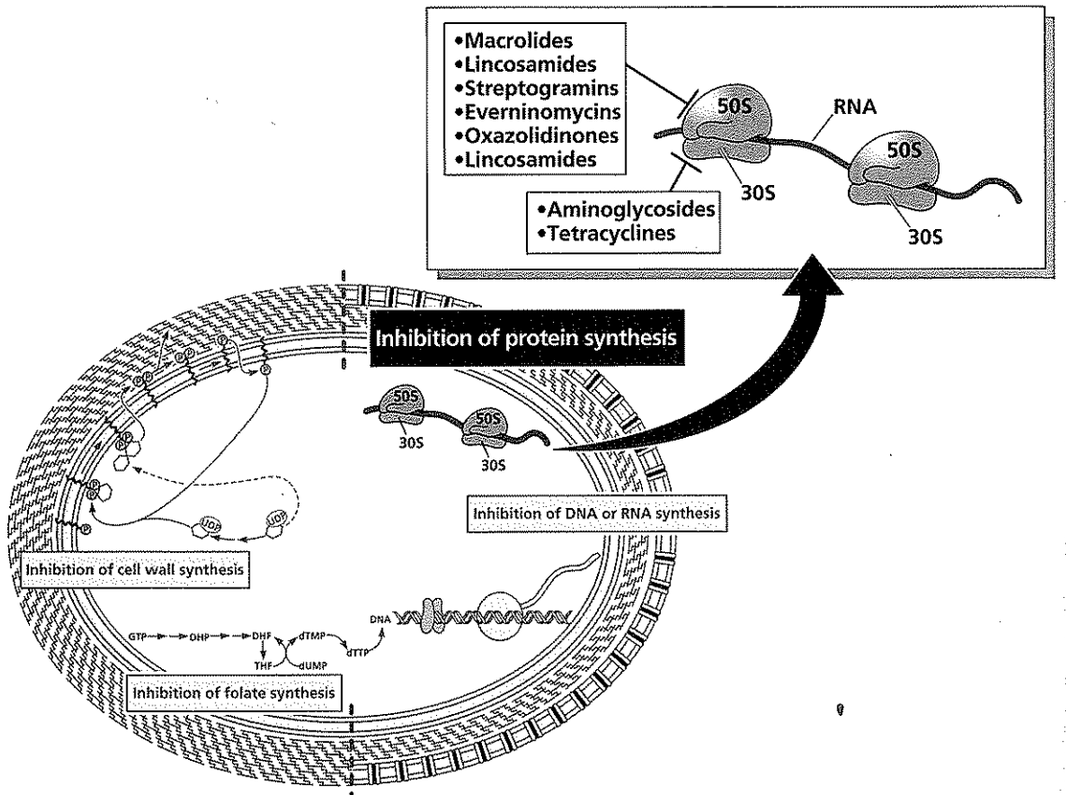


Antibiotics that block bacterial protein biosynthesis.

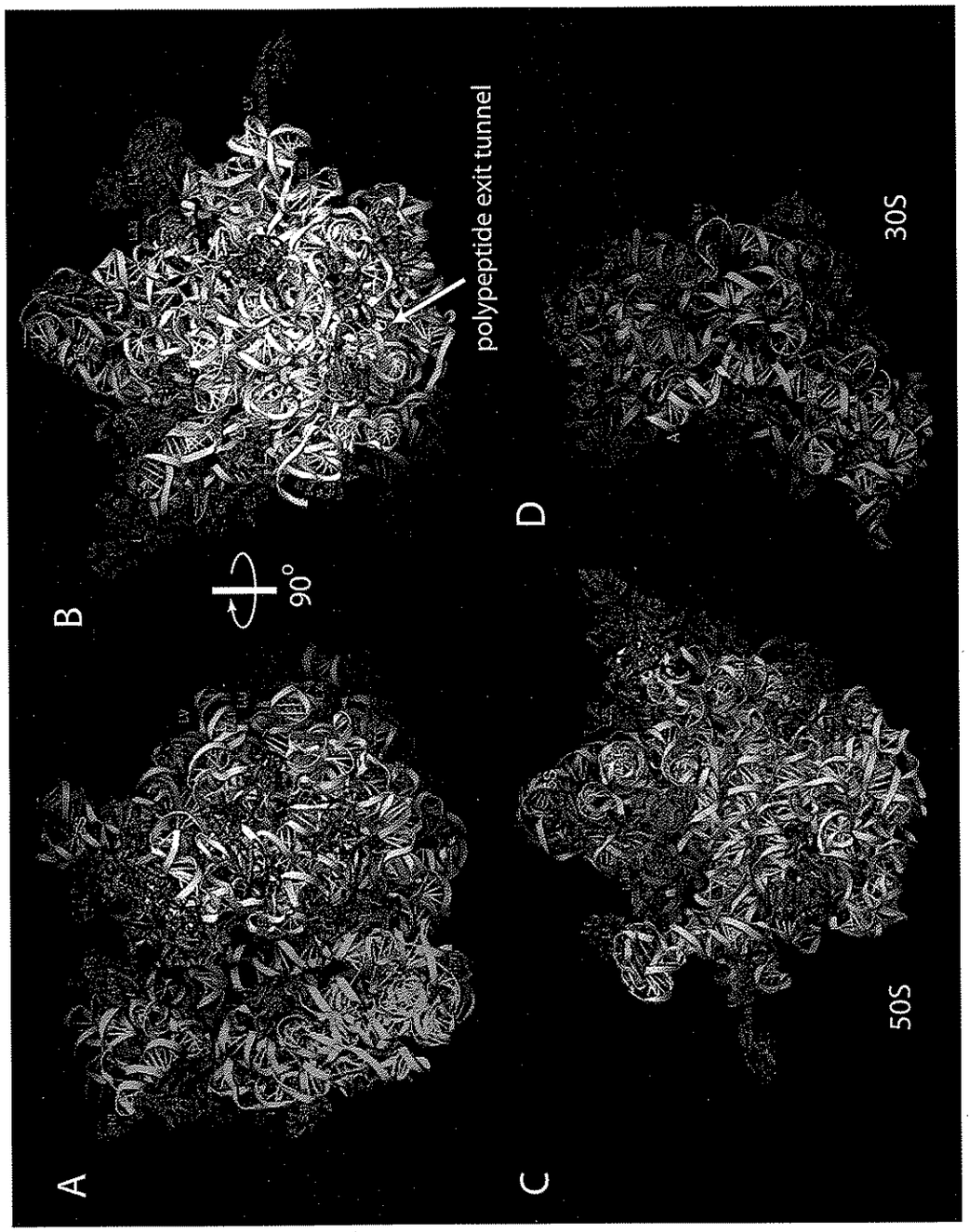
Antibiotics That Block Bacterial Protein Biosynthesis

This chapter deals with the various classes of antibiotics that exert their bacteriostatic or bactericidal action by blockade of one or more of the protein biosynthetic steps that occur on the 30S and 50S subunits of the bacterial ribosome. The figure on the facing page shows a blowup of the relevant portions of Fig. 2.2, emphasizing that some antibiotics block processes at the 50S ribosome and others act at the 30S ribosome.

Bacterial ribosome structure and the peptidyltransferase cycle

Given the centrality of protein biosynthesis to cellular function and the large number of steps involved, from activation of the 20 proteinogenic amino acid monomers by the aminoacyl-tRNA synthetases, to the many steps in chain initiation, chain elongation, and chain termination of the growing polypeptides on the ribosome, it is natural that many natural product antibiotics target one or more steps in protein biosynthesis. Before analyzing the sites and mechanism of action of ribosome-inhibiting antibiotics, a short summary on the ribosome is presented.

In bacteria the ribosome is a two-subunit nucleoprotein particle, about two-thirds RNA and one-third protein, of molecular weight 2.5 to 2.6 MDa. The small subunit, 30S, contains about 20 proteins and a 16S ribosomal rRNA of about 1,500 ribonucleotides. Typically the large, 50S subunit has about 30 proteins, a 23S rRNA of about 2,900 ribonucleotides, and a 5S rRNA (122 nucleotides). The two large rRNAs, with about 4,500 nucleotides, are both scaffold and catalyst for peptide bond formation. The X-ray structure of the 70S ribosome from *Thermus thermophilus* has been reported at a resolution of 5.5 Å, sufficient to reveal the architecture of both the 30S and 50S subunits and their interactions (Yusupov et al., 2001) (**Color Plate 4.1**). **Color Plate 4.1A** shows the 30S subunit on the left, the 50S on the right, and an aminoacyl-tRNA in the interface. A 90° rotation in **Color Plate 4.1B** shows the view from the back of



the 50S subunit with the exit tunnel for the nascent polypeptide chain indicated. **Color Plate 4.1C** gives an interface view of the 50S subunit with three tRNAs, occupying the P, A, and E sites (see below), and **Color Plate 4.1D** shows the corresponding interface view of the 30S subunit.

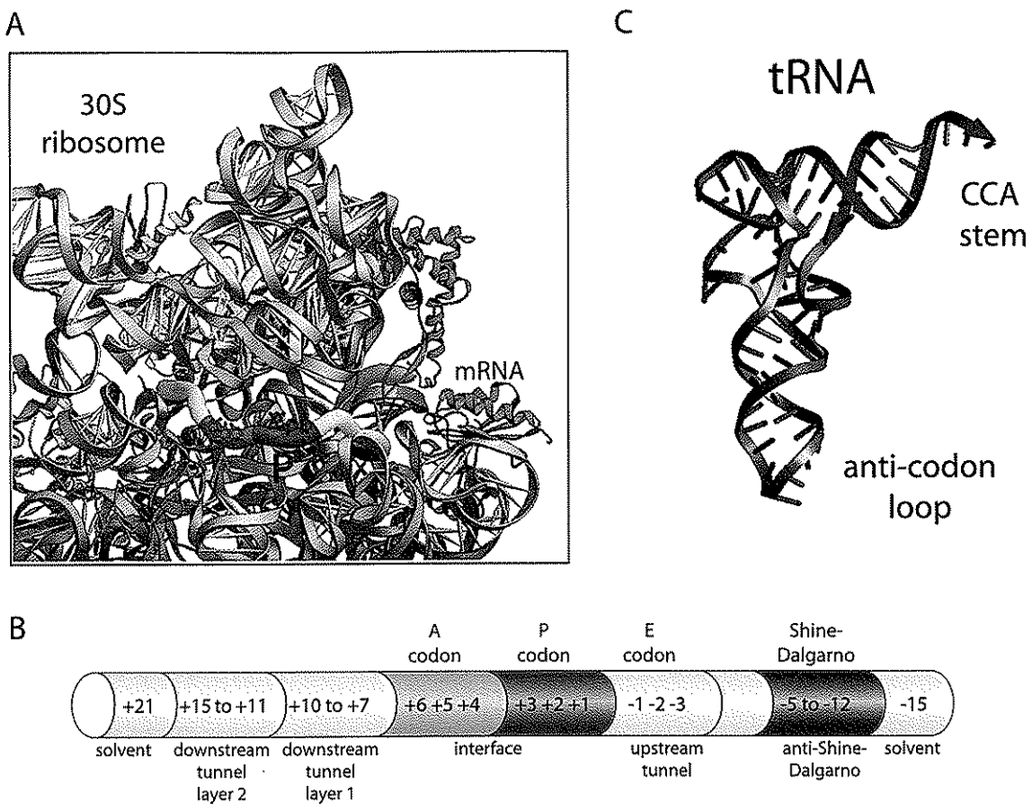
The X-ray structure of the 70S ribosome complements recent structures of the 30S subunit from the same organism (Wimberly et al., 2000), refined to 3 Å resolution, and the structure of the 50S subunit from *Haloarcula marismortui* (Nissen et al., 2000). The ensemble of structures has opened a new chapter in the study of ribosomes as protein-synthesizing machines and the mechanisms of blockade by antibiotics.

In addition to the 16S and 23S rRNA molecules that are crucial structural, recognition, and catalytic elements of the ribosome, two other RNA molecules, mRNA and tRNA, are required for protein synthesis. The mRNA provides the instructional template, and its path through the ribosome has recently been visualized by X-ray analysis (see reviews in Culver, 2001, and Yusupova et al., 2001). mRNA threads through two tunnels in the 30S subunit, with only a short stretch protruding through to the interface between the 30S and 50S subunits (**Color Plate 4.2A**). That stretch of mRNA contains the six nucleotides that comprise the aminoacyl (A) and peptidyl (P) codons (**Color Plate 4.2B**), nucleotides +6 to +1. The exit site codon (E), at −1 to −3 bases, is in the upstream tunnel just in front of the −5 to −12 region, the Shine-Dalgarno sequence, that base pairs with the anti-Shine-Dalgarno sequence at the 3′ end of the 16S rRNA to form the double helix that sets the register for mRNA translation. The tRNA molecules transfer the amino acids to the ribosome and provide the anticodon trinucleotides for Watson Crick base pairing at the A and P sites at the 30S-mRNA interaction site.

The aminoacylated end of the P and A tRNAs reach away from the 30S subunit into the 50S subunit at domain V of the 23S rRNA. The peptidyl chain is translocated onto the aminoacyl-tRNA in the A site by the peptidyltransferase activity in each peptide-chain-elongation cycle of the ribosome. The peptidyltransferase activity derives from the catalytic ribozyme activity of this portion of the 23S rRNA with no obvious assistance from proteins (see Nissen et al., 2000) (**Fig. 4.1**).

In each catalytic cycle of elongation, the 30S subunit functions as a decoding unit to select the proper aminoacyl-tRNA with its anticodon that will fit the A-site codon. Once the correct aminoacyl-tRNA is docked in the A site, the aminoacyl moiety tethered 75 Å away at the CCA end of that tRNA is oriented into a productive conformation to attack the adjacent peptidyl chain, itself oriented at the CCA end of its tRNA, elongating the growing peptidyl chain as it is translocated onto the attacking aminoacyl group. At this juncture this is an

Color Plate 4.1 Interaction of 30S and 50S subunits and location of tRNAs in the A site, P site, and E site. (A) The *T. thermophilus* ribosome 30S subunit is shown on the left, the 50S subunit is at right. (B) 90° rotation from (A) shows the back of the 50S subunit and the location of the exit tunnel for the nascent polypeptide chain. (C) View of the 50S subunit from the 50S/30S subunit interface with tRNAs in P, A, and E sites. (D) View of the 30S subunit interface. (From Yusupov et al. [2001] with permission.)



Color Plate 4.2 (A) Threading of the mRNA into the 30S subunit decoding region; (B) placement of the A, P, and E codons of the mRNA in the decoding site; (C) architecture of a tRNA highlighting the anti-codon loop that recognizes the codons on mRNA and the CCA tail where the amino acid is covalently tethered and activated. (From Culver [2001] with permission.)

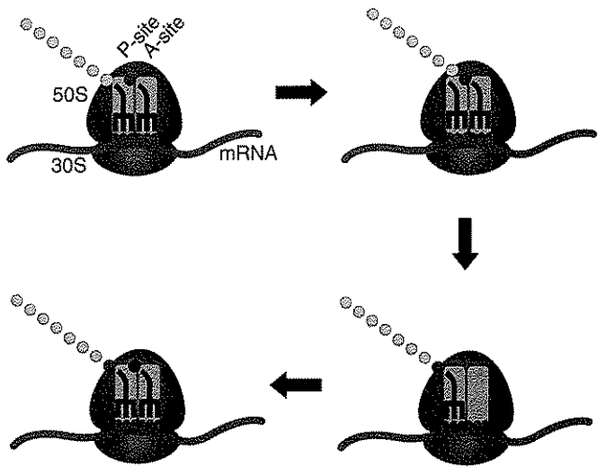


Figure 4.1 Schematic of peptide bond formation at the ribosome.

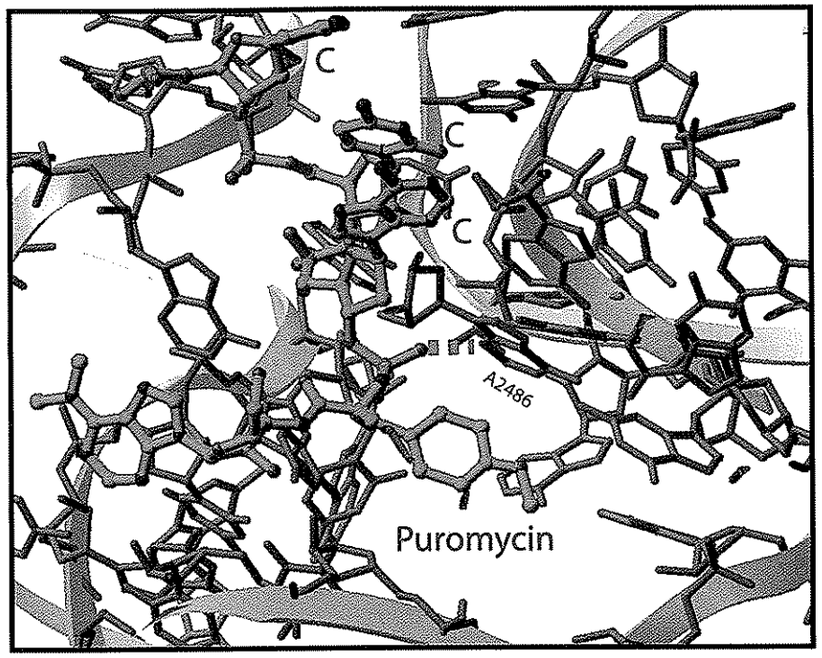
empty, deacylated tRNA in the P site (**Fig. 4.1**) and the peptidyl group is tethered to a tRNA still docked in the A site. For the next round of elongation, the deacylated tRNA moves to the E site, the peptidyl-tRNA relocates to the P site, and the A site becomes open for the next aminoacyl-tRNA to be brought in and decoded by the 30S-mRNA complex. The orchestration of the movement of mRNA, to present a new triplet codon at the A site in the interface between 30S and 50S to allow decoding, and of the three tRNAs, to shuttle between the E, P, and A sites, is not yet understood.

The peptidyltransferase center in the 50S subunit has been defined in domain V of the 23S rRNA by cocrystallization of a transition-state analog, a peptidyl puromycin phophonamidate (**Color Plate 4.3**) that mimics the tetrahedral geometry of the intermediate in peptidyltransferase RNA catalysis. This allows definition of P-site and A-site rRNA nucleotides in relation to the bound analog and has suggested mechanisms for functions of individual RNA bases in the peptide bond-forming steps (Nissen et al., 2000). It has also facilitated definition of the polypeptide exit tunnel, about 100 Å long, that allows passage of the growing polypeptide chain through the 50S subunit to the outside (**Color Plate 4.4**).

It is clear that the low observed error rates in protein synthesis, about one mistake in 10^4 elongation cycles, requires proofreading and editing for maintenance of this high fidelity during amino acid incorporation at the ribosome (see Rodnina and Wintermeyer, 2001, for review). Evidence has accrued over the years for a multistep discrimination to distinguish between the cognate aminoacyl-tRNA in a sea of noncognate aminoacyl-tRNAs. The aminoacyl-tRNAs are brought to the ribosome in complex with a chaperone protein, EF-Tu, which is a latent GTPase. After initial binding to the ribosome, the aminoacyl-tRNA can either dissociate or proceed to base pair in the anticodon-codon helix interaction, leading to reorientation and a longer-lived complex (**Fig. 4.2**). At this point, the GTPase activity of EF-Tu is activated, presumably by conformational change, and GTP cleavage to bound GDP and P_i occurs, followed by product P_i release. The GDP stays bound, promoting a specific conformation of EF-Tu.GDP. When peptide bond formation occurs over in the peptidyltransferase center of the 50S subunit, the now deacylated tRNA is released from the A site by the GDP form of EF-Tu. It is presumed that local structures of rRNA in the 30S and 50S subunits are relaying signals that decoding information has occurred and that the aminoacyl end of the aminacyl-tRNA should orient productively for peptide bond formation.

Antibiotics could interrupt the timing and specificity of any of these steps, and such disruptions are likely to slow down growth and/or be lethal to the bacteria. **Figure 4.3** shows examples of major classes of antibiotics targeted to the 30S subunit (spectinomycin, the aminoglycosides kanamycin and streptomycin, tetracycline) or to the 50S subunit (clindamycin, chloramphenicol, linezolid, and macrolides such as erythromycin, clarithromycin, azithromycin, and tylosin). Structures of antibiotics bound to rRNA target sites in the 30S and 50S subunits have appeared in the past two years (e.g., Carter et al., 2000; Schlunzen et al., 2001). For example, cocrystallization of the three antibiotics paromomycin, spectinomycin, and streptomycin with the target 30S subunits indicated alteration of delicate balances between rRNA conformational states, causing disruption

A



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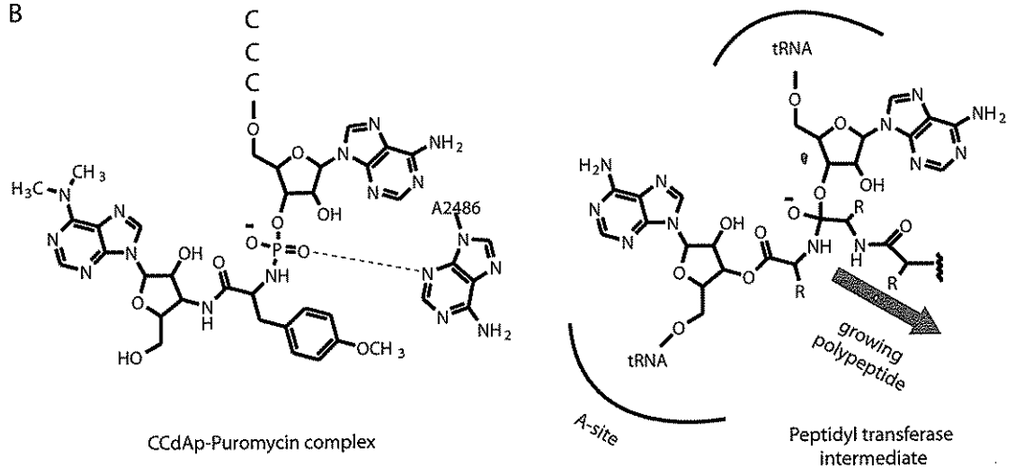
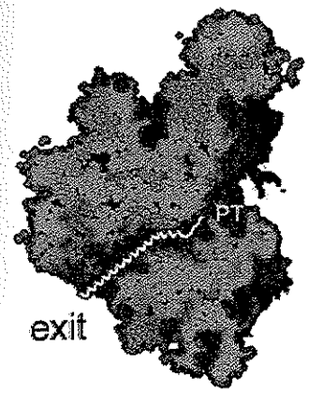


Color Plate 4.3 The peptidyl transferase center on the 50S ribosomal subunit. (A) Docking of the CCdAp-puromycin complex at the peptidyl transferase center of the 50S subunit; (B) two-dimensional projection of the interaction of CCdAp-puromycin with A_{2486} and analogous geometry of the tetrahedral intermediate during peptide bond formation at the same site on the ribosome.



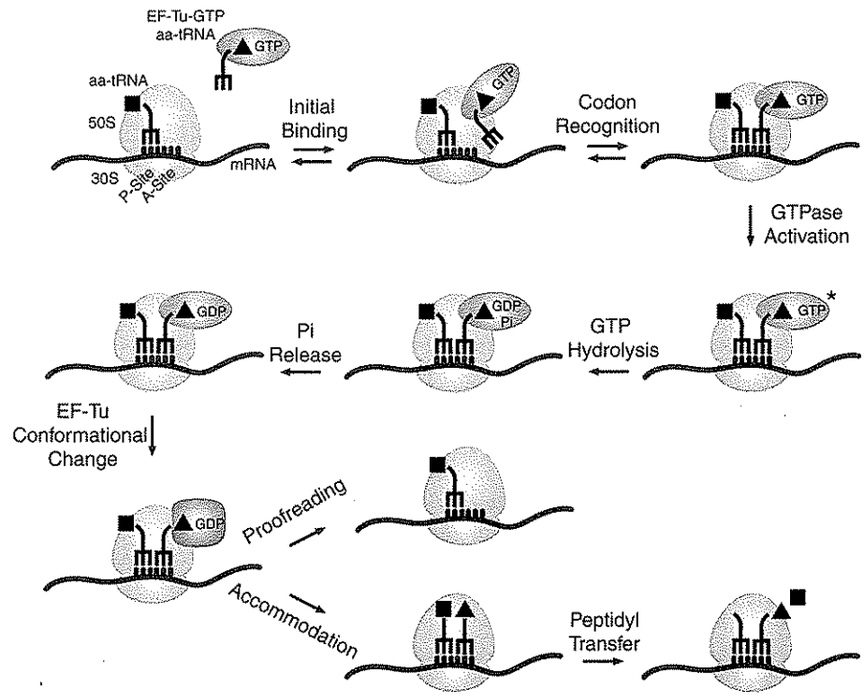
Color Plate 4.4 The polypeptide exit tunnel through the 50S ribosome.

of translocation (spectinomycin), decoding (paromomycin), and translational accuracy (streptomycin) (Carter et al., 2000). This work is likely to be a harbinger of many studies to come that pinpoint the action of antibiotics on one or more of the constituent steps in ribosome function.

The erythromycin class of macrolide antibiotics

Erythromycin is a 14-membered macrocyclic lactone produced by the streptomycete *Saccharopolyspora erythraea*. The aglycone arises from a modular poly-

Figure 4.2 Steps in binding, codon recognition, GTPase activation, proofreading, and peptidyl transfer in peptide bond formation. (Modified from Rodnina and Wintermeyer [2001].)



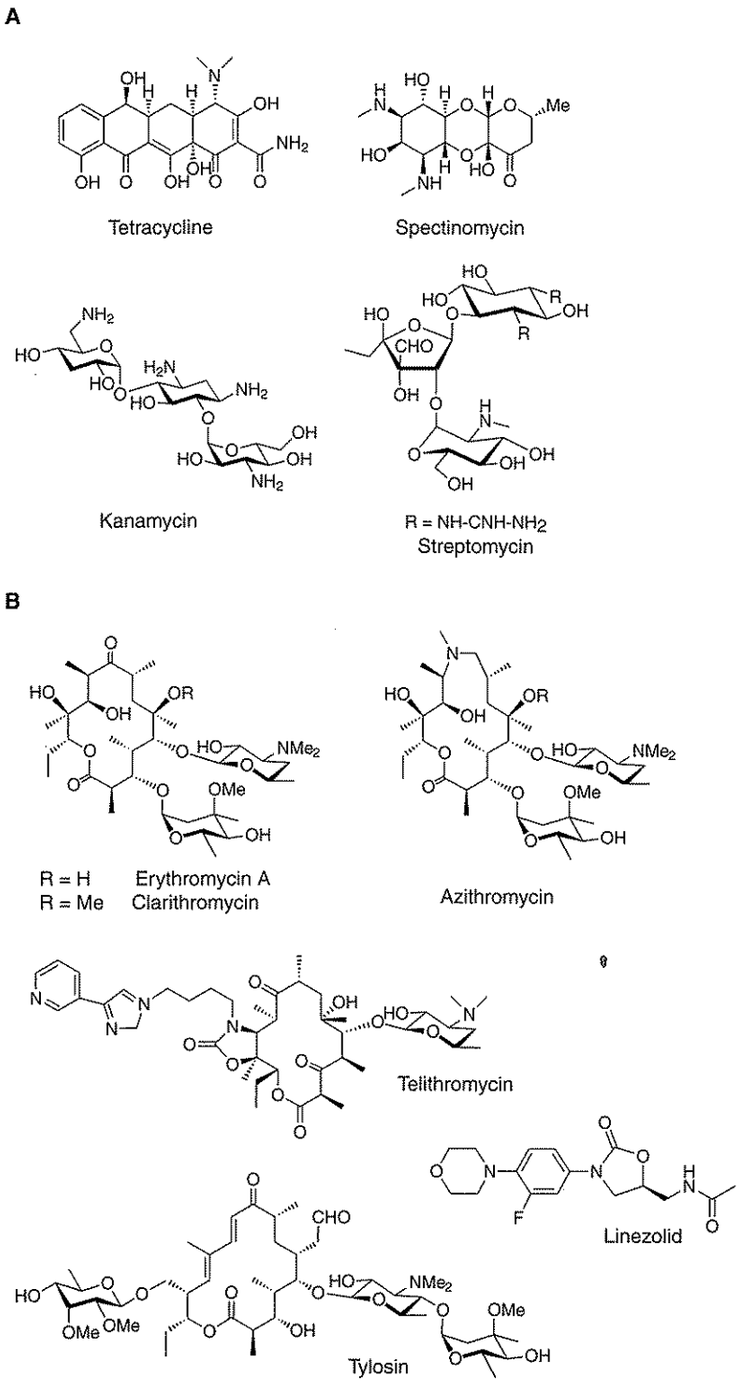


Figure 4.3 Structures of some antibiotics that act at (A) the 30S subunit or (B) the 50S subunit of bacterial ribosomes.

ketide synthase assembly line, as will be examined in chapter 12, and is then bis oxygenated and bis glycosylated to produce the active antibiotic; 15-membered semisynthetic (azithromycin) and 16-membered naturally occurring macrolides such as tylosin are also active (Fig. 4.3). The architecture of the macrolactone and the interactions of the sugars are key determinants of binding and specificity for interaction with the 23S rRNA in the six-nucleotide region 2058-2062. Erythromycin binding blocks polypeptide translation with the net effect of release of peptidyl-tRNA intermediates prematurely, by blocking the approach to the elongating peptide's exit tunnel (Ban et al., 2000; Schlunzen et al., 2001). The drug also blocks assembly of 50S subunits, presumably through this interaction with 23S rRNA. The 50% inhibitory concentartion (IC_{50}) for translation inhibition in *Staphylococcus aureus* cells is about 0.2 μg/ml (Goldman et al., 1990) and the K_d for stoichiometric binding to the 23S rRNA component of the 50S subunit is about 1 nM. Erythromycin has proven safe and effective in adults and children and has been used in both inpatient and outpatient settings.

Expanded-spectrum macrolides such as azithromycin and clarithromycin (Fig. 4.3) fill an important therapeutic niche for treatment of respiratory infections (Scholar and Pratt, 2000). They are semisynthetic molecules—clarithromycin with a methoxy at C_6 and azithromycin with an expanded, 15-membered macrolide and an inserted nitrogen—that have an altered macrolide conformation. Azithromycin and clarithromycin have IC_{50} values about equivalent to that of erythromycin (see Champness, 2000, for review), cause less gastrointestinal tract irritation, are more stable to the acid pH of the stomach, have better tissue penetration, and have longer half-lives, allowing once- or twice-daily dosing. In the narrow- and expanded-spectrum macrolides the cladinose sugar is required for antibiotic activity.

Broad-spectrum macrolides, with the 3-OH oxidized to a ketone, removing the site of attachment of the cladinose sugar, known as ketolides (e.g., telithromycin) (Bronson and Barrett, 2001a), are in late stages of clinical development, and telithromycin has recently been approved in the United States. Such ketolides show about a 1-log improvement in IC_{50} values (0.02 to 0.04 μg/ml) (Capobianco et al., 2000; Douthwaite et al., 2000) and do not induce rRNA methylation resistance gene expression (see chapter 10). The successive generations of macrolides have been optimized for altered properties including acid stability in the stomach and activity against macrolide-resistant pathogens (chapters 9 and 10). All three generations of macrolides must take advantage of architectural differences in the 23S RNA of bacterial ribosomes versus their eukaryotic counterparts to provide selectivity for killing of the bacteria. The recent X-ray analysis of macrolide antibiotics bound to bacterial ribosomes (see below) gives some insight into this selectivity. Members of this class of drugs have also been heavily investigated as targets for diversity generation by combinatorial biosynthesis, as will be discussed in chapter 15.

Tylosin, a 16-membered macrolide with a macrolactone two carbons larger than that of erythromycin and distinct sugars, targets the 23S rRNA at essentially the same site and is used in veterinary medicine. Kinetic analysis of both tylosin and erythromycin binding to the ribosome suggest an initial collisonal complex followed by a slow isomerization step, resulting in tightened binding and slow dissociation. Return from the isomerized RI* complex can be very slow. For

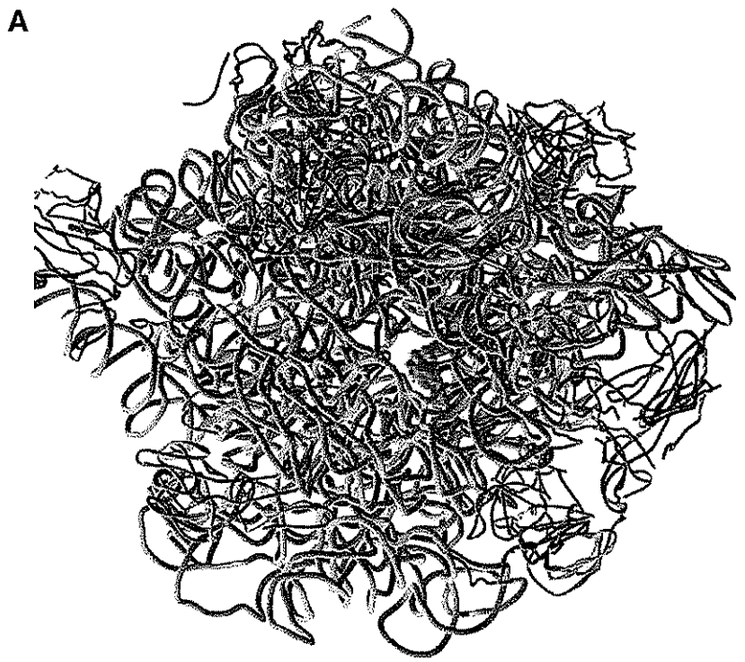
tylosin as inhibitor I, the ribosomeI* complex accumulates over the collisional RI by 600/1.

$$\text{Ribosome} + \text{I} \leftrightarrow \text{Ribosome·I} \leftrightarrow \text{Ribosome·I*}$$

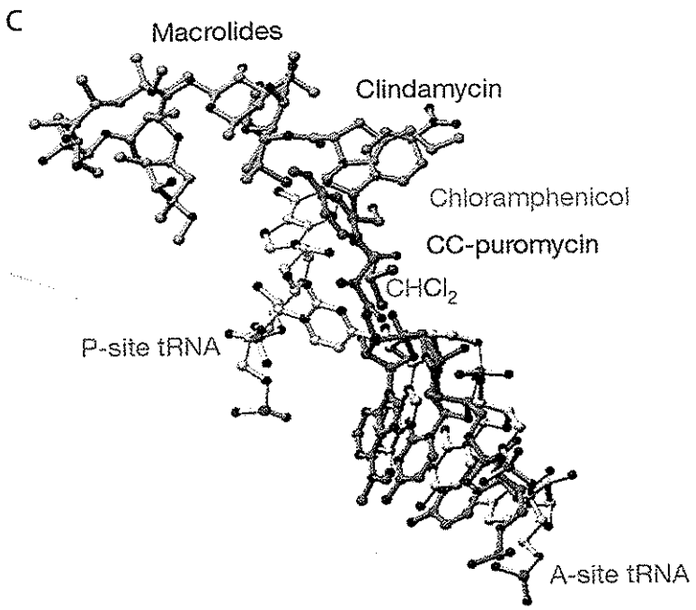
For the ribosome-erythromycin* complex the ratio is 10/1, indicating longer-lived inhibition for the tylosin complex (Dinos and Kalpaxis, 2000). In direct assays of peptidyltransferase activity, erythromycin does not block activity while tylosin does. Footprinting analysis had indicated that erythromycin binds adjacent to the peptidyltransferase center of the 50S and blocks passage of the nascent peptidyl chain into the exit tunnel through the 50S subunit. This has now been directly validated by X-ray analysis of macrolides bound to the 23S rRNA in the 50S subunit of the bacterium *Deinococcus radiodurans* (Schlunzen et al., 2001).

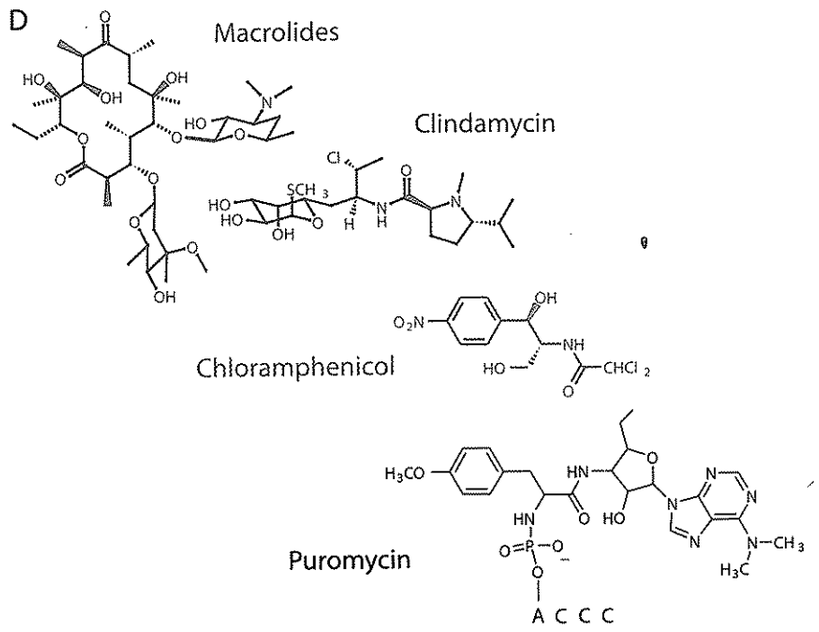
Erythromycin and the expanded-spectrum 14-membered macrolide antibiotics clarithromycin and roxithromycin all bind at the entrance to the polypeptide export tunnel (**Color Plate 4.5A**), allowing about a six- to eight-oligopeptidyl-tRNA buildup before elongation is blocked and prematurely terminated. The narrow- and expanded-spectrum macrolides have three structural elements—macrolactone, desosamine, and cladinose sugars—and this ensemble makes up to seven hydrogen bonds with 23S rRNA. No ribosomal proteins are in molecular contact with the macrolide antibiotics. The 2′-OH of the desosamine makes hydrogen bonds to N_1 and N_6 of A_{2058} in 23S RNA (**Color Plate 4.5B**), explaining the key requirement for A_{2058} for susceptibility to macrolides (Vester and Douthwaite, 2001). In eukaryotes A_{2058} is changed to G_{2058}, explaining at least part of the target selectivity of the erythromycin class of drugs to bacterial ribosomes (Schlunzen et al., 2001). The 6-OH, 11-OH, and 12-OH substituents on the macrolactone may also make hydrogen bond contacts to 23S RNA for orientation of the antibiotic. The cladinose ring makes no crucial interactions and is replaced in the broad-spectrum ketolides with retention and even gain of activity.

Although the macrolides do not directly block the peptide bond-forming step at the peptidyltransferase center of the 50S subunits, it has been known that they are competitive with lincosamide antibiotics that are direct peptidyltransferase inhibitors. Indeed, a single mutation at A_{2058}, to any of the other three bases (G, C, or U), induces a tripartite resistance to macrolides, lincosamides, and streptogramin B family members (MLS_B resistance), suggesting physical overlap (see Vester and Douthwaite, 2001, for review). Schlunzen et al. (2001) have provided direct validation with the cocrystal structure of the lincosamide antibiotic clindamycin (**Color Plate 4.5C and 4.5D**), in which the 2′- and 3′-OH groups of the sugar moiety of the antibiotic form hydrogen bonds to the same exocyclic N_6 amino group of A_{2058}. An overlay of clindamycin binding and erythromycin binding shows partial physical overlap (Color Plate 4.5C). Clindamycin has separately been known to interact with both the A site and the P site of the peptidyltransferase center; thus a model building of the 3′ ends of the A- and P-tRNAs produces the composite in Color Plate 4.5C, showing the placement of clindamycin and erythromycin relative to the two tRNAs which position the peptidyl donor and aminoacyl acceptor in peptide bond formation that is the core reaction of the ribosome. Finally, the antibiotic chloramphenicol, now in restricted usage due to toxicity concerns, has also been cocrystallized



Color Plate 4.5 Mode of action of macrolide antibiotics: (A) binding of macrolides at the 50S polypeptide exit tunnel; (B) interaction with the 23S RNA bases; (C) overlap with the binding sites for clindamycin and chloramphenicol as well as the A-site and P-site tRNAs; (D) inventory of the molecules that are overlapped in panel C. In direct assays of peptidyl transferase activity, erythromycin does not block activity. (From Schlunzen et al. [2001] with permission.)





Color Plate 4.5 (*continued*)

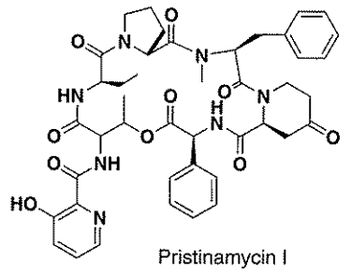
with the *D. radiodurans* 50S subunit by this same research team (Schlunzen et al., 2001). It is known that chloramphenicol blocks aminoacyl-tRNA interaction with the A site of the peptidyltransferase center, and that is indeed where chloramphenicol is bound.

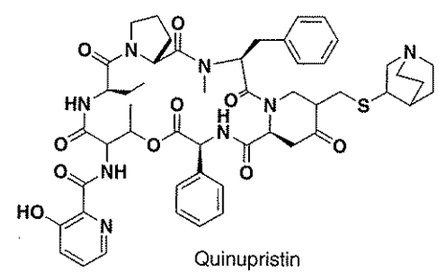
The location of five antibiotics in the cavity of the peptidyltransferase center of the bacterial 50S subunit of the ribosome will certainly enable new efforts in rational design of antibacterials targeted at protein synthesis.

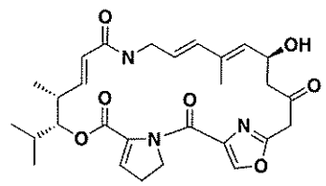
Synergistic nonribosomal peptide combinations: Synercid

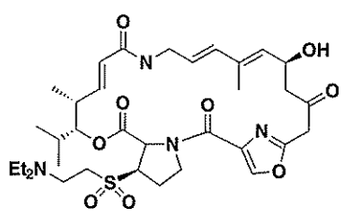
A large variety of streptomycetes and actinoplanes (Champness, 2000) make a pair of antibiotics of the virginiamycin (also called pristinamycin and streptogramin) family, termed group A and group B (Barriere et al., 1998) or, alternatively, group I and II, that work synergistically to block polypeptide translation by the 50S subunit of bacterial ribosomes at 23S rRNA sites partially overlapping those targeted by the macrolides. We will use the term pristinamycins for the therapeutic pair recently approved as Synercid (Livermore, 2000) (**Fig. 4.4**) and the alternate generic term virginiamycins in chapter 11 when discussing regulation of the timing of antibiotic synthesis. The group I pristinamycins are nonribosomal cyclic peptidolactones with the side chain alcohol of an *N*-aryl-Thr_1 connected to the carbonyl of $PheGly_6$. The group II pristinamycins are polyketide/polypeptide hybrids with an oxazole-pro (derived from a ser-pro dipeptide precursor) moiety embedded in a polyketide lactone backbone (chapter

Figure 4.4 Structures of the pristinamycin I (quinuprustin) and pristinamycin IIA (dalfopristin) components of the peptide antibiotic Synercid.









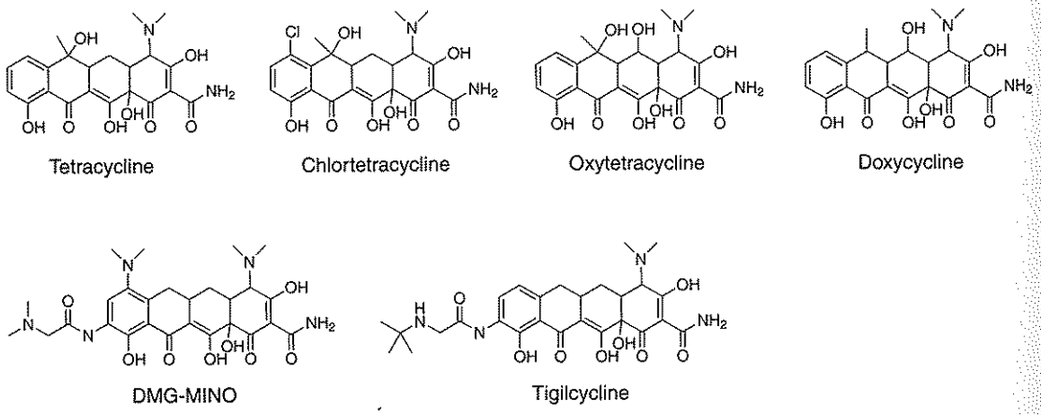
13). The particular pristinamycins I and II of the Synercid combination are semisynthetic versions, with a thioether substitution on the 4-oxopipecolyl residue of the quinupristin and a diethylaminoethylsulfone substituent on the prolyl ring of the dalfopristin component (Fig. 4.4). The modifications improve water solubility and have allowed clinical approval for the significant indication of treatment of vancomycin-resistant enterococcal (VRE) infections.

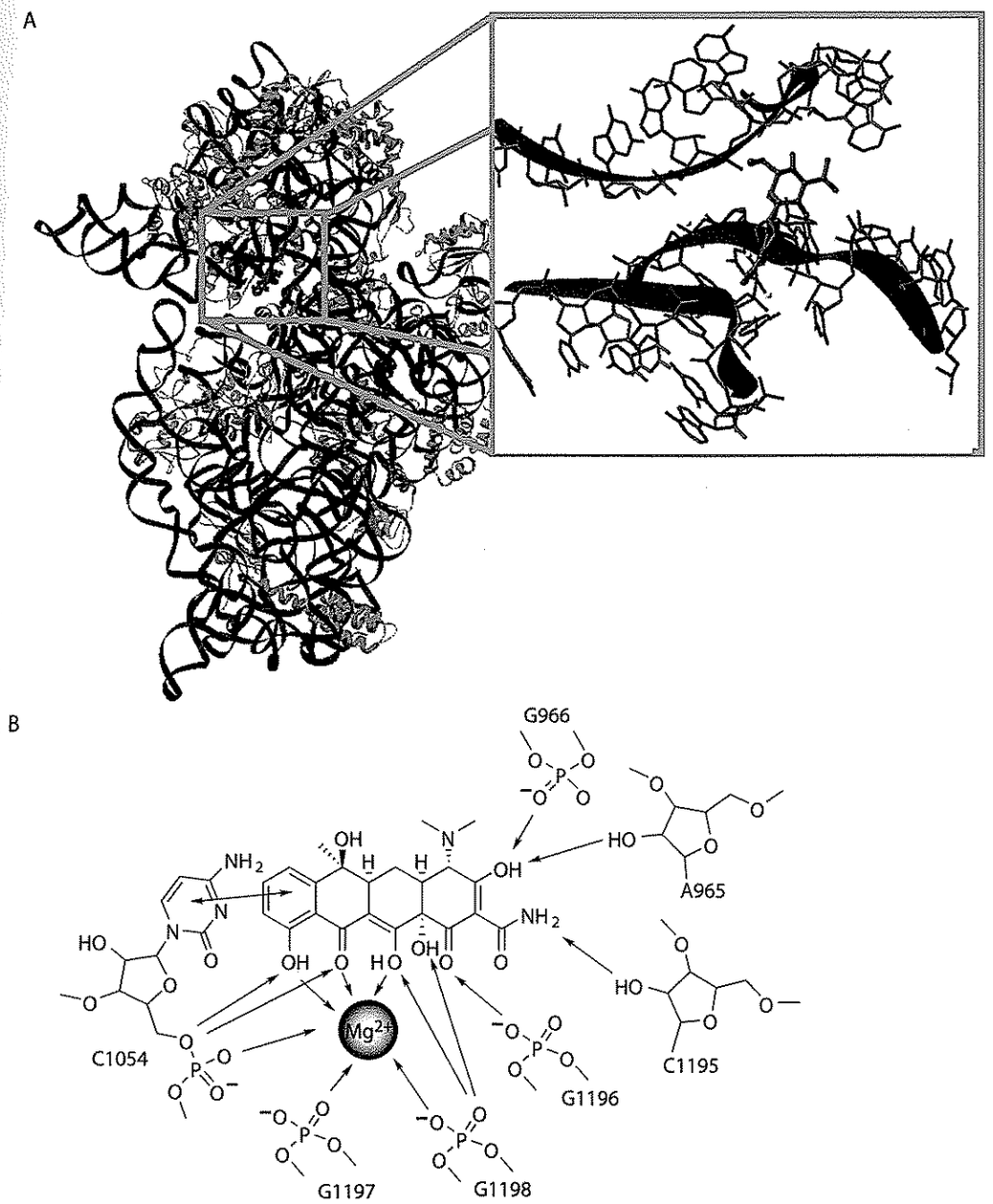
Tetracyclines and glycylcyclines

The tetracyclines have been known since 1948 with the discovery of chlortetracycline and then tetracycline from *Streptomyces aureofaciens* and then oxytetracycline from *S. rimosus* (**Fig. 4.5**). More-recent members of the class include the 6-deoxy-5-hydroxytetracycline (doxycycline), introduced in 1967, and minocycline, introduced in 1972 (Chopra and Roberts, 2001). The lack of new versions of tetracycline in the past 30 years reflects a declining role as front-line therapy in many human infections, but the ongoing clinical development of tigilcycline, a glycylcycline that inhibits efflux, indicates continued interest in this polyketide antibiotic class. The 19-carbon four-ring cyclic skeleton is derived from a starter molecule and eight molecules of malonyl CoA (see chapter 12) by iterative action of a polyketide synthase (Rawlings, 1999). Tetracyclines are largely bacteriostatic, working at the 30S ribosomal subunit to block binding of the incoming aminoacyl-tRNAs to the A site. Selectivity against bacterial versus eukaryotic ribosomes is due to both structural differences in RNA of the ribosomal subunits and selective concentration in susceptible bacterial cells (Chopra and Roberts, 2001). The determination of the structure of the 30S ribosomal subunit from *T. thermophilus* (Brodersen et al., 2000; Pioletti et al., 2001) with bound drug has revealed a major binding site and a lower-affinity binding site for tetracycline (**Color Plate 4.6A**).

The major site has only RNA, not protein, interacting with tetracycline, near the acceptor (A) site for aminoacyl-tRNA binding in a groove 20 Å wide and

Figure 4.5 Structures of tetracycline, chlortetracycline, oxytetracycline, doxycycline, a glycylcycline (DMG-MINO), and tigilcycline.





Color Plate 4.6 (A) Binding site for tetracycline with 16S rRNA on the 30S bacterial subunit of the ribosome; (B) interactions of tetracycline with helix 34 of 16S RNA. (Adapted from Brodersen et al. [2000] with permission.)

7 Å deep. As shown in **Color Plate 4.6B**, the oxygens of internucleotide phosphodiester links in 16S rRNA helix 34 form electrostatic interactions, directly or through a Mg ion to the bottom edge of tetracycline. The bound structure suggests that while tetracycline will not block initial binding of aminoacyl-tRNA and the hydrolysis of GTP by the initiation factor EF-Tu that attends the tRNA delivery, the subsequent rotation of aminoacyl-tRNA into the A site would be blocked by tetracycline. The aminoacyl-tRNA would then be prematurely released, terminating that cycle without peptide bond formation.

Because of gradual development of resistant bacteria through the decades of use of tetracyclines and their derivatives (mechanism discussed in chapter 9), their use in first-line therapy has declined. However, programs to attack the resistance mechanisms are ongoing. For example, substitution of tetracycline at the 9-position with glycine amide functionality produces a compound, DMG-DMDOT (Color Plate 4.6B), with activity against tetracycline-resistant *Escherichia coli* and *S. aureus* as well as against methicillin-resistant *S. aureus* (MRSA) (see Chopra and Roberts, 2001, for reviews; Chu et al., 1996).

In addition to the tetracycline efflux mechanisms to be discussed in chapter 9, a second class of resistance is exemplified by the TetO and TetM proteins, which have been termed ribosomal protection proteins. It is now known that TetO and TetM are structural mimics of the elongation factor EF-G, a GTPase responsible for translocation of aminoacyl- and peptidyl-tRNAs from the A and P sites to the P and E sites in translocation cycles. An electron microscopic analysis (Spahn et al., 2001) of TetO binding shows overlap with the EF-G binding site but failure to induce the ribosome conformational changes that lead to translocation. Instead, the GTP hydrolysis by TetO is proposed to disturb helix 34 in 16S rRNA, leading to a lower-affinity conformation for tetracycline and its release. Thus GTPase action is used to pry tetracycline off the ribosome and relieve inhibition of protein biosynthesis.

Aminoglycoside antibiotics

Aminoglycosides have been widely used for decades, following the discovery of streptomycin in 1944 (see Piepersberg, 1997, for review), in many clinical settings for antibacterial infections due to their bactericidal action and their observed synergy with other antibiotics. It has been suggested that the alternate term aminocyclitols should be used to encompass the broad variation of structures in this antibiotic class (Piepersberg, 1997). They are hydrophilic sugars with multiple amino groups, protonated at physiological pH to function as polycations and target accessible regions of polyanionic 16S rRNA on the 30S ribosome, notably the A site for aminoacyl-tRNA binding (Carter et al., 2000). Several generations of aminoglycosides have been tested clinically, with tobramycin, gentamicin, and amikacin (**Fig. 4.6**) prominent family members in contemporary clinical use. The biosynthesis of the two major classes of aminoglycosides is taken up in chapter 14. The aminoglycosides show renal toxicity and ototoxicity, which is a limiting constraint. The ototoxicity is thought to be through aminoglycoside-iron chelates which reduce O_2 to oxygen radicals that destroy hair cells in the ear. Aminoglycosides are potent drugs against gram-negative bacteria but not

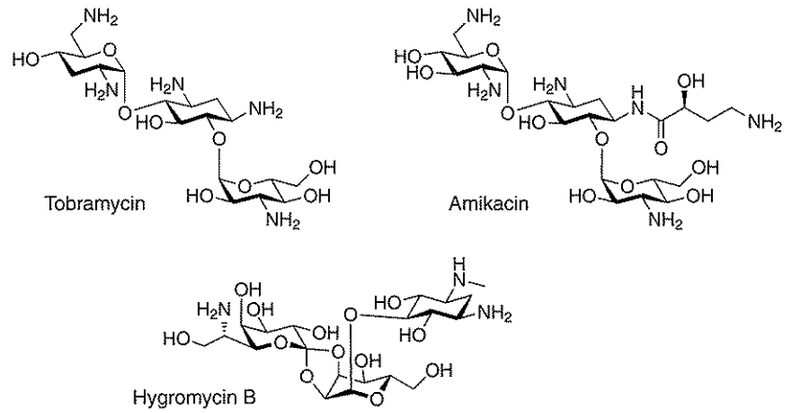


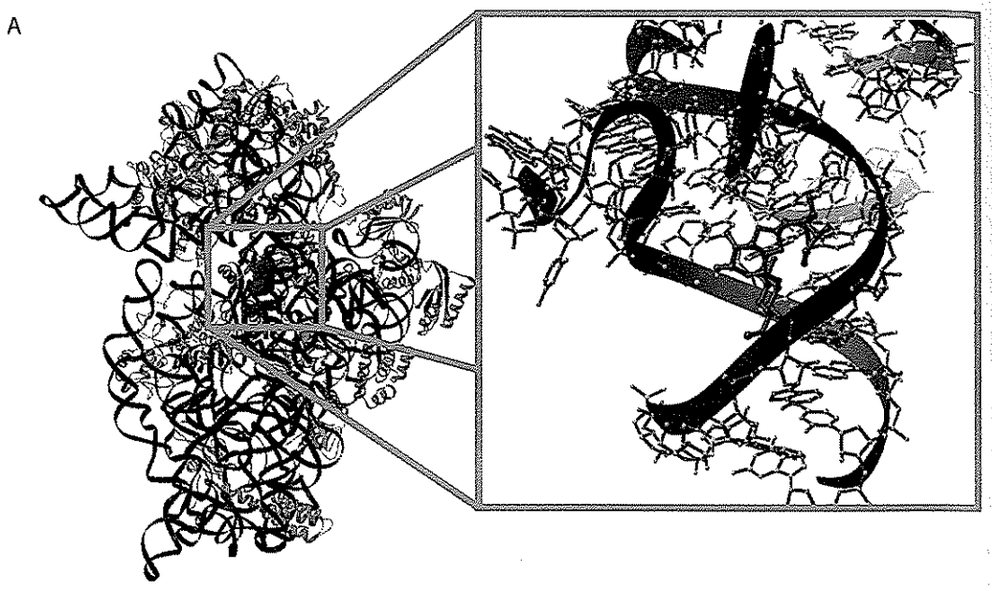
Figure 4.6 Aminoglycoside antibiotics: tobramycin, amikacin, and hygromycin B.

very effective against gram-positive organisms (Scholar and Pratt, 2000), although the combination of aminoglycosides and β-lactams is used to treat enterococcal infections. Synergy with lactam antibiotics is observed, and combinations of gentamicin, tobramycin, or amikacin with ticarcillin or piperacillin are effective against infections caused by *Pseudomonas aeruginosa*. There are several routes of enzymatic deactivation in resistant bacteria, as will be noted in chapter 8.

The structure of an aminocyclitol, hygromycin B, bound to the *T. thermophilus* ribosome 30S subunit has been solved by X-ray analysis (Brodersen et al., 2000) (**Color Plate 4.7A**) and a single binding site at the top of helix 44, near the A, P, and E sites for tRNA, has been observed. The contacts are to the RNA bases rather than backbone atoms, leading to high sequence specificity, in an extended array. Given the fact that hygromycin B has been reported to sequester tRNA at the A site of the ribosome, it is possible that drug binding blocks a required conformational transition during the peptide bond-forming translocation process. Streptomycin binding at the 16S subunit has been similarly characterized by X-ray analysis (**Color Plate 4.7B**) (Brodersen et al., 2000), giving powerful insights into how the aminocyclitols bring the translocation steps of protein biosynthesis in the ribosome to a halt.

Linezolid: a synthetic oxazolidinone antibiotic

The only totally synthetic antibiotic in clinical use that blocks protein synthesis at the ribosome is linezolid (Fig. 4.3), approved by the U.S. Food and Drug Administration in 2000. The core pharmacophore of linezolid is the oxazolidinone ring, and it has been described as the first structurally novel antibiotic to be introduced in three decades (Tally and DeBruin, 2000). It has been reported that linezolid-resistant mutants (Kloss et al., 1999) map to the 23S rRNA sites near the peptidyltransferase center, consistent with recent kinetic studies showing that oxazolidinones are competitive inhibitors of both A-site and P-site sub-



Color Plate 4.7 Binding site for (A) the aminoglycoside hygromycin B and (B) streptomycin with the 16S rRNA of the 30S ribosomal subunit. (Adapted from Brodersen et al., 2000.)

strates (Patel et al., 2001). The mechanism of action has been proposed to be occupancy of the P site in the peptidyltransferase center of the ribosome, blocking the first peptide bond-forming step in protein synthesis (Patel et al., 2001). Linezolid is most active against gram-positive bacteria including VRE and has high oral bioavailability. Its therapeutic niche will be clarified as time from approval lengthens and clinical experience accumulates.