A., and D. Vazquez. 1977a. Cooperative and antagonistic interactions of peptidyl-tRNA and antibiotics with bacterial ribosomes. *Eur J Biochem*. 74:539–547.

Contreras, A., and D. Vazquez. 1977b. Synergistic interaction of the streptogramins with the ribosome. *Eur J Biochem*. 74:549–551.

Craig, W.A. 2011. Optimizing aminoglycoside use. *Crit Care Clin*. 27:107–121.

Czajgucki, Z., R. Andruszkiewicz, and W. Kamysz. 2006. Structure activity relationship studies on the antimicrobial activity of novel edeine A and D analogues. *J Pept Sci*. 12:653–662.

Cundliffe, E. 1989. How antibiotic-producing organisms avoid suicide. *Annu Rev Microbiol*. 43:207–233.

Cundliffe, E., and J. Thompson. 1979. Ribose methylation and resistance to thiostrepton. *Nature*. 278:859–861.

Dam, M., S. Douthwaite, T. Tenson, and A.S. Mankin. 1996. Mutations in domain II of 23 S rRNA facilitate translation of a 23 S rRNA-encoded pentapeptide conferring erythromycin resistance. *J Mol Biol*. 259:1–6.

Dantley, K.A., H.K. Dannelly, and V. Burdett. 1998. Binding interaction between Tet(M) and the ribosome: requirements for binding. *J Bacteriol*. 180:4089–4092.

Das, D., A. Lambert, P. Tulkens, G. Muccioli, and F. van Bambeke. 2012. Study of the Cellular Uptake and Subcellular Distribution of the Oxazolidinone Tedizolid (TZD) in Murine J774 Macrophages: Lack of Association with Mitochondria. 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco), abstr A–1291.

Davidovich, C., A. Bashan, T. Auerbach-Nevo, R.D. Yaggie, R.R. Gontarek, and A. Yonath. 2007. Induced-fit tightens pleuromutilins binding to ribosomes and remote interactions enable their selectivity. *Proc Natl Acad Sci U S A*. 104:4291–4296.

Davis, A.R., D.W. Gohara, and M.N. Yap. 2014. Sequence selectivity of macrolide-induced translational attenuation. *Proc Natl Acad Sci U S A*. 111:15379–15384.

Davis, B.D., L.L. Chen, and P.C. Tai. 1986. Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. *Proc Natl Acad Sci U S A*. 83:6164–6168.

Dean, M., A. Rzhetsky, and R. Allikmets. 2001. The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res*. 11:1156–1166.

Dehoux, P., J. Davies, and M. Cannon. 1993. Natural cycloheximide resistance in yeast. The role of ribosomal protein L41. *Eur J Biochem*. 213:841–848.

Delbecq, P., O. Calvo, R.K. Filipkowski, A. Pierard, and F. Messenguy. 2000. Functional analysis of the leader peptide of the yeast gene CPA1 and heterologous regulation by other fungal peptides. *Curr Genet*. 38:105–112.

Delbecq, P., M. Werner, A. Feller, R.K. Filipkowski, F. Messenguy, and A. Pierard. 1994. A segment of mRNA encoding the leader peptide of the CPA1 gene confers repression by arginine on a heterologous yeast gene transcript. *Mol Cell Biol*. 14:2378–2390.

Delgado, G., Jr., M.M. Neuhauser, D.T. Bearden, and L.H. Danziger. 2000. Quinupristin-dalfopristin: an overview. *Pharmacotherapy*. 20:1469–1485.

Demirci, H., F.t. Murphy, E. Murphy, S.T. Gregory, A.E. Dahlberg, and G. Jogl. 2013. A structural basis for streptomycin-induced misreading of the genetic code. *Nat Commun*. 4:1355.

Depardieu, F., and P. Courvalin. 2001. Mutation in 23S rRNA responsible for resistance to 16-membered macrolides and streptogramins in Streptococcus pneumoniae. *Antimicrob Agents Chemother*. 45:319–323.

Depardieu, F., I. Podglajen, R. Leclercq, E. Collatz, and P. Courvalin. 2007. Modes and modulations of antibiotic resistance gene expression. *Clin Microbiol Rev*. 20:79–114.

Di Giambattista, M., G. Chinali, and C. Cocito. 1989. The molecular basis of the inhibitory activities of type A and type B synergimycins and related antibiotics on ribosomes. *J Antimicrob Chemother*. 24:485–507.

Dinos, G., D.N. Wilson, Y. Teraoka, W. Szaflarski, P. Fucini, D. Kalpaxis, and K.H. Nierhaus. 2004. Dissecting the ribosomal inhibition mechanisms of edeine and pactamycin: the universally conserved residues G693 and C795 regulate P-site RNA binding. *Mol Cell*. 13:113–124.

Doi, Y., and Y. Arakawa. 2007. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. *Clin Infect Dis*. 45:88–94.

Donhofer, A., S. Franckenberg, S. Wickles, O. Berninghausen, R. Beckmann, and D.N. Wilson. 2012. Structural basis for TetM-mediated tetracycline resistance. *Proc Natl Acad Sci U S A*. 109:16900–16905.

Donovick, R., J.F. Pagano, H.A. Stout, and M.J. Weinstein. 1955. Thiostrepton, a new antibiotic. I. In vitro studies. *Antibiot Annu*. 3:554–559.

Douthwaite, S. 1992. Interaction of the antibiotics clindamycin and lincomycin with Escherichia coli 23S ribosomal RNA. *Nucleic Acids Res*. 20:4717–4720.

Douthwaite, S., J.B. Prince, and H.F. Noller. 1985. Evidence for functional interaction between domains II and V of 23S ribosomal RNA from an erythromycin-resistant mutant. *Proc Natl Acad Sci U S A*. 82:8330–8334.

Doyle, D., K.J. McDowall, M.J. Butler, and I.S. Hunter. 1991. Characterization of an oxytetracycline-resistance gene, otrA, of Streptomyces rimosus. *Mol Microbiol*. 5:2923–2933.

Draper, M.P., S. Weir, A. Macone, J. Donatelli, C.A. Trieber, S.K. Tanaka, and S.B. Levy. 2013. Mechanism of action of the novel aminomethylcycline antibiotic omadacycline. *Antimicrob Agents Chemother*. 58:1279–1283.

Drews, J., A. Georgopoulos, G. Laber, E. Schutze, and J. Unger. 1975. Antimicrobial activities of 81.723 hfu, a new pleuromutilin derivative. *Antimicrob Agents Chemother*. 7:507–516.

Drobnica, L., and E. Sturdik. 1980. Antimicrobial activity of 2-vinylfuran derivatives. *Folia Microbiol (Praha)*. 25:467–475.

Drobnica, L., E. Sturdik, J. Kovac, and D. Vegh. 1981. The inhibitory effect of vinyl-furans on the glycolysis in tumor and yeast cells. *Neoplasma*. 28:281–289.

Du, D., H.W. van Veen, S. Murakami, K.M. Pos, and B.F. Luisi. 2015. Structure, mechanism and cooperation of bacterial multidrug transporters. *Curr Opin Struct Biol*. 33:76–91.

Du, D., Z. Wang, N.R. James, J.E. Voss, E. Klimont, T. Ohene-Agyei, H. Venter, W. Chiu, and B.F. Luisi. 2014. Structure of the AcrAB-TolC multidrug efflux pump. *Nature*. 509:512–515.

Dubnau, D. 1985. Induction of ermC requires translation of the leader peptide. *EMBO J*. 4:533–7.

Duggar, B.M. 1948. Aureomycin; a product of the continuing search for new antibiotics. *Ann N Y Acad Sci*. 51:177–181.

Dunkle, J.A., L. Xiong, A.S. Mankin, and J.H. Cate. 2010. Structures of the Escherichia coli ribosome with antibiotics bound near the peptidyl transferase center explain spectra of drug action. *Proc Natl Acad Sci U S A*. 107:17152–17157.

Dupuis, M., and R. Leclercq. 2006. Activity of a new oral streptogramin, XRP2868, against gram-positive cocci harboring various mechanisms of resistance to streptogramins. *Antimicrob Agents Chemother*. 50:237–242.

Egger, H., and H. Reinshagen. 1976. New pleuromutilin derivatives with enhanced antimicrobial activity.II.Structure-activity correlations. *J Antibiot (Tokyo)*. 29:923–927.

Ehrlich, J., Q.R. Bartz, R.M. Smith, D.A. Joslyn, and P.R. Burkholder. 1947. Chloromycetin, a New Antibiotic From a Soil Actinomycete. *Science*. 106:417.

Eliopoulos, G.M., M.J. Ferraro, C.B. Wennersten, and R.C. Moellering, Jr. 2005. In vitro activity of an oral streptogramin antimicrobial, XRP2868, against gram-positive bacteria. *Antimicrob Agents Chemother*. 49:3034–3039.

Eustice, D.C., and J.M. Wilhelm. 1984. Mechanisms of action of aminoglycoside antibiotics in eucaryotic protein synthesis. *Antimicrob Agents Chemother*. 26:53–60.

Fabbretti, A., L. Brandi, D. Petrelli, C.L. Pon, N.R. Castanedo, R. Medina, and C.O. Gualerzi. 2012. The antibiotic Furvina(R) targets the P-site of 30S ribosomal subunits and inhibits translation initiation displaying start codon bias. *Nucleic Acids Res*. 40:10366–10374.

Fabbretti, A., A.M. Giuliodori, and L. Brandi. 2013. Inhibitors of bacterial elngation factor EF-Tu. *In* Antibiotics: Targets, Mechanisms and Resistance. B.L. Gualerzi C.Q., Fabbretti A., editor. Wiley-VCH.

Farrell, D.J., M. Castanheira, and I. Chopra. 2011. Characterization of global patterns and the genetics of fusidic acid resistance. *Clin Infect Dis*. 52 Suppl 7:S487–492.

Fernandez, L., and R.E. Hancock. 2012. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev*. 25:661–681.

Fernandez-Munoz, R., R.E. Monro, R. Torres-Pinedo, and D. Vazquez. 1971. Substrate- and antibiotic-binding sites at the peptidyl-transferase centre of Escherichia coli ribosomes. Studies on the chloramphenicol. lincomycin and erythromycin sites. *Eur J Biochem*. 23:185–193.

Finlay, A.C., G.L. Hobby, S.Y. P'an, P.P. Regna, J.B. Routien, D.B. Seeley, G.M. Shull, B.A. Sobin, I.A. Solomons, J.W. Vinson, and J.H. Kane. 1950. Terramycin, a new antibiotic. *Science*. 111:85.

Foster, T.J., and W.V. Shaw. 1973. Chloramphenicol acetyltransferases specified by fi minus R factors. *Antimicrob Agents Chemother*. 3:99–104.

Franceschi, F., Z. Kanyo, E.C. Sherer, and J. Sutcliffe. 2004. Macrolide resistance from the ribosome perspective. *Curr Drug Targets Infect Disord*. 4:177–191.

Francois, B., R.J. Russell, J.B. Murray, F. Aboul-ela, B. Masquida, Q. Vicens, and E. Westhof. 2005. Crystal structures of complexes between aminoglycosides and decoding A site oligonucleotides: role of the number of rings and positive charges in the specific binding leading to miscoding. *Nucleic Acids Res*. 33:5677–5690.

Fredrick, K., and H.F. Noller. 2003. Catalysis of ribosomal translocation by sparsomycin. *Science*. 300:1159–1162.

Fugitt, R.B., Luckenbaugh, R. W. 1978. 5-halomethyl-3-phenyl-2-oxazolidinones. US patent 4,128, 654.December 5,1978.

Fussenegger, M., R.P. Morris, C. Fux, M. Rimann, B. von Stockar, C.J. Thompson, and J.E. Bailey. 2000. Streptogramin-based gene regulation systems for mammalian cells. *Nat Biotechnol*. 18:1203–1208.

Gagnon, M.G., R.N. Roy, I.B. Lomakin, T. Florin, A.S. Mankin, and T.A. Steitz. 2016. Structures of proline-rich peptides bound to the ribosome reveal a common mechanism of protein synthesis inhibition. *Nucleic Acids Res*. 44:2439–2450.

Gale, E.F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., Waring, M. 1981. The molecular basis of antibiotic action. John Wiley & Sons, London.

Ganguly, A.K., J.L. McCormick, T.M. Chan, A.K. Saksena, and P.R. Das. 1997. Determination of the absolute stereochemistry at the C16 orthoester of everninomycin antibiotics: a novel acidcatalyzed isomerization of orthoesters. *Tetrahedron Letters*. 38:7989–7992.

Gao, Y.G., M. Selmer, C.M. Dunham, A. Weixlbaumer, A.C. Kelley, and V. Ramakrishnan. 2009. The structure of the ribosome with elongation factor G trapped in the posttranslocational state. *Science*. 326:694–699.

Garreau de Loubresse, N., I. Prokhorova, W. Holtkamp, M.V. Rodnina, G. Yusupova, and M. Yusupov. 2014. Structural basis for the inhibition of the eukaryotic ribosome. *Nature*. 513:517–522.

Geissmann, T., S. Marzi, and P. Romby. 2009. The role of mRNA structure in translational control in bacteria. *RNA Biol*. 6:153–160.

Gennaro, R., B. Skerlavaj, and D. Romeo. 1989. Purification, composition, and activity of two bactenecins, antibacterial peptides of bovine neutrophils. *Infect Immun*. 57:3142–3146.

Gentry, D.R., S.F. Rittenhouse, L. McCloskey, and D.J. Holmes. 2007. Stepwise exposure of Staphylococcus aureus to pleuromutilins is associated with stepwise acquisition of mutations in rplC and minimally affects susceptibility to retapamulin. *Antimicrob Agents Chemother*. 51:2048–2052.

Gerrits, M.M., M.R. de Zoete, N.L. Arents, E.J. Kuipers, and J.G. Kusters. 2002. 16S rRNA mutation-mediated tetracycline resistance in Helicobacter pylori. *Antimicrob Agents Chemother*. 46:2996–3000.

Godtfredsen, W.O., S. Jahnsen, H. Lorck, K. Roholt, and L. Tybring. 1962. Fusidic acid: a new antibiotic. *Nature*. 193:987.

Godtfredsen, W.O., W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Melera. 1965. The stereochemistry of fusidic acid. *Tetrahedron*. 21:3505–3530.

Gong, F., K. Ito, Y. Nakamura, and C. Yanofsky. 2001. The mechanism of tryptophan induction of tryptophanase operon expression: tryptophan inhibits release factor-

mediated cleavage of TnaC-peptidyl-tRNA(Pro). *Proc Natl Acad Sci U S A*. 98:8997–9001.
Gong, F., and C. Yanofsky. 2002. Instruction of translating ribosome by nascent peptide. *Science*. 297:1864–1867.
Gordon, J. 1969. Hydrolysis of guanosine 5'-triphosphate associated with binding of aminoacyl transfer ribonucleic acid to ribosomes. *J Biol Chem*. 244:5680–5686.
Gossen, M., and H. Bujard. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci U S A*. 89:5547–5551.
Gould, D.J., and P. Favorov. 2003. Vectors for the treatment of autoimmune disease. *Gene Ther*. 10:912–927.
Gregory, S.T., and A.E. Dahlberg. 1999. Erythromycin resistance mutations in ribosomal proteins L22 and L4 perturb the higher order structure of 23 S ribosomal RNA. *J Mol Biol*. 289:827–834.
Grkovic, S., M.H. Brown, N.J. Roberts, I.T. Paulsen, and R.A. Skurray. 1998. QacR is a repressor protein that regulates expression of the Staphylococcus aureus multidrug efflux pump QacA. *J Biol Chem*. 273:18665–18673.
Grkovic, S., M.H. Brown, and R.A. Skurray. 2002. Regulation of bacterial drug export systems. *Microbiol Mol Biol Rev*. 66:671–701.
Grollman, A.P. 1967. Inhibitors of protein biosynthesis. II. Mode of action of anisomycin. *J Biol Chem*. 242:3226–3233.
Gromadski, K.B., and M.V. Rodnina. 2004. Streptomycin interferes with conformational coupling between codon recognition and GTPase activation on the ribosome. *Nat Struct Mol Biol*. 11:316–322.
Grunberg-Manago, M., J. Dondon, and M. Graffe. 1972. Inhibition by thiostrepton of the IF-2-dependent ribosomal GTPase. *FEBS Lett*. 22:217–221.
Gryczan, T.J., G. Grandi, J. Hahn, R. Grandi, and D. Dubnau. 1980. Conformational alteration of mRNA structure and the posttranscriptional regulation of erythromycin-induced drug resistance. *Nucleic Acids Res*. 8:6081–6097.
Gupta, P., B. Liu, D. Klepacki, V. Gupta, K. Schulten, A.S. Mankin, and N. Vazquez-Laslop. 2016. Nascent peptide assists the ribosome in recognizing chemically distinct small molecules. *Nat Chem Biol*. 12:153–158.
Gupta, P., S. Sothiselvam, N. Vazquez-Laslop, and A.S. Mankin. 2013. Deregulation of translation due to post-transcriptional modification of rRNA explains why erm genes are inducible. *Nat Commun*. 4:1984.
Hamada, M., T. Takeuchi, S. Kondo, Y. Ikeda, and H. Naganawa. 1970. A new antibiotic, negamycin. *J Antibiot (Tokyo)*. 23:170–171.
Hansen, J.L., J.A. Ippolito, N. Ban, P. Nissen, P.B. Moore, and T.A. Steitz. 2002. The structures of four macrolide antibiotics bound to the large ribosomal subunit. *Mol Cell*. 10:117–128.
Hansen, J.L., P.B. Moore, and T.A. Steitz. 2003. Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. *J Mol Biol*. 330:1061–1075.
Harms, J.M., F. Schlunzen, P. Fucini, H. Bartels, and A. Yonath. 2004. Alterations at the peptidyl transferase centre of the ribosome induced by the synergistic action of the streptogramins dalfopristin and quinupristin. *BMC Biol*. 2:4.
Harms, J.M., D.N. Wilson, F. Schluenzen, S.R. Connell, T. Stachelhaus, Z. Zaborowska, C.M. Spahn, and P. Fucini. 2008. Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin. *Mol Cell*. 30:26–38.

Helser, T.L., J.E. Davies, and J.E. Dahlberg. 1972. Mechanism of kasugamycin resistance in Escherichia coli. *Nat New Biol*. 235:6–9.

Hensens, O.D., and G. Albers-Schonberg. 1983. Total structure of the highly modified peptide antibiotic components of thiopeptin. *J Antibiot (Tokyo)*. 36:814–831.

Hermann, T. 2005. Drugs targeting the ribosome. *Curr Opin Struct Biol*. 15:355–366.

Herrera, F., N. Moreno, and J.A. Martinez. 1984. Increased ribosomal affinity for mRNA causes resistance to edeine in a mutant of Saccharomyces cerevisiae. *Eur J Biochem*. 145:339–344.

Hierowski, M., and Z. Kurylo-Borowska. 1965. On the mode of action of edeine. I. Effect of edeine on the synthesis of polyphenylalanine in a cell-free system. *Biochim Biophys Acta*. 95:578–589.

Hildebrandt, J.F., Berner, H., Laber, G., Turnowsky, F., Schutze, E. 1982. A new semisynthetic pleuromutilin derivative with antibacterial activity: in vitro evaluation. *Curr Chemother Immunother*. 1:346–347.

Hinchliffe, P., M.F. Symmons, C. Hughes, and V. Koronakis. 2013. Structure and operation of bacterial tripartite pumps. *Annu Rev Microbiol*. 67:221–242.

Hobbie, S.N., S. Akshay, S.K. Kalapala, C.M. Bruell, D. Shcherbakov, and E.C. Bottger. 2008. Genetic analysis of interactions with eukaryotic rRNA identify the mitoribosome as target in aminoglycoside ototoxicity. *Proc Natl Acad Sci U S A*. 105:20888–20893.

Hogenauer, G. 1975. The mode of action of pleuromutilin derivatives. Location and properties of the pleuromutilin binding site on Escherichia coli ribosomes. *Eur J Biochem*. 52:93–98.

Horinouchi, S., and B. Weisblum. 1980. Posttranscriptional modification of mRNA conformation: mechanism that regulates erythromycin-induced resistance. *Proc Natl Acad Sci U S A*. 77:7079–7083.

Hughes, D., and D.I. Andersson. 2012. Selection of resistance at lethal and non-lethal antibiotic concentrations. *Curr Opin Microbiol*. 15:555–560.

Hummel, H., and A. Bock. 1987a. 23S ribosomal RNA mutations in halobacteria conferring resistance to the anti-80S ribosome targeted antibiotic anisomycin. *Nucleic Acids Res*. 15:2431–2443.

Hummel, H., and A. Bock. 1987b. Thiostrepton resistance mutations in the gene for 23S ribosomal RNA of halobacteria. *Biochimie*. 69:857–861.

Ingolia, N.T., S. Ghaemmaghami, J.R. Newman, and J.S. Weissman. 2009. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science*. 324:218–223.

Ippolito, J.A., Z.F. Kanyo, D. Wang, F.J. Franceschi, P.B. Moore, T.A. Steitz, and E.M. Duffy. 2008. Crystal structure of the oxazolidinone antibiotic linezolid bound to the 50S ribosomal subunit. *J Med Chem*. 51:3353–3356.

Itoh, J., S. Omoto, T. Shomura, N. Nishizawa, S. Miyado, Y. Yuda, U. Shibata, and S. Inouye. 1981. Amicoumacin-A, a new antibiotic with strong antiinflammatory and antiulcer activity. *J Antibiot (Tokyo)*. 34:611–613.

Jackson, R.J., C.U. Hellen, and T.V. Pestova. 2010. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol*. 11:113–127.

Jacquet, E., J.M. Girard, O. Ramaen, O. Pamlard, H. Levaique, J.M. Betton, E. Dassa, and O. Chesneau. 2008. ATP hydrolysis and pristinamycin IIA inhibition of the Staphylococcus aureus Vga(A), a dual ABC protein involved in streptogramin A resistance. *J Biol Chem*. 283:25332–25339.

Jana, S., and J.K. Deb. 2006. Molecular understanding of aminoglycoside action and resistance. *Appl Microbiol Biotechnol*. 70:140–150.

Jenkins, G., and E. Cundliffe. 1991. Cloning and characterization of two genes from Streptomyces lividans that confer inducible resistance to lincomycin and macrolide antibiotics. *Gene*. 108:55–62.

Jenner, L., A.L. Starosta, D.S. Terry, A. Mikolajka, L. Filonava, M. Yusupov, S.C. Blanchard, D.N. Wilson, and G. Yusupova. 2013. Structural basis for potent inhibitory activity of the antibiotic tigecycline during protein synthesis. *Proc Natl Acad Sci U S A*. 110:3812–3816.

Jones, R.N., and M.S. Barrett. 1995. Antimicrobial activity of SCH 27899, oligosaccharide member of the everninomycin class with a wide Gram-positive spectrum. *Clin Microbiol Infect*. 1:35–43.

Just-Baringo, X., F. Albericio, and M. Alvarez. 2014. Thiopeptide antibiotics: retrospective and recent advances. *Mar Drugs*. 12:317–351.

Kannan, K., P. Kanabar, D. Schryer, T. Florin, E. Oh, N. Bahroos, T. Tenson, J.S. Weissman, and A.S. Mankin. 2014. The general mode of translation inhibition by macrolide antibiotics. *Proc Natl Acad Sci U S A*. 111:15958–15963.

Kannan, K., N. Vazquez-Laslop, and A.S. Mankin. 2012. Selective protein synthesis by ribosomes with a drug-obstructed exit tunnel. *Cell*. 151:508–520.

Kappen, L.S., and I.H. Goldberg. 1976. Analysis of the two steps in polypeptide chain initiation inhibited by pactamycin. *Biochemistry*. 15:811–818.

Karlsson, M., A. Gunnarsson, and A. Franklin. 2001. Susceptibility to pleuromutilins in Brachyspira (Serpulina) hyodysenteriae. *Anim Health Res Rev*. 2:59–65.

Katz, L., and G.W. Ashley. 2005. Translation and protein synthesis: macrolides. *Chem Rev*. 105:499–528.

Kavanagh, F., A. Hervey, and W.J. Robbins. 1951. Antibiotic substances from Basidiomycetes: VIII. Pleurotus multilus (Fr.) sacc. and Pleurotus passeckerianus pilat. *Proc Natl Acad Sci U S A*. 37:570–574.

Kehrenberg, C., S. Schwarz, L. Jacobsen, L.H. Hansen, and B. Vester. 2005. A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. *Mol Microbiol*. 57:1064–1073.

Kelly, W.L., L. Pan, and C. Li. 2009. Thiostrepton biosynthesis: prototype for a new family of bacteriocins. *J Am Chem Soc*. 131:4327–4334.

Kerr, I.D. 2004. Sequence analysis of twin ATP binding cassette proteins involved in translational control, antibiotic resistance, and ribonuclease L inhibition. *Biochem Biophys Res Commun*. 315:166–173.

Kerridge, D. 1958. The effect of actidione and other antifungal agents on nucleic acid and protein synthesis in Saccharomyces carlsbergensis. *J Gen Microbiol*. 19:497–506.

Kirst, A.H. 2014. Macrolide antibiotics. *In* Antimicrobials. new and old molecules in the fight against multi-resistant bacteria. O.G. Flavia Marinelli, editor. Springer Berlin Heidelberg. 211–230.

Klinge, S., F. Voigts-Hoffmann, M. Leibundgut, S. Arpagaus, and N. Ban. 2011. Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6. *Science*. 334:941–948.

Knauseder, F., and E. Brandl. 1976. Pleuromutilins. Fermentation, structure and biosynthesis. *J Antibiot (Tokyo)*. 29:125–131.

Kobayashi, N., K. Nishino, and A. Yamaguchi. 2001. Novel macrolide-specific ABC-type efflux transporter in Escherichia coli. *J Bacteriol*. 183:5639–5644.

Kozak, M. 2005. Regulation of translation via mRNA structure in prokaryotes and eukaryotes. *Gene*. 361:13–37.

Krause, K.M., A.W. Serio, T.R. Kane, and L.E. Connolly. 2016. Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med*. 6.

Kucers, A. 1980. Current position of chloramphenicol chemotherapy. *J Antimicrob Chemother*. 6:1–4.

Kuroda, T., and T. Tsuchiya. 2009. Multidrug efflux transporters in the MATE family. *Biochim Biophys Acta*. 1794:763–768.

Kurylo-Borowska, Z. 1959. Isolation and properties of pure edeine, an antibiotic of the strain Bacillus brevis Vm4. *Biul Inst Med Morsk Gdansk*. 10:151–163.

Kurylo-Borowska, Z. 1975. Biosynthesis of edeine: II. Localization of edeine synthetase within Bacillus brevis Vm4. *Biochim Biophys Acta*. 399:31–41.

Kurylo-Borowska, Z., and W. Szer. 1972. Inhibition of bacterial DNA synthesis by edeine. Effect on Escherichia coli mutants lacking DNA polymerase I. *Biochim Biophys Acta*. 287:236–245.

La Teana, A., C.O. Gualerzi, and A.E. Dahlberg. 2001. Initiation factor IF 2 binds to the alpha-sarcin loop and helix 89 of Escherichia coli 23S ribosomal RNA. *RNA*. 7:1173–1179.

LaMarre, J., R.E. Mendes, T. Szal, S. Schwarz, R.N. Jones, and A.S. Mankin. 2013. The genetic environment of the cfr gene and the presence of other mechanisms account for the very high linezolid resistance of Staphylococcus epidermidis isolate 426-3147L. *Antimicrob Agents Chemother*. 57:1173–1179.

LaMarre, J.M., J.B. Locke, K.J. Shaw, and A.S. Mankin. 2011. Low fitness cost of the multidrug resistance gene cfr. *Antimicrob Agents Chemother*. 55:3714–3719.

Lambert, T. 2012. Antibiotics that affect the ribosome. *Rev Sci Tech*. 31:57–64.

Landini, P., M. Bandera, A. Soffientini, and B.P. Goldstein. 1993. Sensitivity of elongation factor Tu (EF-Tu) from different bacterial species to the antibiotics efrotomycin, pulvomycin and MDL 62879. *J Gen Microbiol*. 139:769–774.

Lazaro, E., E. Sanz, M. Remacha, and J.P. Ballesta. 2002. Characterization of sparsomycin resistance in Streptomyces sparsogenes. *Antimicrob Agents Chemother*. 46:2914–2919.

Lazaro, E., L.A. van den Broek, A. San Felix, H.C. Ottenheijm, and J.P. Ballesta. 1991a. Biochemical and kinetic characteristics of the interaction of the antitumor antibiotic sparsomycin with prokaryotic and eukaryotic ribosomes. *Biochemistry*. 30:9642–9648.

Lazaro, E., L.A. Van den Broek, A. San Felix, H.C. Ottenheijm, and J.P. Ballesta. 1991b. Chemical, biochemical and genetic endeavours characterizing the interaction of sparsomycin with the ribosome. *Biochimie*. 73:1137–1143.

Law, G.L., A. Raney, C. Heusner, and D.R. Morris. 2001. Polyamine regulation of ribosome pausing at the upstream open reading frame of S-adenosylmethionine decarboxylase. *J Biol Chem*. 276:38036–38043.

Lawrence, M.G., L. Lindahl, and J.M. Zengel. 2008. Effects on translation pausing of alterations in protein and RNA components of the ribosome exit tunnel. *J Bacteriol*. 190:5862–5869.

Leach, K.L., S.M. Swaney, J.R. Colca, W.G. McDonald, J.R. Blinn, L.M. Thomasco, R.C. Gadwood, D. Shinabarger, L. Xiong, and A.S. Mankin. 2007. The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. *Mol Cell*. 26:393–402.

Lee, J., M.K. Shin, D.K. Ryu, S. Kim, and W.S. Ryu. 2010. Insertion and deletion mutagenesis by overlap extension PCR. *Methods Mol Biol*. 634:137–146.

Leipe, D.D., Y.I. Wolf, E.V. Koonin, and L. Aravind. 2002. Classification and evolution of P-loop GTPases and related ATPases. *J Mol Biol*. 317:41–72.

Lell, B., and P.G. Kremsner. 2002. Clindamycin as an antimalarial drug: review of clinical trials. *Antimicrob Agents Chemother*. 46:2315–2320.

Lenart, J., V. Vimberg, L. Vesela, J. Janata, and G. Balikova Novotna. 2015. Detailed mutational analysis of Vga(A) interdomain linker: implication for antibiotic resistance specificity and mechanism. *Antimicrob Agents Chemother*. 59:1360–1364.

Lentzen, G., R. Klinck, N. Matassova, F. Aboul-ela, and A.I. Murchie. 2003. Structural basis for contrasting activities of ribosome binding thiazole antibiotics. *Chem Biol*. 10:769–778.

Lewis, C. 1974. Clinically useful antibiotics obtained by directed chemical modification-lincomycins. *Fed Proc*. 33:2303–2306.

Lewis, K. 1994. Multidrug resistance pumps in bacteria: variations on a theme. *Trends Biochem Sci*. 19:119–123.

Li, W., J. Tailhades, N.M. O'Brien-Simpson, F. Separovic, L. Otvos, Jr., M.A. Hossain, and J.D. Wade. 2014. Proline-rich antimicrobial peptides: potential therapeutics against antibiotic-resistant bacteria. *Amino Acids*. 46:2287–2294.

Li, X.Z., and H. Nikaido. 2009. Efflux-mediated drug resistance in bacteria: an update. *Drugs*. 69:1555–1623.

Lin, A.H., R.W. Murray, T.J. Vidmar, and K.R. Marotti. 1997. The oxazolidinone eperezolid binds to the 50S ribosomal subunit and competes with binding of chloramphenicol and lincomycin. *Antimicrob Agents Chemother*. 41:2127–2131.

Littlejohn, T.G., I.T. Paulsen, M.T. Gillespie, J.M. Tennent, M. Midgley, I.G. Jones, A.S. Purewal, and R.A. Skurray. 1992. Substrate specificity and energetics of antiseptic and disinfectant resistance in Staphylococcus aureus. *FEMS Microbiol Lett*. 74:259–265.

Locke, J.B., J. Finn, M. Hilgers, G. Morales, S. Rahawi, C.K. G, J.J. Picazo, W. Im, K.J. Shaw, and J.L. Stein. 2010. Structure-activity relationships of diverse oxazolidinones for linezolid-resistant Staphylococcus aureus strains possessing the cfr methyltransferase gene or ribosomal mutations. *Antimicrob Agents Chemother*. 54:5337–5343.

Locke, J.B., M. Hilgers, and K.J. Shaw. 2009. Mutations in ribosomal protein L3 are associated with oxazolidinone resistance in staphylococci of clinical origin. *Antimicrob Agents Chemother*. 53:5275–5278.

Lodato, P.B., E.J. Rogers, and P.S. Lovett. 2006. A variation of the translation attenuation model can explain the inducible regulation of the pBC16 tetracycline resistance gene in Bacillus subtilis. *J Bacteriol*. 188:4749–4758.

Lolk, L., J. Pohlsgaard, A.S. Jepsen, L.H. Hansen, H. Nielsen, S.I. Steffansen, L. Sparving, A.B. Nielsen, B. Vester, and P. Nielsen. 2008. A click chemistry approach to pleuromutilin conjugates with nucleosides or acyclic nucleoside derivatives and their binding to the bacterial ribosome. *J Med Chem*. 51:4957–4967.

Long, K.S., J. Poehlsgaard, C. Kehrenberg, S. Schwarz, and B. Vester. 2006. The Cfr rRNA methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. *Antimicrob Agents Chemother*. 50:2500–2505.

Long, K.S., and B.T. Porse. 2003. A conserved chloramphenicol binding site at the entrance to the ribosomal peptide exit tunnel. *Nucleic Acids Res*. 31:7208–7215.

Lovett, P.S. 1990. Translational attenuation as the regulator of inducible cat genes. *J Bacteriol*. 172:1–6.
Lovett, P.S., and E.J. Rogers. 1996. Ribosome regulation by the nascent peptide. *Microbiol Rev*. 60:366–385.
Lovmar, M., K. Nilsson, V. Vimberg, T. Tenson, M. Nervall, and M. Ehrenberg. 2006. The molecular mechanism of peptide-mediated erythromycin resistance. *J Biol Chem*. 281:6742–6750.
Lu, J., and C. Deutsch. 2008. Electrostatics in the ribosomal tunnel modulate chain elongation rates. *J Mol Biol*. 384:73–86.
Lubelski, J., W.N. Konings, and A.J. Driessen. 2007. Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. *Microbiol Mol Biol Rev*. 71:463–476.
Ma, D., D.N. Cook, M. Alberti, N.G. Pon, H. Nikaido, and J.E. Hearst. 1995. Genes acrA and acrB encode a stress-induced efflux system of Escherichia coli. *Mol Microbiol*. 16:45–55.
Macdonald, J.S., and I.H. Goldberg. 1970. An effect of pactamycin on the initiation of protein synthesis in reticulocytes. *Biochem Biophys Res Commun*. 41:1–8.
Magerlein, B.J. 1971. Modification of lincomycin. *Adv Appl Microbiol*. 14:185–229.
Malbruny, B., A.M. Werno, D.R. Murdoch, R. Leclercq, and V. Cattoir. 2011. Cross-resistance to lincosamides, streptogramins A, and pleuromutilins due to the lsa(C) gene in Streptococcus agalactiae UCN70. *Antimicrob Agents Chemother*. 55:1470–1474.
Malcolmson, S.J., T.S. Young, J.G. Ruby, P. Skewes-Cox, and C.T. Walsh. 2013. The posttranslational modification cascade to the thiopeptide berninamycin generates linear forms and altered macrocyclic scaffolds. *Proc Natl Acad Sci U S A*. 110:8483–8488.
Mankin, A.S. 1997. Pactamycin resistance mutations in functional sites of 16 S rRNA. *J Mol Biol*. 274:8–15.
Mann, P.A., L. Xiong, A.S. Mankin, A.S. Chau, C.A. Mendrick, D.J. Najarian, C.A. Cramer, D. Loebenberg, E. Coates, N.J. Murgolo, F.M. Aarestrup, R.V. Goering, T.A. Black, R.S. Hare, and P.M. McNicholas. 2001. EmtA, a rRNA methyl-transferase conferring high-level evernimicin resistance. *Mol Microbiol*. 41:1349–1356.
Margus, T., M. Remm, and T. Tenson. 2007. Phylogenetic distribution of translational GTPases in bacteria. *BMC Genomics*. 8:15.
Marks, J., K. Kannan, E.J. Roncase, D. Klepacki, A. Kefi, C. Orelle, N. Vazquez-Laslop, and A.S. Mankin. 2016. Context-specific inhibition of translation by ribosomal antibiotics targeting the peptidyl transferase center. *Proc Natl Acad Sci U S A*. 113:12150–12155.
Mason, D.J., A. Dietz, and C. DeBoer. 1962. Lincomycin, a new antibiotic I. Discovery and biological properties. *Antimicrobial Agents and Chemotherapy*:554–559.
Mast, Y., and W. Wohlleben. 2014. Streptogramins – two are better than one! *Int J Med Microbiol*. 304:44–50.
Matassova, N.B., M.V. Rodnina, R. Endermann, H.P. Kroll, U. Pleiss, H. Wild, and W. Wintermeyer. 1999. Ribosomal RNA is the target for oxazolidinones, a novel class of translational inhibitors. *RNA*. 5:939–946.
May, T., H. Hauser, and D. Wirth. 2006. Current status of transcriptional regulation systems. *Cytotechnology*. 50:109–119.

Mayford, M., and B. Weisblum. 1990. The ermC leader peptide: amino acid alterations leading to differential efficiency of induction by macrolide-lincosamide-streptogramin B antibiotics. *J Bacteriol*. 172:3772–3779.

McConkey, G.A., M.J. Rogers, and T.F. McCutchan. 1997. Inhibition of Plasmodium falciparum protein synthesis. Targeting the plastid-like organelle with thiostrepton. *J Biol Chem*. 272:2046–2049.

McCormick, J.R.D., U. Hirsch, N.O. Sjolander, and A.P. Doerschuk. 1960. Cosynthesis of tetracyclines by pairs of Streptomyces aureofaciens mutants. *J Am Chem Soc*. 82:5006–5007.

McCoy, L.S., Y. Xie, and Y. Tor. 2011. Antibiotics that target protein synthesis. *Wiley Interdiscip Rev RNA*. 2:209–232.

McGuire, J.M., R.L. Bunch, R.C. Anderson, H.E. Boaz, E.H. Flynn, H.M. Powell, and J.W. Smith. 1952. Ilotycin, a new antibiotic. *Antibiot Chemother (Northfield Ill)*. 2:281–283.

McIntosh, J.A., M.S. Donia, and E.W. Schmidt. 2009. Ribosomal peptide natural products: bridging the ribosomal and nonribosomal worlds. *Nat Prod Rep*. 26:537–559.

McMurry, L., R.E. Petrucci, Jr., and S.B. Levy. 1980. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in Escherichia coli. *Proc Natl Acad Sci U S A*. 77:3974–3977.

McNicholas, P.M., D.J. Najarian, P.A. Mann, D. Hesk, R.S. Hare, K.J. Shaw, and T.A. Black. 2000. Evernimicin binds exclusively to the 50S ribosomal subunit and inhibits translation in cell-free systems derived from both gram-positive and gram-negative bacteria. *Antimicrob Agents Chemother*. 44:1121–1126.

Menninger, J.R., and D.P. Otto. 1982. Erythromycin, carbomycin, and spiramycin inhibit protein synthesis by stimulating the dissociation of peptidyl-tRNA from ribosomes. *Antimicrob Agents Chemother*. 21:811–818.

Mikolajka, A., H. Liu, Y. Chen, A.L. Starosta, V. Marquez, M. Ivanova, B.S. Cooperman, and D.N. Wilson. 2011. Differential effects of thiopeptide and orthosomycin antibiotics on translational GTPases. *Chem Biol*. 18:589–600.

Milenkovski, S., E. Baath, P.E. Lindgren, and O. Berglund. 2010. Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. *Ecotoxicology*. 19:285–294.

Miller, G.H., F.J. Sabatelli, R.S. Hare, Y. Glupczynski, P. Mackey, D. Shlaes, K. Shimizu, and K.J. Shaw. 1997. The most frequent aminoglycoside resistance mechanisms – changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. *Clin Infect Dis*. 24 Suppl 1:S46–62.

Milller, J.H. 1992. A Short Course in Bacterial Genetics. CSH Laboratory Press, Cold Spring Harbor, New York.

Monod, M., C. Denoya, and D. Dubnau. 1986. Sequence and properties of pIM13, a macrolide-lincosamide-streptogramin B resistance plasmid from Bacillus subtilis. *J Bacteriol*. 167:138–147.

Monro, R.E., M.L. Celma, and D. Vazquez. 1969. Action of sparsomycin on ribosome-catalysed peptidyl transfer. *Nature*. 222:356–358.

Moore, I.F., D.W. Hughes, and G.D. Wright. 2005. Tigecycline is modified by the flavin-dependent monooxygenase TetX. *Biochemistry*. 44:11829–11835.

Moore, R.A., D. DeShazer, S. Reckseidler, A. Weissman, and D.E. Woods. 1999. Efflux-mediated aminoglycoside and macrolide resistance in Burkholderia pseudomallei. *Antimicrob Agents Chemother*. 43:465–470.

Morimoto, S., Y. Takahashi, Y. Watanabe, and S. Omura. 1984. Chemical modification of erythromycins. I. Synthesis and antibacterial activity of 6-O-methylerythromycins A. *J Antibiot (Tokyo)*. 37:187–189.

Morita, Y., A. Kataoka, S. Shiota, T. Mizushima, and T. Tsuchiya. 2000. NorM of vibrio parahaemolyticus is an Na(+)-driven multidrug efflux pump. *J Bacteriol*. 182:6694–6697.

Mosher, R.H., D.J. Camp, K. Yang, M.P. Brown, W.V. Shaw, and L.C. Vining. 1995. Inactivation of chloramphenicol by O-phosphorylation. A novel resistance mechanism in Streptomyces venezuelae ISP5230, a chloramphenicol producer. *J Biol Chem*. 270:27000–27006.

Moussatova, A., C. Kandt, M.L. O'Mara, and D.P. Tieleman. 2008. ATP-binding cassette transporters in Escherichia coli. *Biochim Biophys Acta*. 1778:1757–1771.

Mulhall, A., J. de Louvois, and R. Hurley. 1983. Chloramphenicol toxicity in neonates: its incidence and prevention. *Br Med J (Clin Res Ed)*. 287:1424–1427.

Mullis, K.B., and F.A. Faloona. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol*. 155:335–350.

Munita, J.M., and C.A. Arias. 2016. Mechanisms of Antibiotic Resistance. *Microbiol Spectr*. 4.

Murray, I.A., J.V. Martinez-Suarez, T.J. Close, and W.V. Shaw. 1990. Nucleotide sequences of genes encoding the type II chloramphenicol acetyltransferases of Escherichia coli and Haemophilus influenzae, which are sensitive to inhibition by thiol-reactive reagents. *Biochem J*. 272:505–510.

Murray, I.A., and W.V. Shaw. 1997. O-Acetyltransferases for chloramphenicol and other natural products. *Antimicrob Agents Chemother*. 41:1–6.

Nakatogawa, H., and K. Ito. 2002. The ribosomal exit tunnel functions as a discriminating gate. *Cell*. 108:629–636.

Nathans, D. 1967. Puromycin. *In* Antibiotics Vol I mechanism of action. D. Gottlieb, Shaw, P. D., editor. Springer Berlin Heidelberg. 259–277.

Neddermann, P., C. Gargioli, E. Muraglia, S. Sambucini, F. Bonelli, R. De Francesco, and R. Cortese. 2003. A novel, inducible, eukaryotic gene expression system based on the quorum-sensing transcription factor TraR. *EMBO Rep*. 4:159–165.

Nguyen, F., A.L. Starosta, S. Arenz, D. Sohmen, A. Donhofer, and D.N. Wilson. 2014. Tetracycline antibiotics and resistance mechanisms. *Biol Chem*. 395:559–575.

Nicolaou, K.C., M. Zak, S. Rahimipour, A.A. Estrada, S.H. Lee, A. O'Brate, P. Giannakakou, and M.R. Ghadiri. 2005. Discovery of a biologically active thiostrepton fragment. *J Am Chem Soc*. 127:15042–15044.

Nierhaus, K.H. 1993. Solution of the ribosome riddle: how the ribosome selects the correct aminoacyl-tRNA out of 41 similar contestants. *Mol Microbiol*. 9:661–669.

Nikaido, H. 1996. Multidrug efflux pumps of gram-negative bacteria. *J Bacteriol*. 178:5853–5859.

Nissen, P., J. Hansen, N. Ban, P.B. Moore, and T.A. Steitz. 2000. The structural basis of ribosome activity in peptide bond synthesis. *Science*. 289:920–930.

Noeske, J., J. Huang, N.B. Olivier, R.A. Giacobbe, M. Zambrowski, and J.H. Cate. 2014. Synergy of streptogramin antibiotics occurs independently of their effects on translation. *Antimicrob Agents Chemother*. 58:5269–5279.

Norstrom, T., J. Lannergard, and D. Hughes. 2007. Genetic and phenotypic identification of fusidic acid-resistant mutants with the small-colony-variant phenotype in Staphylococcus aureus. *Antimicrob Agents Chemother*. 51:4438–4446.

Novak, R. 2011. Are pleuromutilin antibiotics finally fit for human use? *Ann N Y Acad Sci*. 1241:71–81.

Novak, R., and D.M. Shlaes. 2010. The pleuromutilin antibiotics: a new class for human use. *Curr Opin Investig Drugs*. 11:182–191.

Nyssen, E., M. Di Giambattista, and C. Cocito. 1989. Analysis of the reversible binding of virginiamycin M to ribosome and particle functions after removal of the antibiotic. *Biochim Biophys Acta*. 1009:39–46.

Obrig, T., J. Irvin, W. Culp, and B. Hardesty. 1971. Inhibition of peptide initiation on reticulocyte ribosomes by edeine. *Eur J Biochem*. 21:31–41.

Oehler, R., N. Polacek, G. Steiner, and A. Barta. 1997. Interaction of tetracycline with RNA: photoincorporation into ribosomal RNA of Escherichia coli. *Nucleic Acids Res*. 25:1219–1224.

O'Farrell, H.C., Z. Xu, G.M. Culver, and J.P. Rife. 2008. Sequence and structural evolution of the KsgA/Dim1 methyltransferase family. *BMC Res Notes*. 1:108.

O'Gorman, S., D.T. Fox, and G.M. Wahl. 1991. Recombinase-mediated gene activation and site-specific integration in mammalian cells. *Science*. 251:1351–1355.

O'Hara, K., T. Kanda, K. Ohmiya, T. Ebisu, and M. Kono. 1989. Purification and characterization of macrolide 2'-phosphotransferase from a strain of Escherichia coli that is highly resistant to erythromycin. *Antimicrob Agents Chemother*. 33:1354–1357.

Olsthoorn-Tieleman, L.N., R.J. Palstra, G.P. van Wezel, M.J. Bibb, and C.W. Pleij. 2007. Elongation factor Tu3 (EF-Tu3) from the kirromycin producer Streptomyces ramocissimus Is resistant to three classes of EF-Tu-specific inhibitors. *J Bacteriol*. 189:3581–3590.

O'Neill, A.J., and I. Chopra. 2006. Molecular basis of fusB-mediated resistance to fusidic acid in Staphylococcus aureus. *Mol Microbiol*. 59:664–676.

O'Neill, A.J., A.R. Larsen, A.S. Henriksen, and I. Chopra. 2004. A fusidic acid-resistant epidemic strain of Staphylococcus aureus carries the fusB determinant, whereas fusA mutations are prevalent in other resistant isolates. *Antimicrob Agents Chemother*. 48:3594–3597.

O'Neill, A.J., F. McLaws, G. Kahlmeter, A.S. Henriksen, and I. Chopra. 2007. Genetic basis of resistance to fusidic acid in staphylococci. *Antimicrob Agents Chemother*. 51:1737–1740.

Orelle, C., S. Carlson, B. Kaushal, M.M. Almutairi, H. Liu, A. Ochabowicz, S. Quan, V.C. Pham, C.L. Squires, B.T. Murphy, and A.S. Mankin. 2013. Tools for characterizing bacterial protein synthesis inhibitors. *Antimicrob Agents Chemother*. 57:5994–6004.

Ottenheijm, H.C., L.A. van den Broek, J.P. Ballesta, and Z. Zylicz. 1986. Chemical and biological aspects of sparsomycin, an antibiotic from Streptomyces. *Prog Med Chem*. 23:219–268.

Ounissi, H., and P. Courvalin. 1985. Nucleotide sequence of the gene ereA encoding the erythromycin esterase in Escherichia coli. *Gene*. 35:271–278.

Owen, S.P., A. Dietz, and G.W. Camiener. 1962. Sparsomycin, a new antitumor antibiotic. I. Discovery and biological properties. *Antimicrobial Agents and Chemotherapy*:772–779.

Parfait, R., and C. Cocito. 1980. Lasting damage to bacterial ribosomes by reversibly bound virginiamycin M. *Proc Natl Acad Sci U S A*. 77:5492–5496.

Paukner, S., and R. Riedl. 2016. Pleuromutilins: potent drugs for resistant bugs. Mode of action and resistance. *Cold Spring Harb Perspect Med*.

Paulsen, I.T., M.H. Brown, and R.A. Skurray. 1996. Proton-dependent multidrug efflux systems. *Microbiol Rev*. 60:575–608.

Perlman, D., L.J. Heuser, J.D. Dutcher, J.M. Barrett, and J.A. Boska. 1960. Biosynthesis of tetracycline by 5-hydroxy-tetracycline-producing cultures of Streptomyces rimosus. *J Bacteriol*. 80:419–420.

Pestka, S. 1969. Studies on the formation of transfer ribonucleic acid-ribosome complexes. XI. Antibiotic effects on phenylalanyl-oligonucleotide binding to ribosomes. *Proc Natl Acad Sci U S A*. 64:709–714.

Pestka, S. 1977. Inhibitors of protein synthesis. *In* Molecular mechanisms of protein biosynthesis. H. Weisbach and S. Pestka, editors. Academic Press, New York. 467–553.

Peterson, L.R. 2008. A review of tigecycline – the first glycylcycline. *Int J Antimicrob Agents*. 32 Suppl 4:S215–222.

Phillips, O.A., Sharaf, L. H. 2007. Pleuromutilin antibacterial agents: patent review 2001–2006. *Expert Opin Thera Pat*. 17:429–435.

Pickens, L.B., and Y. Tang. 2009. Decoding and engineering tetracycline biosynthesis. *Metab Eng*. 11:69–75.

Piddock, L.J. 2006a. Multidrug-resistance efflux pumps – not just for resistance. *Nat Rev Microbiol*. 4:629–636.

Piddock, L.J. 2006b. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev*. 19:382–402.

Pioletti, M., F. Schlunzen, J. Harms, R. Zarivach, M. Gluhmann, H. Avila, A. Bashan, H. Bartels, T. Auerbach, C. Jacobi, T. Hartsch, A. Yonath, and F. Franceschi. 2001. Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3. *Embo J*. 20:1829–1839.

Poehlsgaard, J., and S. Douthwaite. 2003. Macrolide antibiotic interaction and resistance on the bacterial ribosome. *Curr Opin Investig Drugs*. 4:140–148.

Poehlsgaard, J., and S. Douthwaite. 2005. The bacterial ribosome as a target for antibiotics. *Nat Rev Microbiol*. 3:870–881.

Poehlsgaard, J., P. Pfister, E.C. Bottger, and S. Douthwaite. 2005. Molecular mechanisms by which rRNA mutations confer resistance to clindamycin. *Antimicrob Agents Chemother*. 49:1553–1555.

Polikanov, Y.S., I.A. Osterman, T. Szal, V.N. Tashlitsky, M.V. Serebryakova, P. Kusochek, D. Bulkley, I.A. Malanicheva, T.A. Efimenko, O.V. Efremenkova, A.L. Konevega, K.J. Shaw, A.A. Bogdanov, M.V. Rodnina, O.A. Dontsova, A.S. Mankin, T.A. Steitz, and P.V. Sergiev. 2014a. Amicoumacin a inhibits translation by stabilizing mRNA interaction with the ribosome. *Mol Cell*. 56:531–540.

Polikanov, Y.S., T. Szal, F. Jiang, P. Gupta, R. Matsuda, M. Shiozuka, T.A. Steitz, N. Vazquez-Laslop, and A.S. Mankin. 2014b. Negamycin interferes with decoding and translocation by simultaneous interaction with rRNA and tRNA. *Mol Cell*. 56:541–550.

Politano, A.D., and R.G. Sawyer. 2010. NXL-103, a combination of flopristin and linopristin, for the potential treatment of bacterial infections including community-acquired pneumonia and MRSA. *Curr Opin Investig Drugs*. 11:225–236.

Poole, K. 2005. Efflux-mediated antimicrobial resistance. *J Antimicrob Chemother*. 56:20–51.

Poole, K. 2008. Bacteria multidrug efflux pumps serve other functions. *Micobes*. 3:179–185.

Poole, K., K. Krebes, C. McNally, and S. Neshat. 1993. Multiple antibiotic resistance in Pseudomonas aeruginosa: evidence for involvement of an efflux operon. *J Bacteriol*. 175:7363–7372.

Porse, B.T., S.V. Kirillov, M.J. Awayez, H.C. Ottenheijm, and R.A. Garrett. 1999. Direct crosslinking of the antitumor antibiotic sparsomycin, and its derivatives, to A2602 in the peptidyl transferase center of 23S-like rRNA within ribosome-tRNA complexes. *Proc Natl Acad Sci U S A*. 96:9003–9008.

Porse, B.T., I. Leviev, A.S. Mankin, and R.A. Garrett. 1998. The antibiotic thiostrepton inhibits a functional transition within protein L11 at the ribosomal GTPase centre. *J Mol Biol*. 276:391–404.

Porter, J.N., R.I. Hewitt, C.W. Hesseltine, G. Krupka, J.A. Lowery, W.S. Wallace, N. Bohonos, and J.H. Williams. 1952. Achromycin: a new antibiotic having trypanocidal properties. *Antibiot Chemother (Northfield Ill)*. 2:409–410.

Poulet, F.M., R. Veneziale, P.M. Vancutsem, P. Losco, K. Treinen, and R.E. Morrissey. 2005. Ziracin-induced congenital urogenital malformations in female rats. *Toxicol Pathol*. 33:320–328.

Poulsen, S.M., M. Karlsson, L.B. Johansson, and B. Vester. 2001. The pleuromutilin drugs tiamulin and valnemulin bind to the RNA at the peptidyl transferase centre on the ribosome. *Mol Microbiol*. 41:1091–1099.

Prince, W.T., Z. Ivezic-Schoenfeld, C. Lell, K.J. Tack, R. Novak, F. Obermayr, and G.H. Talbot. 2013. Phase II clinical study of BC-3781, a pleuromutilin antibiotic, in treatment of patients with acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother*. 57:2087–2094.

Pringle, M., J. Poehlsgaard, B. Vester, and K.S. Long. 2004. Mutations in ribosomal protein L3 and 23S ribosomal RNA at the peptidyl transferase centre are associated with reduced susceptibility to tiamulin in Brachyspira spp. isolates. *Mol Microbiol*. 54:1295–1306.

Rahal, J.J., Jr., and M.S. Simberkoff. 1979. Bactericidal and bacteriostatic action of chloramphenicol against memingeal pathogens. *Antimicrob Agents Chemother*. 16:13–18.

Ramirez, M.S., and M.E. Tolmasky. 2010. Aminoglycoside modifying enzymes. *Drug Resist Updat*. 13:151–171.

Ramu, H., A. Mankin, and N. Vazquez-Laslop. 2009. Programmed drug-dependent ribosome stalling. *Mol Microbiol*. 71:811–824.

Ramu, H., N. Vazquez-Laslop, D. Klepacki, Q. Dai, J. Piccirilli, R. Micura, and A.S. Mankin. 2011. Nascent peptide in the ribosome exit tunnel affects functional properties of the A-site of the peptidyl transferase center. *Mol Cell*. 41:321–330.

Raney, A., G.L. Law, G.J. Mize, and D.R. Morris. 2002. Regulated translation termination at the upstream open reading frame in s-adenosylmethionine decarboxylase mRNA. *J Biol Chem*. 277:5988–5994.

Ranger, L. 2004. Creation of a novel class: the oxazolidinone antibiotics. Innova Institute for Medical Education, Tampa.

Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Mason. 1987. Spectrum and mode of action of azithromycin

(CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. *Antimicrob Agents Chemother*. 31:1939–1947.
Rheinberger, H.J., and K.H. Nierhaus. 1990. Partial release of AcPhe-Phe-tRNA from ribosomes during poly(U)-dependent poly(Phe) synthesis and the effects of chloramphenicol. *Eur J Biochem*. 193:643–650.
Roberts, M.C. 2005. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett*. 245:195–203.
Roberts, M.C. 2011. Environmental macrolide-lincosamide-streptogramin and tetracycline resistant bacteria. *Front Microbiol*. 2:40.
Roberts, M.C., W.O. Chung, D. Roe, M. Xia, C. Marquez, G. Borthagaray, W.L. Whittington, and K.K. Holmes. 1999. Erythromycin-resistant Neisseria gonorrhoeae and oral commensal Neisseria spp. carry known rRNA methylase genes. *Antimicrob Agents Chemother*. 43:1367–1372.
Rodriguez-Fonseca, C., R. Amils, and R.A. Garrett. 1995. Fine structure of the peptidyl transferase centre on 23 S-like rRNAs deduced from chemical probing of antibiotic-ribosome complexes. *J Mol Biol*. 247:224–235.
Rosendahl, G., and S. Douthwaite. 1993. Ribosomal proteins L11 and L10.(L12)4 and the antibiotic thiostrepton interact with overlapping regions of the 23 S rRNA backbone in the ribosomal GTPase centre. *J Mol Biol*. 234:1013–1020.
Ross, J.I., E.A. Eady, J.H. Cove, and W.J. Cunliffe. 1998. 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. *Antimicrob Agents Chemother*. 42:1702–1705.
Saidijam, M., G. Benedetti, Q. Ren, Z. Xu, C.J. Hoyle, S.L. Palmer, A. Ward, K.E. Bettaney, G. Szakonyi, J. Meuller, S. Morrison, M.K. Pos, P. Butaye, K. Walravens, K. Langton, R.B. Herbert, R.A. Skurray, I.T. Paulsen, J. O'Reilly, N.G. Rutherford, M.H. Brown, R.M. Bill, and P.J. Henderson. 2006. Microbial drug efflux proteins of the major facilitator superfamily. *Curr Drug Targets*. 7:793–811.
Saier, M.H., Jr., I.T. Paulsen, M.K. Sliwinski, S.S. Pao, R.A. Skurray, and H. Nikaido. 1998. Evolutionary origins of multidrug and drug-specific efflux pumps in bacteria. *Faseb J*. 12:265–274.
Sanchez-Pescador, R., J.T. Brown, M. Roberts, and M.S. Urdea. 1988. Homology of the TetM with translational elongation factors: implications for potential modes of tetM-conferred tetracycline resistance. *Nucleic Acids Res*. 16:1218.
Sauer, B., and N. Henderson. 1989. Cre-stimulated recombination at loxP-containing DNA sequences placed into the mammalian genome. *Nucleic Acids Res*. 17:147–161.
Savic, M., J. Lovric, T.I. Tomic, B. Vasiljevic, and G.L. Conn. 2009. Determination of the target nucleosides for members of two families of 16S rRNA methyltransferases that confer resistance to partially overlapping groups of aminoglycoside antibiotics. *Nucleic Acids Res*. 37:5420–5431.
Schatz, A., E. Bugie, and S.A. Waksman. 1944. Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Proc.Soc. Exptl.Biol. & Med.* 65:66–69.
Schindler, B.D., E. Frempong-Manso, C.E. DeMarco, C. Kosmidis, V. Matta, S.M. Seo, and G.W. Kaatz. 2015. Analyses of multidrug efflux pump-like proteins encoded on the Staphylococcus aureus chromosome. *Antimicrob Agents Chemother*. 59:747–748.
Schindler, B.D., and G.W. Kaatz. 2016. Multidrug efflux pumps of Gram-positive bacteria. *Drug Resist Updat*. 27:1–13.

Schlunzen, F., J.M. Harms, F. Franceschi, H.A. Hansen, H. Bartels, R. Zarivach, and A. Yonath. 2003. Structural basis for the antibiotic activity of ketolides and azalides. *Structure*. 11:329–338.

Schlunzen, F., E. Pyetan, P. Fucini, A. Yonath, and J.M. Harms. 2004. Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from Deinococcus radiodurans in complex with tiamulin. *Mol Microbiol*. 54:1287–1294.

Schlunzen, F., R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath, and F. Franceschi. 2001. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature*. 413:814–821.

Schmeing, T.M., K.S. Huang, S.A. Strobel, and T.A. Steitz. 2005. An induced-fit mechanism to promote peptide bond formation and exclude hydrolysis of peptidyl-tRNA. *Nature*. 438:520–524.

Schmid, B., T. Anke, and H. Wolf. 1978. Action of pulvomycin and kirromycin on eukaryotic cells. *FEBS Lett*. 96:189–191.

Schneider-Poetsch, T., J. Ju, D.E. Eyler, Y. Dang, S. Bhat, W.C. Merrick, R. Green, B. Shen, and J.O. Liu. 2010. Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. *Nat Chem Biol*. 6:209–217.

Scholz, T., C.L. Heyl, D. Bernardi, S. Zimmermann, L. Kattner, and C.D. Klein. 2012. Chemical, biochemical and microbiological properties of a brominated nitro-vinylfuran with broad-spectrum antibacterial activity. *Bioorg Med Chem*. 21:795–804.

Schrag, S.J., and V. Perrot. 1996. Reducing antibiotic resistance. *Nature*. 381:120–121.

Schrag, S.J., V. Perrot, and B.R. Levin. 1997. Adaptation to the fitness costs of antibiotic resistance in Escherichia coli. *Proc Biol Sci*. 264:1287–1291.

Schwarz, S., C. Kehrenberg, B. Doublet, and A. Cloeckaert. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev*. 28:519–542.

Schwarz, S., C. Werckenthin, and C. Kehrenberg. 2000. Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in Staphylococcus sciuri. *Antimicrob Agents Chemother*. 44:2530–2533.

Scocchi, M., B. Skerlavaj, D. Romeo, and R. Gennaro. 1992. Proteolytic cleavage by neutrophil elastase converts inactive storage proforms to antibacterial bactenecins. *Eur J Biochem*. 209:589–595.

Seefeldt, A.C., M. Graf, N. Perebaskine, F. Nguyen, S. Arenz, M. Mardirossian, M. Scocchi, D.N. Wilson, and C.A. Innis. 2016. Structure of the mammalian antimicrobial peptide Bac7(1–16) bound within the exit tunnel of a bacterial ribosome. *Nucleic Acids Res*. 44:2429–2438.

Seefeldt, A.C., F. Nguyen, S. Antunes, N. Perebaskine, M. Graf, S. Arenz, K.K. Inampudi, C. Douat, G. Guichard, D.N. Wilson, and C.A. Innis. 2015. The proline-rich antimicrobial peptide Onc112 inhibits translation by blocking and destabilizing the initiation complex. *Nat Struct Mol Biol*. 22:470–475.

Seo, H.S., S. Abedin, D. Kamp, D.N. Wilson, K.H. Nierhaus, and B.S. Cooperman. 2006. EF-G-dependent GTPase on the ribosome. conformational change and fusidic acid inhibition. *Biochemistry*. 45:2504–2514.

Sharkey, L.K., T.A. Edwards, and A.J. O'Neill. 2016. ABC-F Proteins Mediate Antibiotic Resistance through Ribosomal Protection. *MBio*. 7:e01975.

Shaw, K.J., and M.R. Barbachyn. 2011. The oxazolidinones: past, present, and future. *Ann N Y Acad Sci*. 1241:48–70.

Shaw, K.J., S. Poppe, R. Schaadt, V. Brown-Driver, J. Finn, C.M. Pillar, D. Shinabarger, and G. Zurenko. 2008. In vitro activity of TR-700, the antibacterial moiety of the prodrug TR-701, against linezolid-resistant strains. *Antimicrob Agents Chemother*. 52:4442–4447.

Shaw, K.J., P.N. Rather, R.S. Hare, and G.H. Miller. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev*. 57:138–163.

Shaw, W.V. 1967. The enzymatic acetylation of chloramphenicol by extracts of R factor-resistant Escherichia coli. *J Biol Chem*. 242:687–693.

Shaw, W.V., and D.A. Hopwood. 1976. Chloramphenicol acetylation in Streptomyces. *J Gen Microbiol*. 94:159–166.

Shaw, W.V., and J. Unowsky. 1968. Mechanism of R factor-mediated chloramphenicol resistance. *J Bacteriol*. 95:1976–1978.

Shirai, A., M. Sadaie, K. Shinmyozu, and J. Nakayama. 2010. Methylation of ribosomal protein L42 regulates ribosomal function and stress-adapted cell growth. *J Biol Chem*. 285:22448–22460.

Shlykov, M.A., W.H. Zheng, E. Wang, J.D. Nguyen, and M.H. Saier. 2013. Transmembrane Molecular Transporters Facilitating Export of Molecules from Cells and Organelles. *In* Microbial Efflux Pumps: Current Research. E.W. Yu, Q. Zhang, and M.H. Brown, editors. Caister Academic Press. 1–20.

Skinner, R.H., and E. Cundliffe. 1982. Dimethylation of adenine and the resistance of Streptomyces erythraeus to erythromycin. *J Gen Microbiol*. 128:2411–2416.

Slatter, J.G., L.A. Adams, E.C. Bush, K. Chiba, P.T. Daley-Yates, K.L. Feenstra, S. Koike, N. Ozawa, G.W. Peng, J.P. Sams, M.R. Schuette, and S. Yamazaki. 2002. Pharmacokinetics, toxicokinetics, distribution, metabolism and excretion of linezolid in mouse, rat and dog. *Xenobiotica*. 32:907–924.

Slee, A.M., M.A. Wuonola, R.J. McRipley, I. Zajac, M.J. Zawada, P.T. Bartholomew, W.A. Gregory, and M. Forbes. 1987. Oxazolidinones, a new class of synthetic antibacterial agents: in vitro and in vivo activities of DuP 105 and DuP 721. *Antimicrob Agents Chemother*. 31:1791–1797.

Smith, L.K., and A.S. Mankin. 2008. Transcriptional and translational control of the mlr operon, which confers resistance to seven classes of protein synthesis inhibitors. *Antimicrob Agents Chemother*. 52:1703–1712.

Sobin, B.A., Tanner, F.W. 1954. Anisomycin, a new antiprotozoan antibiotic. *J Am Chem Soc* 76:4053.

Sofia, H.J., G. Chen, B.G. Hetzler, J.F. Reyes-Spindola, and N.E. Miller. 2001. Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. *Nucleic Acids Res*. 29:1097–1106.

Sothiselvam, S., B. Liu, W. Han, H. Ramu, D. Klepacki, G.C. Atkinson, A. Brauer, M. Remm, T. Tenson, K. Schulten, N. Vazquez-Laslop, and A.S. Mankin. 2014. Macrolide antibiotics allosterically predispose the ribosome for translation arrest. *Proc Natl Acad Sci U S A*. 111:9804–9809.

Spahn, C.M., G. Blaha, R.K. Agrawal, P. Penczek, R.A. Grassucci, C.A. Trieber, S.R. Connell, D.E. Taylor, K.H. Nierhaus, and J. Frank. 2001. Localization of the ribosomal protection protein Tet(O) on the ribosome and the mechanism of tetracycline resistance. *Mol Cell*. 7:1037–1045.

Speer, B.S., and A.A. Salyers. 1989. Novel aerobic tetracycline resistance gene that chemically modifies tetracycline. *J Bacteriol*. 171:148–153.

Spizek, J., and T. Rezanka. 2004. Lincomycin, clindamycin and their applications. *Appl Microbiol Biotechnol*. 64:455–464.

Srinivasan, V.B., G. Rajamohan, and W.A. Gebreyes. 2009. Role of AbeS, a novel efflux pump of the SMR family of transporters, in resistance to antimicrobial agents in Acinetobacter baumannii. *Antimicrob Agents Chemother*. 53:5312–5316.

Stanton, T.B., S.B. Humphrey, K.P. Scott, and H.J. Flint. 2005. Hybrid tet genes and tet gene nomenclature: request for opinion. *Antimicrob Agents Chemother*. 49:1265–1266.

Stark, H., M.V. Rodnina, J. Rinke-Appel, R. Brimacombe, W. Wintermeyer, and M. van Heel. 1997. Visualization of elongation factor Tu on the Escherichia coli ribosome. *Nature*. 389:403–406.

Stocklein, W., W. Piepersberg, and A. Bock. 1981. Amino acid replacements in ribosomal protein YL24 of Saccharomyces cerevisiae causing resistance to cycloheximide. *FEBS Lett*. 136:265–268.

Sturdik, E., L. Drobnica, S. Balaz, and V. Marko. 1979. Reaction of vinylfurans with sulfhydryl and amino groups. *Biochem Pharmacol*. 28:2525–2530.

Su, T.L. 1948. Micrococcin, an antibacterial substance formed by a strain of Micrococcus. *Br J Exp Pathol*. 29:473–481.

Sun, C., D.K. Hunt, C.L. Chen, Y. Deng, M. He, R.B. Clark, C. Fyfe, T.H. Grossman, J.A. Sutcliffe, and X.Y. Xiao. 2015. Design, Synthesis, and Biological Evaluation of Hexacyclic Tetracyclines as Potent, Broad Spectrum Antibacterial Agents. *J Med Chem*. 58:4703–4712.

Sun, J., Z. Deng, and A. Yan. 2014. Bacterial multidrug efflux pumps: Mechanisms, physiology and pharmacological exploitations. *Biochem Biophys Res Commun*. 453:254–267.

Zaher, H.S., and R. Green. 2009. Fidelity at the molecular level: lessons from protein synthesis. *Cell*. 136:746–762.

Zechini, B., and I. Versace. 2009. Inhibitors of multidrug resistant efflux systems in bacteria. *Recent Pat Antiinfect Drug Discov*. 4:37–50.

Zgurskaya, H.I., and H. Nikaido. 1999. Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of Escherichia coli. *Proc Natl Acad Sci U S A*. 96:7190–7195.

Zurenko, G.E., C.W. Ford, D.K. Hutchinson, S.J. Brickner, and M.R. Barbachyn. 1997. Oxazolidinone antibacterial agents: development of the clinical candidates eperezolid and linezolid. *Expert Opin Investig Drugs*. 6:151–158.

Tai, P.C., B.J. Wallace, and B.D. Davis. 1973. Actions of aurintricarboxylate, kasugamycin, and pactamycin on Escherichia coli polysomes. *Biochemistry*. 12:616–620.

Tanner, D.R., D.A. Cariello, C.J. Woolstenhulme, M.A. Broadbent, and A.R. Buskirk. 2009. Genetic identification of nascent peptides that induce ribosome stalling. *J Biol Chem*. 284:34809–34818.

Taylor, D.E. 1986. Plasmid-mediated tetracycline resistance in Campylobacter jejuni: expression in Escherichia coli and identification of homology with streptococcal class M determinant. *J Bacteriol*. 165:1037–1039.

Taylor, D.E., and A. Chau. 1996. Tetracycline resistance mediated by ribosomal protection. *Antimicrob Agents Chemother*. 40:1–5.

Tennent, J.M., B.R. Lyon, M. Midgley, I.G. Jones, A.S. Purewal, and R.A. Skurray. 1989. Physical and biochemical characterization of the qacA gene encoding antiseptic and disinfectant resistance in Staphylococcus aureus. *J Gen Microbiol*. 135:1–10.

Tenson, T., A. DeBlasio, and A. Mankin. 1996. A functional peptide encoded in the Escherichia coli 23S rRNA. *Proc Natl Acad Sci U S A*. 93:5641–5646.
Tenson, T., and M. Ehrenberg. 2002. Regulatory nascent peptides in the ribosomal tunnel. *Cell*. 108:591–594.
Tenson, T., M. Lovmar, and M. Ehrenberg. 2003. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *J Mol Biol*. 330:1005–1014.
Tenson, T., and A.S. Mankin. 2001. Short peptides conferring resistance to macrolide antibiotics. *Peptides*. 22:1661–1668.
Tenson, T., L. Xiong, P. Kloss, and A.S. Mankin. 1997. Erythromycin resistance peptides selected from random peptide libraries. *J Biol Chem*. 272:17425–17430.
Thakor, N.S., R. Nechifor, P.G. Scott, M. Keelan, D.E. Taylor, and K.S. Wilson. 2008. Chimeras of bacterial translation factors Tet(O) and EF-G. *FEBS Lett*. 582:1386–1390.
Thanassi, D.G., L.W. Cheng, and H. Nikaido. 1997. Active efflux of bile salts by Escherichia coli. *J Bacteriol*. 179:2512–2518.
Thibault, G., M. Guitard, and R. Daigneault. 1980. A study of the enzymatic inactivation of chloramphenicol by highly purified chloramphenicol acetyltransferase. *Biochim Biophys Acta*. 614:339–342.
Thompson, J., F. Schmidt, and E. Cundliffe. 1982. Site of action of a ribosomal RNA methylase conferring resistance to thiostrepton. *J Biol Chem*. 257:7915–7917.
Torocsik, B., and J. Szeberenyi. 2000. Anisomycin uses multiple mechanisms to stimulate mitogen-activated protein kinases and gene expression and to inhibit neuronal differentiation in PC12 phaeochromocytoma cells. *Eur J Neurosci*. 12:527–532.
Trieber, C.A., N. Burkhardt, K.H. Nierhaus, and D.E. Taylor. 1998. Ribosomal protection from tetracycline mediated by Tet(O): Tet(O) interaction with ribosomes is GTP-dependent. *Biol Chem*. 379:847–855.
Tripathi, S., P.S. Kloss, and A.S. Mankin. 1998. Ketolide resistance conferred by short peptides. *J Biol Chem*. 273:20073–20077.
Tu, D., G. Blaha, P.B. Moore, and T.A. Steitz. 2005. Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. *Cell*. 121:257–270.
Udekwu, K.I., N. Parrish, P. Ankomah, F. Baquero, and B.R. Levin. 2009. Functional relationship between bacterial cell density and the efficacy of antibiotics. *J Antimicrob Chemother*. 63:745–757.
Waller, C.W., P. W. Fryth, B. L. Hutchings, J. H. Williams. 1953. Achromycin. The structure of the antibiotic puromycin. *I J Am Chem Soc*. 75:2025.
Walsh, C.T., M.G. Acker, and A.A. Bowers. 2010. Thiazolyl peptide antibiotic biosynthesis: a cascade of post-translational modifications on ribosomal nascent proteins. *J Biol Chem*. 285:27525–27531.
Walter, J.D., M. Hunter, M. Cobb, G. Traeger, and P.C. Spiegel. 2011. Thiostrepton inhibits stable 70S ribosome binding and ribosome-dependent GTPase activation of elongation factor G and elongation factor 4. *Nucleic Acids Res*. 40:360–370.
van Hoek, A.H., D. Mevius, B. Guerra, P. Mullany, A.P. Roberts, and H.J. Aarts. 2011. Acquired antibiotic resistance genes: an overview. *Front Microbiol*. 2:203.
Vanek, Z., and M. Vondracek. 1965. Biogenesis of cycloheximide and of related compounds. *Antimicrob Agents Chemother (Bethesda)*. 5:982–991.

Vasquez, D. 1975. The streptogramin family of antibiotics. *In* Antibiotics, Vol. III. J.W. Corcoran, Hahn, F. E., editor. Springer-Verlag, Berlin. 521–534.
Vazquez, D. 1966. Studies on the mode of action of the streptogramin antibiotics. *J Gen Microbiol*. 42:93–106.
Vazquez-Laslop, N., and A.S. Mankin. 2011. Picky nascent peptides do not talk to foreign ribosomes. *Proc Natl Acad Sci U S A*. 108:5931–5932.
Watts, J.L., and R.J. Yancey, Jr. 1994. Identification of veterinary pathogens by use of commercial identification systems and new trends in antimicrobial susceptibility testing of veterinary pathogens. *Clin Microbiol Rev*. 7:346–356.
Weber, T., K.J. Laiple, E.K. Pross, A. Textor, S. Grond, K. Welzel, S. Pelzer, A. Vente, and W. Wohlleben. 2008. Molecular analysis of the kirromycin biosynthetic gene cluster revealed beta-alanine as precursor of the pyridone moiety. *Chem Biol*. 15:175–188.
Weber, W., W. Bacchus, M. Daoud-El Baba, and M. Fussenegger. 2007. Vitamin H-regulated transgene expression in mammalian cells. *Nucleic Acids Res*. 35:e116.
Weber, W., C. Fux, M. Daoud-el Baba, B. Keller, C.C. Weber, B.P. Kramer, C. Heinzen, D. Aubel, J.E. Bailey, and M. Fussenegger. 2002. Macrolide-based transgene control in mammalian cells and mice. *Nat Biotechnol*. 20:901–907.
Weinstein, M.J., G.M. Luedemann, E.M. Oden, and G.H. Wagman. 1964. Everninomicin, a new antibiotic complex from Micromonospora carbonacea. *Antimicrob Agents Chemother (Bethesda)*. 10:24–32.
Weisblum, B. 1995a. Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother*. 39:577–585.
Weisblum, B. 1995b. Insights into erythromycin action from studies of its activity as inducer of resistance. *Antimicrob Agents Chemother*. 39:797–805.
Weisblum, B. 1998. Macrolide resistance. *Drug Resist Updat*. 1:29–41.
Weisblum, B., C. Siddhikol, C.J. Lai, and V. Demohn. 1971. Erythromycin-inducible resistance in Staphylococcus aureus: requirements for induction. *J Bacteriol*. 106:835–847.
Verdier, L., J. Gharbi-Benarous, G. Bertho, P. Mauvais, and J.P. Girault. 2002. Antibiotic resistance peptides: interaction of peptides conferring macrolide and ketolide resistance with Staphylococcus aureus ribosomes: conformation of bound peptides as determined by transferred NOE experiments. *Biochemistry*. 41:4218–4229.
Whiffen, A.J. 1947. Actidione, an antibiotic produced by Streptomyces griseus. *J Bacteriol*. 54:41.
White, J.P., and C.R. Cantor. 1971. Role of magnesium in the binding of tetracycline to Escherichia coli ribosomes. *J Mol Biol*. 58:397–400.
Vicens, Q., and E. Westhof. 2001. Crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site. *Structure*. 9:647–658.
Vicens, Q., and E. Westhof. 2003. Molecular recognition of aminoglycoside antibiotics by ribosomal RNA and resistance enzymes: an analysis of x-ray crystal structures. *Biopolymers*. 70:42–57.
Wiegand, I., K. Hilpert, and R.E. Hancock. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*. 3:163–175.
Wiley, P.F., H.K. Jahnke, F. MacKellar, R.B. Kelly, and A.D. Argoudelis. 1970. The structure of pactamycin. *J Org Chem*. 35:1420–1425.

Wiley, P.F., and F.A. MacKellar. 1976. Sparsomycin, structure and chemistry. *J Org Chem*. 41:1858–1862.
Wilson, D.N. 2009. The A-Z of bacterial translation inhibitors. *Crit Rev Biochem Mol Biol*. 44:393–433.
Wilson, D.N. 2014. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat Rev Microbiol*. 12:35–48.
Wilson, D.N. 2016. The ABC of Ribosome-Related Antibiotic Resistance. *MBio*. 7.
Wilson, D.N., S. Arenz, and R. Beckmann. 2016. Translation regulation via nascent polypeptide-mediated ribosome stalling. *Curr Opin Struct Biol*. 37:123–133.
Wilson, D.N., J.M. Harms, K.H. Nierhaus, F. Schlunzen, and P. Fucini. 2005. Species-specific antibiotic-ribosome interactions: implications for drug development. *Biol Chem*. 386:1239–1252.
Wilson, D.N., F. Schluenzen, J.M. Harms, A.L. Starosta, S.R. Connell, and P. Fucini. 2008. The oxazolidinone antibiotics perturb the ribosomal peptidyl-transferase center and effect tRNA positioning. *Proc Natl Acad Sci U S A*. 105:13339–13344.
Vimberg, V., L. Xiong, M. Bailey, T. Tenson, and A. Mankin. 2004. Peptide-mediated macrolide resistance reveals possible specific interactions in the nascent peptide exit tunnel. *Mol Microbiol*. 54:376–385.
Vogeley, L., G.J. Palm, J.R. Mesters, and R. Hilgenfeld. 2001. Conformational change of elongation factor Tu (EF-Tu) induced by antibiotic binding. Crystal structure of the complex between EF-Tu.GDP and aurodox. *J Biol Chem*. 276:17149–17155.
Wolf, H., H. Zahner, and K. Nierhaus. 1972. Kirromycin, an inhibitor of the 30 S ribosomal subunits function. *FEBS Lett*. 21:347–350.
Volkers, G., G.J. Palm, M.S. Weiss, G.D. Wright, and W. Hinrichs. 2011. Structural basis for a new tetracycline resistance mechanism relying on the TetX mono-oxygenase. *FEBS Lett*. 585:1061–1066.
Wong, A., S.P. Reddy, D.S. Smyth, M.E. Aguero-Rosenfeld, G. Sakoulas, and D.A. Robinson. 2010. Polyphyletic emergence of linezolid-resistant staphylococci in the United States. *Antimicrob Agents Chemother*. 54:742–748.
Woo, P.W.K., H.W. Dion, and Q.R. Bartz. 1971. Butirosins A and B, aminoglycoside antibiotics. I. Structural Units. *Tertahedron Lett.* . 28:2617–2620.
Woodcock, J., D. Moazed, M. Cannon, J. Davies, and H.F. Noller. 1991. Interaction of antibiotics with A- and P-site-specific bases in 16S ribosomal RNA. *Embo J*. 10:3099–3103.
Woodford, N., and M.J. Ellington. 2007. The emergence of antibiotic resistance by mutation. *Clin Microbiol Infect*. 13:5–18.
Vos, C. 1972. Antibiotic MYC 8003. *Chem Abstr*. 77: 32742.
Vos, C., and P.E.J. Verwiel. 1973. The total structure of the novel antibiotic mocimycin (MYC 8003). *Tetrahedron Letters*. 52:5173–5176.
Wright, G.D. 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv Drug Deliv Rev*. 57:1451–1470.
Wu, J.Y., J.J. Kim, R. Reddy, W.M. Wang, D.Y. Graham, and D.H. Kwon. 2005. Tetracycline-resistant clinical Helicobacter pylori isolates with and without mutations in 16S rRNA-encoding genes. *Antimicrob Agents Chemother*. 49:578–583.
Yamanaka, R. 2004. Alphavirus vectors for cancer gene therapy (review). *Int J Oncol*. 24:919–923.
Yan, F., J.M. LaMarre, R. Rohrich, J. Wiesner, H. Jomaa, A.S. Mankin, and D.G. Fujimori. 2010. RlmN and Cfr are radical SAM enzymes involved in methylation of ribosomal RNA. *J Am Chem Soc*. 132:3953–3964.

Yan, K., L. Madden, A.E. Choudhry, C.S. Voigt, R.A. Copeland, and R.R. Gontarek. 2006. Biochemical characterization of the interactions of the novel pleuromutilin derivative retapamulin with bacterial ribosomes. *Antimicrob Agents Chemother*. 50:3875–3881.

Yang, W., I.F. Moore, K.P. Koteva, D.C. Bareich, D.W. Hughes, and G.D. Wright. 2004. TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. *J Biol Chem*. 279:52346–52352.

Yates, J.D., and P.J. Schaible. 1962. Virginiamycin as an antibiotic for poultry feeds. *Nature*. 194:183–184.

Yong, D., M.A. Toleman, C.G. Giske, H.S. Cho, K. Sundman, K. Lee, and T.R. Walsh. 2009. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob Agents Chemother*. 53:5046–5054.

You, P., F. Xing, J. Huo, B. Wang, J. Di, S. Zeng, and J. Liu. 2013. In vitro and in vivo evaluation of anisomycin against Ehrlich ascites carcinoma. *Oncol Rep*. 29:2227–2236.

SUMMARY IN ESTONIAN

Ribosoomiga seonduvad antibiootikumid ja antibiootikumiresistentsuse mehhanismid

Antibiootikumid on nüüdisaegse meditsiini alustugi ja nende väärtust inimelude päästmisel peetakse mõõtmatuks. Üks tähtsamaid antibiootikumide sihtmärke on prokarüootne 70S ribosoom (Arenz ja Wilson, 2016; Poehlsgaard ja Douthwaite, 2005; McCoy et al., 2011). Antibiootikumide abil on võimalik pärssida erinevaid valgusünteesi etappe (Wilson, 2009). Kuigi antibiootikumide molekulid on ehituse poolest väga erinevad, seonduvad nad suhteliselt väheste ribosoomi piirkondadega. Tetratsükliinid ja aminoglükosiidid seonduvad ribosoomi 30S alaühiku dekodeerivasse keskusesse ning takistavad mRNA poolt kodeeritud geneetilise informatsiooni korrektset tõlgendamist. 50S alaühikul leidub kolm olulist antibiootikumide seondumiskohta (Wilson, 2011). Neist esimene on peptidüültransferaasne keskus. See on seondumiskohaks klooramfenikoolidele, oksasolidinoonidele ja tiamuliinile, mis pärsivad peptiidsideme moodustumist. Teine tähtis 50S alaühikul paiknev antibiootikumide sihtmärk on peptiidi väljumistunnel, kuhu seonduvad makroliidid, linkoosamiidid ja streptogramiin B. Need ravimid takistavad sünteesitava peptiidahela kasvu ribosoomis. Mõned antibiootikumid, näiteks tiostreptoon, seonduvad ribosoomi GTPaasse regiooniga (Harms et al., 2008).

Suur osa valgusünteesi regulatsioonist toimub initsiatsiooni tasemel ja vajab mitmesuguste valkude või RNA molekulide osavõttu (Babitzke et al., 2009; Jackson et al., 2010). Praeguseks on teada, et teatud juhtudel võib valgusüntees olla reguleeritud ka polüpeptiidahela poolt, mida ribosoom parajasti sünteesib. Teatud peptiidide transleerimine võib põhjustada ribosoomi seiskumist ja ühtlasi blokeerida mRNA'l allavoolu paiknevate järjestuste translatsiooni (Lovett ja Rogers, 1996; Wilson, 2016). Mõned ribosoomi seiskumist põhjustavad peptiidid toimivad iseseisvalt, kuid teatud juhtudel vajatakse seisaku toimumiseks väikese ligandmolekuli (antibiootikum, aminohape vms) juuresolekut (Ramu et al., 2009). Üks paremini uuritud looduslikke ligand-sõltuvalt ribosoomi seiskavaid peptiide on ErmCL, mis vajab funktsioneerimiseks erütromütsiini juuresolekut. Kui keskkonnas on erütromütsiin, siis jääb ribosoom ErmCL mRNA'd transleerides seisma ja selle tulemusena saab võimalikuks allavoolu asetseva erütromütsiini resistentsust tagava geeni ekspressioon (Weisblum, 1995b).

Tänaseks on teada mitmeid ribosoomi seisakut põhjustavaid peptiide (Weisblum, 1995b; Lovett ja Rogers, 1996; Gong et al., 2001; Delbecq et al., 2000; Law et al., 2001). Huvitaval kombel pole neid võrreldes suudetud leida ühtset konsensusjärjestust. Samas võib oletada, et teatud reeglid on järjestuste hulgas siiski olemas ja nende tuvastamine on võimalik, kui analüüsida suuremat hulka peptiide. Seetõttu otsustasime töötada välja universaalse meetodi valgusünteesi peatavate peptiide leidmiseks. Spetsiifiliselt soovisime leida järjestusi, mis peataksid ribosoomi kas erütromütsiini, troleandomütsiini, klooramfenikooli, meta-toluaadi või homoseriinlaktooni juuresolekul.

Peptiidide selekteerimiseks konstrueerisime plasmiidse raamatukogu, kus ampitsilliinile resistentsust tagava β-laktamaasi lugemisraami ette oli sisestatud 21 juhuslikku nukleotiidi. Selektsioon põhines eeldusel, et kui ribosoom peatub mRNA'l ligandmolekuli tõttu enne β-laktamaasi transleerimist, siis muutuvad rakud tundlikuks ampitsilliinile. Sellised rakud lüüsiti hiljem ampitsillini kasutades ja nendes sisaldunud plasmiidid koguti sadestamise teel.

Oma meetodit kasutades suutsime identifitseerida järjestused, mis spetsiifiliselt peatasid translatsiooni kõigi viie testitud ühendi juuresolekul. Meil ei õnnestunud peptiidide aminohappeliste järjestuste võrdlemisel tuvastada universaalset konsensusjärjestust. See tulemus pole siiski üllatav, sest ka varasemad uuringud pole konsensusjärjestust avastanud. Siiski märkasime järjestuste võrdlemisel teatud seaduspärasusi. Erütromütsiini juuresolekul selekteeritud järjestused sisaldasid sageli seriini või leutsiini, troleandomütsiini juuresolekul tuvastatud järjestused arginiini ja klooramfenikoolile vastavad järjestused olid rikkad glütsiini poolest (artikkel I, tabel 1). Erütromütsiinile vastavad peptiidid kaldusid olema hüdrofoobsed, mis ühtib teiste autorite tulemustega. Kui me muteerisime peptiide veelgi hüdrofoobsemaks või hüdrofiilsemaks, siis nende aktiivsus vastavalt kasvas või vähenes (artikkel I, joonis 2). Seega vajab erütromütsiini juuresolekul toimuv ribosoomi seisak eelistatult hüdrofoobset kasvavat peptiidi. Me ei välista, et meie meetodit laialdasemalt rakendades ja peptiidide valimit suurendades on võimalik tuvastada konkreetsemaid ribosoomi peatumist põhjustavaid järjestusmotiive.

Teoreetiliselt on võimalik, et meie poolt selekteeritud järjestused ei mõjuta otseselt ribosoomi, vaid hoopis rikuvad ära β-laktamaasi struktuuri ja muudavad seetõttu rakud ampitsilliinile tundlikuks. Et seda võimalust kontrollida, mõõtsime järjestuste aktiivsust erinevates reportersüsteemides. Selleks paigutasime selekteeritud järjestused kas β-galaktosidaasi või GFP lugemisraami ette ja mõõtsime nende valkude ekspressioonitasemeid. Selgus, et ainult erütromütsiini ja klooramfenikooli juuresolekul selekteeritud peptiidid peatasid ribosoomi kõikides reportersüsteemides (artikkel I, joonised 3 ja 4). Me järeldame, et translatsioonilist seisakut põhjustavate peptiidide funktsionaalsus võib olla tugevasti mõjutatud mRNA kontekstist.

Kontrollisime ka võimalust, et meie poolt selekteeritud järjestused ei funktsioneeri mitte peptiidi vaid hoopis mRNA tasemel. Selekteeritud järjestus võiks toimida ribolülitina, sidudes selektsioonil kasutatavaid ühendeid ja pärssides valgusünteesi mRNA sekundaarstruktuuri muutumise kaudu. Selle võimaluse testimiseks asendasime mõned koodonid järjestustes sünonüümsete koodonitega ning mõõtsime nende funktsionaalsust β-galaktosidaasi reportersüsteemis. mRNA järjestuse muutumine rikub suure tõenäosusega võimaliku ligandi seondumiskoha. Ilmnes, et sünonüümsed koodonid ei muutnud järjestuste funktsionaalsust (artikkel I, joonis 1). Seega on meie poolt testitud järjestused aktiivsed peptiididena.

Meie selektsioonimeetodi abil leitud järjestustel võib olla kasutusvõimalusi biotehnoloogia valdkondades. Üheks rakendusvõimaluseks võiksid olla uudsed

ekspressioonisüsteemid, kus geeniekspressioon on kontrollitud ribosoomi, kasvava polüpeptiidi ja spetsiifilise ligandmolekuli vahelise interaktsiooni kaudu.

Käesoleva väitekirja teine publikatsioon on seotud antibiootikumiresistentsuse valdkonnaga. Tavaliseks antibiootikumiresistentsuse tekkemehhanismiks on antibiootikumi märklaua modifitseerimine või mutatsioon, mistõttu ravim ei saa sihtmärgiga seonduda. Sageli sünteesivad bakterid ensüüme, mis modifitseerivad antibiootikumi molekule või lagundavad neid (Wright, 2005). Levinud on ka antibiootikumide rakust eemaldamine nende väljapumpamise teel (Schindler ja Kaatz, 2016; Blair et al., 2014). Sageli paiknevad resistentsust tagavad geenid mobiilsetel elementidel, mistõttu nad saavad horisontaalse geeniülekande teel kiiresti keskkonnas levida.

Üheks unikaalseks nähtuseks on nn ribsoomi kaitsevalkude vahendusel toimuv resistentsusmehhanism, mis tagab resistentsuse tetratsükliinile (Taylor ja Chau, 1996; Connell et al., 2003). Ribosoomi kaitsevalgud on levinud nii Gram-negatiivsete kui ka Gram-positiivsete bakterite hulgas. Juba mõnda aega on teada, et kaitsevalgud on võimelised tetratsükliini seondumist ribosoomile takistama, kuid mehhanismi üksikasjad on hakanud ilmema alles hiljuti tänu kõrglahutus-mikroskoopia abil saadud struktuuride analüüsile (Donhofer et al., 2012).

Meie uuringute eesmärk oli heita valgust ribosoomi kaitsevalgu Tet(O)-vahendatud resistentsusmehhanismile. Töö aluseks oli 9.6 Å lahutavusega 70S ribosoomi ja Tet(O) kompleksi atomaarne mudel. Struktuuri uuringud näitasid, et Tet(O) interakteerub nii väikese kui suure ribosoomi alaühikuga (artikkel II, joonis 3). Tet(O) koosneb viiest struktuursest domäänist. Domään IV tipus eristuvad kolm iseloomulikku lingu (lingud 465, 438 ja 507) (artikkel II, joonis 4a). Struktuuriandmetele tuginedes püstitasime hüpoteesi, et need lingud on olulised tetratsükliini molekuli eemaldamisel ribosoomilt.

Me tegime asendus- ja deletsioonimutatsioone Tet(O) neljanda domääni lingude regioonidesse, mis vastavalt atomaarsele mudelile interakteeruvad otseselt tetratsükliini seondumiskohaga või asuvad selle lähedal. Mutantsete ja metsik-tüüpi Tet(O) variantide analüüs näitas, et kõik kolm lingu on Tet(O) tööks hädavajalikud, sest mutatsioonid YSP507-509G (lingus 507), LGY466-468G (lingus 465) ja VPP436-438G (lingus 438) kaotasid täielikult Tet(O) aktiivsuse. Ühe aminohappe asendused lingudes vähendasid Tet(O) aktiivsust, kuid ei kaotanud seda täielikult (artikkel II, tabel 1).

Struktuursete uuringute ja mutatsioonanalüüsi tulemusi kõrvutades pakkusime välja mudeli Tet(O) vahendusel toimuva tetratsükliiniresistentsuse mehhanismi selgitamiseks. Meie arvates muudab Tet(O) ling 465 tetratsükliini seondumiskoha juures 16S rRNA struktuuri. See nõrgendab interaktsiooni tetratsükliini molekuli ja 16S rRNA vahel ning võimaldab lingul 507 ravim ribosoomilt eemale tõugata. Me oletasime, et ling 438 koos 16S rRNA nukleotiididega moodustab kanali, mille kaudu tetratsükliin ribosoomilt lahkub.

Väitekirja kolmas publikatsioon puudutab probleeme, mis on seotud uute turule ilmuvate antimikroobsete ainetega. Seoses multiresistentsete patogeenide levikuga on väga oluliseks muutunud uute antibiootikumide leidmine ja

kasutusele võtmine. Alates aastast 2000 on turule ilmunud 30 uut antibiootikumi (Butler et al., 2017). Paljud ravimikandidaadid on sisenenud kliinilistesse katsetesse, kuid nende arendamine on erinevatel põhjustel pooleli jäänud. Samas leidub ka antimikroobseid aineid, mida kasutatakse meditsiinis, kuigi nende toimemehhanism pole veel selge. Üheks selliseks näiteks on nitrovinüülfuraanide hulka kuuluv aine G1 ehk Furvina®, mida juba mõnda aega kasutatakse Kuubal nahainfektsioonide ravis.

On teada, et G1 laguneb vees ja tioolrühmi sisaldavate ühendite (näiteks tsüsteiini) olemasolu keskkonnas kiirendab seda protsessi. Me iseloomustasime G1 lagunemist spektrofotomeetriliselt, kasutades G1 neeldumisspektri maksimumi (390 nm) muutumist G1 lagunemisel. Tsüsteiinhüdrokloriidi lisamisel G1 lahusele toimus neeldumisspektri maksimumi kiire nihkumine ultravioleti suunas (artikkel III, joonis 2a). Reaktsiooni kiirus vähenes koos lahuse pH vähenemisega (artikkel III, joonis 2c). G1 oli stabiilsem tsüsteiini mittesisaldavas M9 minimaalsöötmes (poolestusaeg 58 minutit), kuid ühend lagunes kiiremini tioole sisaldavates CAMHB ja IMDM söötmetes (poolestusajad vastavalt 11 ja 6 minutit) (artikkel III, joonis S1a lisamaterjalides). G1 lagunemise reaktsiooniskeem koos selle käigus tekkivate produktidega (artikkel III, joonis 3) selgitati välja tuumamagnetresonantsi ja kõrgefektiivse vedelikukromatograafia abil.

On teada, et vähemalt mõned G1 lagunemise käigus tekkivatest produktidest säilitavad antimikroobse aktiivsuse (Scholz et al., 2013). Standardsed testid, näiteks tavaline MIC määramine, ei võimalda kindlaks teha, milline osa antimikroobsest aktiivsusest tuleneb lagunemata ainest ja milline osa on põhjustatud laguproduktidest. Selleks, et teha vahet G1 ja selle laguproduktide põhjustatud antimikroobsetel efektidel, inkubeerisime uuritavat ainet enne MIC määramist vedelsöötmes. Katsealusteks organismideks olid *E. coli* tüved BW25113 ja CFT073 ning pärm *Candida albicans*. Nagu võis ennustada, olid MIC väärtused tsüsteiinivabas söötmes väiksemad kui tioolrühmi sisaldavas söötmes (artikkel III, tabel 1). Kui lisasime söötmetele G1 lagunemist soodustavat tsüsteiinhüdrokloriidi (lõppkontsentratsioon 100 μg/ml), suurenesid MIC väärtused. Samas jäid G1 antimikroobsed omadused alles isegi pärast kahe tunni pikkust testile eelnenud inkubatsiooniaega. Me järeldame, et G1 aktiivsus on aine kohese reaktiivsuse ja laguproduktide antibakteriaalsete aktiivsuste summa.

Soovides iseloomustada G1 toksilist mõju jagunevatele *E. coli* rakkudele, viisime läbi inhibitsioonikatse erinevate G1 kontsentratsioonidega. Leidsime, et G1 kontsentratsioon 8 μg/ml pidurdab bakterite kasvu, kuid ei peata seda täielikult. G1 kontsentratsioon 16 μg/ml mõjus rakkudele bakteriostaatiliselt ja kõrgemad kontsentratsioonid olid jagunevatele bakteritele bakteritsiidsed (artikkel III, joonised 4a ja 4b). Enamik antibiootikume takistavad vaid jagunevate bakterirakkude elutegevust ning on väheefektiivsed mittejagunevate rakkude vastu. Seetõttu uurisime G1 mõju statsionaarses faasis olevale *E. coli* rakukultuurile. Ilmnes, et mittejagunevate bakterite surmamine G1 abil on võimalik, kuid selleks on vajalik kõrge G1 kontsentratsioon (80 μg/ml) ja vähemalt 100-kordne bakterikultuuri lahjendamine (10^7 CFU/ml) (artikkel III, joonis 5). Seetõttu ei ole G1 efektiivne vahend mittejagunevate bakterite hävitamiseks.

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PUBLICATIONS

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Work

Since 2000	free-lance DJ and writer
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2004–2005	Tartu Health Care College, teacher
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My research field has been related to mechanisms of prokaryotic translation and antibacterials that inhibit its functionality. I've been investigating ribosome biogenesis, rRNA maturation and tRNA aminoacylation processes in Prof. Jaanus Remme's lab. In Prof. Tanel Tenson's lab I've been focused on the study of protein synthesis inhibitors and antibiotic resistance mechanisms

List of scientific publications

Allas Ü, Toom L, Selyutina A, Mäeorg U, Medina R, Merits A, Rinken A, Hauryliuk V, Kaldalu N, Tenson T. (2016). Antibacterial activity of the nitrovinylfuran G1 (Furvina) and its conversion products. Sci Rep. 6:36844.

Li W, Atkinson GC, Thakor NS, **Allas Ü**, Lu CC, Chan KY, Tenson T, Schulten K, Wilson KS, Hauryliuk V, Frank J. (2013). Mechanism of tetracycline resistance by ribosomal protection protein Tet(O). Nat Commun 4: 1477.

Allas Ü, Tenson T. (2010). A method for selecting cis-acting regulatory sequences that respond to small molecule effectors. BMC Mol Biol 11: 56.

Allas Ü, Liiv A, Remme J. (2003). Functional interaction between RNase III and the Escherichia coli ribosome. BMC Mol Biol 4: 8.

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Uurimisvaldkond on olnud prokarüootse valgusünteesi mehhanismid ja antibiootikumid, mis mõjutavad selle funktsionaalsust. Professor Jaanus Remme laboris olen tegelenud ribosoomide biogeneesi, rRNA küpsemise ja tRNA aminoatsüleerimise uuringutega. Professor Tanel Tensoni laboris olen keskendunud valgusünteesi inhibiitorite ja antibiootikumresistentsuse mehhanismide uuringutele.

Teaduspublikatsioonid

Allas Ü, Toom L, Selyutina A, Mäeorg U, Medina R, Merits A, Rinken A, Hauryliuk V, Kaldalu N, Tenson T. (2016). Antibacterial activity of the nitrovinylfuran G1 (Furvina) and its conversion products. Sci Rep. 6:36844.

Li W, Atkinson GC, Thakor NS, **Allas Ü**, Lu CC, Chan KY, Tenson T, Schulten K, Wilson KS, Hauryliuk V, Frank J. (2013). Mechanism of tetracycline resistance by ribosomal protection protein Tet(O). Nat Commun 4: 1477.

Allas Ü, Tenson T. (2010). A method for selecting cis-acting regulatory sequences that respond to small molecule effectors. BMC Mol Biol 11: 56.

Allas Ü, Liiv A, Remme J. (2003). Functional interaction between RNase III and the Escherichia coli ribosome. BMC Mol Biol 4: 8.

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