A Bright Future for Antibiotics?

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Abstract

Multidrug resistance is a global threat as the clinically available potent antibiotic drugs are becoming exceedingly scarce. For example, increasing drug resistance among gram-positive bacteria is responsible for approximately one-third of nosocomial infections. As ribosomes are a major target for these drugs, they may serve as suitable objects for novel development of next-generation antibiotics. Three-dimensional structures of ribosomal particles from *Staphylococcus aureus* obtained by X-ray crystallography have shed light on fine details of drug binding sites and have revealed unique structural motifs specific for this pathogenic strain, which may be used for the design of novel degradable pathogen-specific, and hence, environmentally friendly drugs.

Contents

1. HISTORICAL NOTES

This article addresses a major problem in modern medicine: resistance of pathogens to antibiotics. It focuses on how antibiotics paralyze ribosomes, the universal multicomponent cellular particles that translate the genetic code into proteins. It highlights conventional and nonconventional suggestions that may relieve, to some extent, the current problematic medical situation and shows how we may benefit from the vast amount of available structural information. Notably, understanding the mechanisms of resistance to antibiotics could not even be dreamt about when a project aimed at the determination of the atomic structure of ribosomes was started during the last two weeks of November 1979.

The way to the incredible current situation was far from being trivial or easy. The first obstacle was crystallization of intact ribosomes, which was considered formidable owing to the repeating failures of numerous attempts performed worldwide by leading scientists. At that time, a rather inexperienced young scientist, Ada Yonath, presumed that the main reason for the extreme difficulties in ribosome crystallization was their fast deterioration, in addition to the trivial reasons mentioned worldwide, namely the complexity, huge size, mobility, and functional flexibility of ribosomes. She was inspired by the finding that ribosome preparations were regularly only partially functional in protein biosynthesis, hence indicating nonhomogeneous populations, which obviously are not suitable for the production of crystals, namely periodic organization of identical objects.

The finding that ribosomes in the cells of winter-sleeping bears are periodically packed in monolayers on the inner side of the cell membranes (1) inspired her and triggered the assumption that ribosomes tend to pack tightly under stressful conditions to maintain pools of active ribosomes for the post-stressful period, the spring. Consequently, she started her crystallization attempts using the ribosomes from bacteria that live under extreme conditions, such as the Dead Sea bacteria, *Haloarcula marismortui*, which exist at high temperature and high salt concentrations; *Thermus thermophilus* and *Bacillus stearothermophilus* (2), which live at elevated temperatures; as well as the ultimate survivor, *Deinococcus radiodurans*, which withstands hot and cold temperatures and survives hunger, dust, and irradiation.

Despite the growing skepticism of most of the scientific community and the slow progress of her studies, she kept pushing this project as she observed minimal although continuous progress, which

was hard to explain as she often developed unconventional scientific methodologies. Consequently, almost two decades were required for reaching the goal: the determination of the ribosome's high-resolution structure. Among the methodological advances introduced by her group is cryo bio-crystallography (3). This method minimizes the radiation damage of the ribosome crystals, which are extremely sensitive to X-irradiation, and therefore became routine in biological crystallography worldwide within a few months. She also visualized the nascent protein exit tunnel (4), a feature that continued to be controversial until it was rediscovered almost a decade later by low-resolution cryo–electron microscopy (cryo-EM) and identified in the high-resolution crystal structures.

It took 15 years to prove the feasibility of ribosomal crystallography. Then, a few leading scientific groups joined her "running wagon" by repeating her procedures and even using the same ribosomal sources that she identified. Hence, she was not anymore the only "crazy" or "dreamer," and some of her credibility was restored. Consequently, and with the progress of high-resolution single-particle cryo-EM, more than two dozen structures of the ribosome have recently been determined. Thus, the previously ridiculed studies, led by a "treeless" scientist (5), became the center of active, fascinating, and relevant research. Ironically, owing to the dramatic improvement of single-particle three-dimensional (3D) cryo-EM, which can now be performed at high resolution, structural biology is undergoing a technical and conceptual revolution. Hence, detailed structures, showing protein side chains and nucleic acid bases as well as their methylations, can be obtained even from eukaryotic ribosomes (6) in a relatively short timeframe, because crystals are not needed. In fact, ribosomes became the object of choice for this method, as their size and density are ideal for detection by cryo-EM, and their overall structure, which has been determined crystallographically, is being used as the starting model. Consequently, within her own history as a structural biologist she has gone through a major transformation: from challenging the most difficult objects, to investigating the most suitable entities.

Throughout she collaborated intensively with the Max Planck Institute for Molecular Genetics in Berlin and directed two research groups with wonderful young researchers and students in two locations: the Weizmann Institute of Science in Rehovot, Israel, and the Max Planck Research Unit in the Deutsches Elektronen-Synchrotron (DESY) in Hamburg, Germany. The first German student, Dr. Klaus von Bohlen, lost his life in an accident. The first Israeli student, Dr. Anat Bashan, who is currently the senior scientist of the Ribosome Group at the Weizmann Institute, and Ms. Donna Matzov, one of the most recent Israeli students, are coauthors of this article.

2. INTRODUCTION: PIONEERING STRUCTURAL STUDIES ON CURRENT RIBOSOMAL ANTIBIOTICS

The increasing appearance of multidrug-resistant strains, together with the minimal (actually negligible) number of new antibiotic drugs that are presently undergoing development and/or clinical trials, is becoming a colossal health threat. Thus, it seems that we will soon revert back to the pre-antibiotic era, during which diseases caused by parasites or by simple as well as severe infections (such as tuberculosis, pneumonia, wounds, etc.) were almost untreatable and resulted in frequent deaths.

Ribosomes are complex ribonucleoproteins that translate the genetic code into proteins in all living cells. They comprise two structurally independent subunits of unequal sizes. When functional, the two subunits associate to form the active ribosome, in which the small subunit binds messenger RNAs (mRNAs) and provides three sites for decoding by the association of the anticodons of the aminoacyl, peptidyl, and exiting transfer RNA (A-tRNA, P-tRNA, and E-tRNA) molecules. The large subunit contains the ribosomal catalytic site, namely the peptidyl transferase center (PTC), which catalyzes the formation of peptide bonds between the amino acid of the

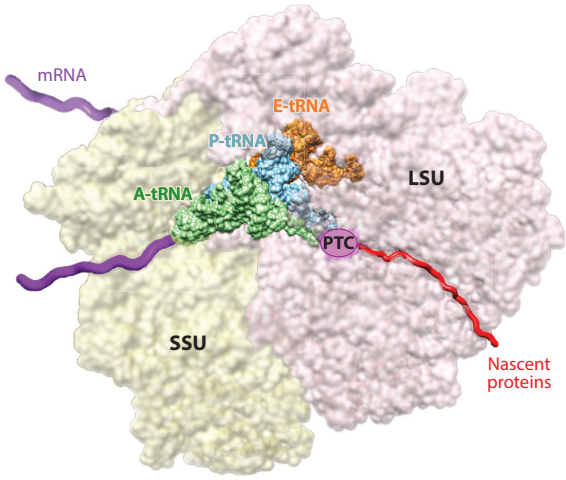

Figure 1

The structure of a bacterial ribosome. The small and large subunits are shown in light yellow and light pink, respectively. Several functional sites (PTC, tunnel, decoding region) as well as the positions of the three transfer RNA molecules are depicted. Abbreviations: A-tRNA, aminoacyl transfer RNA; E-tRNA, exiting transfer RNA; LSU, large subunit; mRNA, messenger RNA; PTC, peptidyl transferase center; P-tRNA, peptidyl transfer RNA; SSU, small subunit.

A-tRNA and the growing protein on P-tRNA, thereby acting as a polymerase. The nascent proteins progress through a tunnel in the large subunit until exiting from the ribosome (**Figure 1**).

Sixteen years of increasingly available high-resolution structures of ribosomal particles from various sources, including prokaryotes, archaea, and eukaryotes, have revolutionized our insights into translation and their inhibition. Furthermore, owing to their key role in life, ribosomes are targeted by many antimicrobial drugs. The high-resolution crystal structures of ribosomes, from bacteria suitable to serve as pathogen models in complex with antibiotics and their semisynthetic derivatives, provide matchless insights into common properties of antibiotic action (6, 7–51). Thus, almost all of the structural elements involved in antibiotic binding and of the mechanisms underlying the inhibition action of antimicrobial therapeutics have been identified.

Currently, almost all of the clinically useful ribosomal antibiotic therapeutics are derived from natural compounds produced by microorganisms for inhibiting the growth of other bacteria types, thus defending themselves. Many of these natural antibiotics that were shown to be medically useful have undergone subsequent chemical modifications to improve their effectiveness. In addition to the natural and semisynthetic substances, a few synthetic drugs are currently in use. Among these are the oxazolidinone linezolid and several selective aminoglycoside variants capable of "fixing" damaged genes during the translation process and thus may provide a general tool for the treatment of genetic diseases caused by nonsense mutations (52, 53) as well as inhibit protein biosynthesis in parasites such as *Leishmania* spp. (6, 54).

Resistance to antibiotics is a basic process for the survival of many microorganisms, regardless of their exposure to modern clinical treatment and/or nutrition (55–59). Resistance is generally acquired by molecular mechanisms, some of which, such as activation of cellular efflux pumps, are common to almost all antibiotics (60). In addition, in some cases, such as ribosomal antibiotics, bacteria developed specific molecular pathways that cause resistance. Prominent processes acquiring resistance include modifications of the antibiotic binding pockets by mutations (e.g.,

macrolide resistance by modification of a component crucial for their binding, A2058G). Other frequently used mechanisms include activation of key enzymatic processes (e.g., methylation of the binding components of macrolide and aminoglycosides by methylases); enzymatic inactivation of the drug, such as the macrolide molecule by esterases (61); removal of the antibiotic drug from its target (i.e., resistance to tetracycline by disturbing the ribosomal protection proteins) (62–65); and modification of ribosomal proteins essential for ribosomal functionality at the PTC and tunnel entrance, such as rpL3, which is associated with resistance to linezolid, tiamulin, and anisomycin (66, 67), or disruption of the interactions between proteins that play key roles in protein biosynthesis (68).

Cross-resistance occurs when each of a chemically heterogeneous group of antibiotics (e.g., macrolides, lincosamides, streptogramins B, and ketolides, termed MLSBK) that bind to ribosomes in close proximity trigger resistance to all other members of the group. Importantly, in several cases resistant mechanisms are involved in cellular regulation, such as translation arrest, that can be triggered by the activation of antibiotic-resistance genes (69). Also, alterations in the locations of ribosomal components may cause resistance by reshaping antibiotic binding pockets or their environments (e.g., 69–72). Importantly, macrolide binding seems to be involved in cellular processes. Thus, stalling by specific nascent peptides, a cellular mechanism used for regulation of expression of several bacterial and eukaryotic genes, is sensitive to signals connected with macrolide binding (73–83).

Staphylococcus aureus possess capabilities to respond rapidly to many antibiotics by acquiring resistance. Recent additions to the long list of its resistant mutants have been described in several review articles (84–86). The methicillin-resistant *S. aureus* (MRSA) strain is considered to be one of the most common and problematic bacteria associated with increasing antimicrobial resistance. Consequently, continuous efforts are required to discover lead compounds for antistaphylococcal therapy (87). Popular among these efforts are attempts to create libraries of potential drugs by mining underexplored microbial niches or designing chemical probes for improving known molecular scaffolds. Hence, most clinically used antibiotics originate from a small set of molecular scaffolds (88–99).

Extensive efforts are also being made for the development of practical systems for production of new antibiotics. An interesting example is the fully synthetic platform for the discovery and manufacture of new macrolide antibiotics by the convergent assembly of simple chemical building blocks, which has already yielded more than 300 new macrolide antibiotic candidates, some of which showed antibiotic activity even in strains resistant to commonly used macrolides (90). Likewise, extensive efforts are being made for establishing molecular genetic tools for manipulation of biosynthetic pathways that are expected to yield state-of-the-art targeted methods for understanding and manipulating antibiotic binding sites (91–92) and for exploiting different segments, such as polyketides in conjugates with peptides (e.g., bactobolin) (49).

A crucial environmental issue, which is linked to antibiotic resistance, results from the chemical nature of the molecular scaffolds of most ribosomal antibiotics, which are composed of organic metabolites that cannot be fully digested by humans or animals. These nondigestible, rather toxic compounds are also nonbiodegradable and thus contaminate the environment (93). Furthermore, by penetrating into agricultural irrigation systems (e.g., milk via the cows that eat the grass), these compounds are increasingly being consumed by humans and thereby spreading antibiotic resistance (94).

As most of the studies on ribosomal antimicrobial drugs (e.g., macrolides, ketolides, pleuromutilins, streptogramins, lincosamides, aminoglycosides, orthosomycins, etc.) and the resistance to them have been critically described in several recent comprehensive reviews (84, 89, 95–103),

in this article we relate mainly to species specificity in susceptibility to antimicrobial drugs of multidrug-resistant bacteria alongside vicious parasites. We elaborate on directions that could be suitable for the design and the creation of future antimicrobial therapeutics with better distinction between pathogens and useful bacterial species in the microbiome, on ecological aspects of antibiotic resistance, and on the pros and cons of species-specific drugs. We also underline our views on future experimental methods that should be suitable for future exploitation.

3. RECENT STUDIES FOCUSING ON RIBOSOMES FROM PATHOGENS

Careful interpretations of the structures of the above discussed complexes alongside the recently determined structures of ribosomes from pathogenic bacteria and the ongoing structural studies on ribosomes from resistant strains have led to a deeper understanding of the main problems in contemporary medicine. Thus, the high-resolution structures of ribosomes shed light on their specific structural elements and on the mechanisms underlying the action of the antimicrobial therapeutics that paralyze them.

Recent years have been very fruitful in terms of research on the structure and function of ribosomes as well as on their inhibitors. However, much less was done in terms of manufacturing new clinically useful antibiotic drugs. Here we describe some of the current studies, their outcomes, and the lessons learned from crystal structures of the large ribosomal subunit from the problematic pathogen *S. aureus* and its complexes with various antibiotics.

3.1. Increasing the Potency of Existing Antibiotics

The pleuoromutilins, a family of antibiotics that bind to the PTC, include several potent compounds, among them retapamulin, which was developed for treatment of skin infections and demonstrates activity against clinical isolates *Streptococcus pyogenes*, *Streptococcus agalactiae*, β-hemolytic streptococci, viridans streptococci, *S. aureus*, coagulase-negative staphylococci (including *Staphylococcus epidermidis*), *Propionibacterium* spp. (including *Propionibacterium acnes*), *Prevotella* spp., *Porphyromonas* spp., and *Fusobacterium* spp. (104). New, more potent lead molecules, belonging to a series of pleuoromutilins, have been recently produced by Nabriva Therapeutics (47). Structural studies have showed that the increase in the antibiotic potency of BC-3205 by a factor of 16 was achieved by the addition of a single hydrogen bond to the interactions between the pleuoromutilins with the PTC (47, 104). Moreover, recent crystallographic and biochemical studies indicated that a more sophisticated chemical design yielded a potential antibiotic drug with even higher potency (105).

3.2. Better Discrimination and Specificity

Comparison between the structures of complexes of erythromycin with the large ribosomal subunit from the nonpathogenic bacteria *D. radiodurans* and *T. thermophilus* and those of the pathogens *Escherichia coli* and *S. aureus* indicated clearly available free space adjacent to several antibiotic binding sites. Among them is a reasonable large space at the rims of the erythromycin binding pockets of both pathogenic bacteria, due to the length of the chain of protein rpL32 of the *S. aureus* and *E. coli* ribosome (shorter compared with its length in the other ribosomes) (47, 103). This special arrangement provides space for extending erythromycin in a fashion that should allow binding specifically to the pathogenic species studied so far, namely *S. aureus* and *E. coli*, and not to many nonpathogens, represented by *D. radiodurans* and *T. thermophilus* (**Figure 2**).

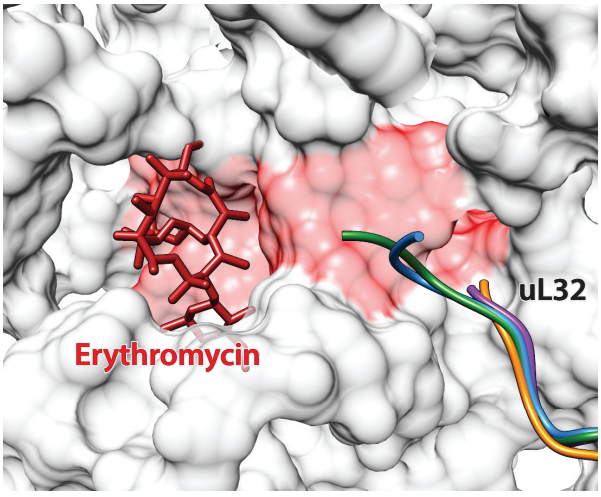

Figure 2

Erythromycin (*dark red*) binding pocket and its proximity. Protein uL32 is shorter in the pathogens *Staphylococcus aureus* (*orange*) and *Escherichia coli* (*purple*) compared with its length in the ribosomes of the nonpathogenic bacteria *Deinococcus radiodurans* (*green*) and *Thermus thermophilus* (*teal*). Hence, an extra region is available for extension design only in the ribosomes from those pathogens. Light pink covers the total space for the future design of extended erythromycin. **Figure 2** modified from Reference 47.

3.3. Meaningful Core Alterations and Binding Pocket Extensions Benefiting from Multiple Binding Sites

The findings described above encouraged revisiting previous studies on tetracycline binding to the small ribosomal subunit that revealed six binding sites (**Figure 3**) of various occupancies (14). Among those, actual interference of protein biosynthesis could be assigned to only two sites with the highest occupancies (namely the strongest binding). One of these sites is located in the vicinity of the decoding center, and the second is at a strategic location for the mobility required for ribosomal functionality. The other sites seem to be somewhat remote from any ribosomal centers of action but in positions that in principle can be chemically connected to the ribosomal functional sites while increasing their binding strength. As accurate structural information is available, the chemical nature of the extensions, as well as the alterations of the tetracycline cores required for increasing their binding strengths, can also be optimized in terms of toxicity and degradability, benefiting from considerations similar to those shown to be suitable for the peripheral novel sites (see below). It should be mentioned that multiple binding sites were also observed recently in other systems. An appropriate example is the broad-spectrum antibacterial activity of negamycin that interferes with decoding and translocation by simultaneous interaction with ribosomal RNA (rRNA) and transfer RNA (tRNA) (44, 45).

3.4. Novel Potential Binding Sites

Potential antibiotic binding sites, of diverse nature, have been identified. Some of them are common to eubacteria, others seem to be species specific. Many are located in the periphery, whereas a few are in the ribosomal core.

The binding sites and the modes of action of most known antibiotics are common to all eubacteria, including those comprising the microbiome (106). Thus, an unintentional consequence

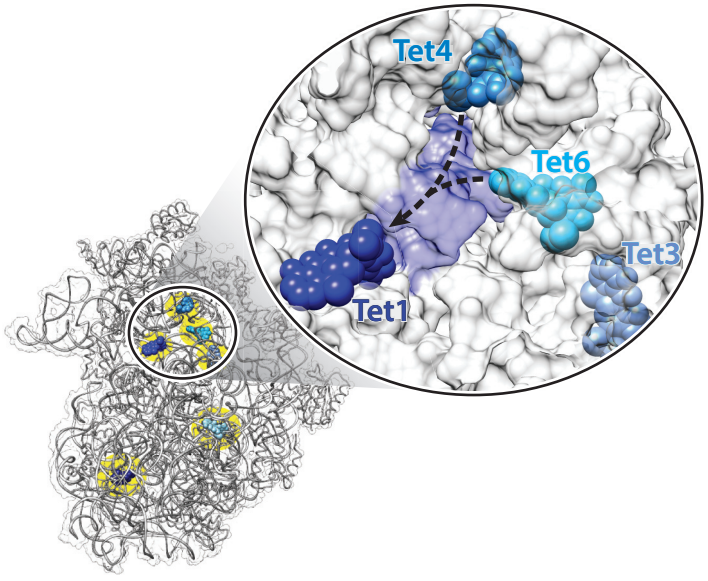

Figure 3

The six tetracycline binding pockets in the small subunit of *Thermus thermophilus* (17). The main site, at the decoding center (to which there is resistance), is shown in blue. Sites 4 and 6, which are distal to the main active site (14), can in principle be extended toward the decoding center, utilizing channels within the region covered by light blue, approximately along the black arrows.

of the use of currently preferred broad-spectrum antibiotics is the alteration of the delicate composition of the microbiome, which may cause several symptoms or disease, such as diabetics cases linked to the usage of antibiotics. Furthermore, it is conceivable that responsiveness to the species-specific differences in drug action should minimize uncontrolled microbiome alterations. Indeed, careful comparisons between the structures of ribosomes from the genuine pathogen, *S. aureus* (47), and from nonpathogenic species, identified unique structural motifs that may be exploited for the design of innovative species-specific antibiotics (47).

Considerable differences between the 3D structures of ribosomes from nonpathogenic bacteria and that of *S. aureus* were detected. These differences are located mainly, but not exclusively, within the particle's periphery, particularly in stem loops of rRNA helices (**Figure 4**). Some of these features seem to be involved in various ribosomal interactions with diverse cellular components. Hence, these extended and exposed rRNA helices can, in principle, be exploited for the design of species-specific potent antibiotics.

In fact, surface-exposed rRNA features that seem to be involved in ribosomal functions have also been identified in a model system, namely the ribosome of *D. radiodurans* (107). Most of these regions contain unpaired single helices or stem loops, a feature that should be exploited for binding potential base-pairing donors by antisense technology. Preliminary studies showed that ribosomal function can be hampered in vitro by attaching compounds such as complementary DNA to the exposed rRNA regions. Thus, in vitro targeting of 16 among the 25 such exposed rRNA regions hampered protein biosynthesis.

Notably, oligonucleotides were used as ribosomal inhibitors and as tools for structural and functional studies, even before the 3D structures of the ribosomes were determined. Short DNA oligonucleotides were used as in vitro antisense DNA to probe rRNA accessibility (108) and

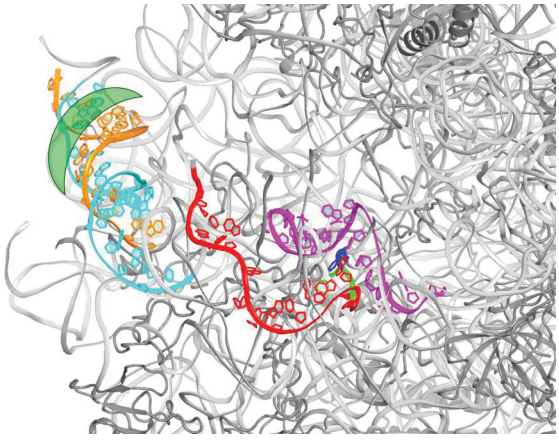

Figure 4

Part of the backbone of the large ribosomal subunit of *Staphylococcus aureus* (*gray*). The ribosomal RNA regions with fold variability compared with all other known structures on the large subunit surface are shown in different colors. The light green crescent adjacent to helix H68 in *S. aureus* (shown in *orange*) symbolizes a possible location for antisense technology.

to locate specific functional regions (109). Furthermore, antisense peptide nucleic acid (PNA) conjugates were used for sensitization of MRSA and methicillin-resistant *Staphylococcus pseudintermedius*, indicating that even antibiotic-resistance species can be targeted by antisense agents (110).

The oligonucleotides that were used as inhibitors can potentially serve as bases for future antibacterial drugs alone and in conjunction with other components, such as the DNA analog PNA, amino acids, or short peptides. Examples are those used recently to target Helix 69 (111), the first 16 residues of the proline-rich antimicrobial peptide mammalian Bac7 (112), the thiazolyl peptide antibiotics (113), and the small peptides that were shown to inhibit translation in prokaryotes (75, 114).

In short, some of the exposed rRNA chains and the species-specific protein loop regions (47) can become binding sites to a new generation of antibiotics, built of sophisticated antisense compounds designed from molecules containing various combinations of organic molecules, such as nucleic acids, PNA, short peptides, aliphatic chain, etc. These can be optimized in terms of their chemical properties, length, toxicity, and degradability; hence, they should cause very little ecological or environmental contamination, which contributes to the increase of antibiotic resistance.

3.5. CTC, a Multidomain Protein Containing a Domain Typical to Many Pathogenic Bacteria

Avilamycin (avi) and evernimicin (evn), of the orthosomycins family, were discovered in the 1960s and are active against gram-positive bacteria, including vancomycin-resistant enterococci, MRSA, and penicillin-resistant pneumococci (115, 116). Both were shown by biochemical methods to bind to a unique site at the large subunit and to affect translation (117, 118).

Two recent structural studies shed more light on their mechanism of inhibitory action. One was performed by 3D cryo-EM using two complexes of *E. coli* ribosome each with one of them (119), which indicated binding to the large ribosomal subunit at the entrance of the A-site tRNA corridor. The second (50) was performed by X-ray crystallography at somewhat higher resolution and

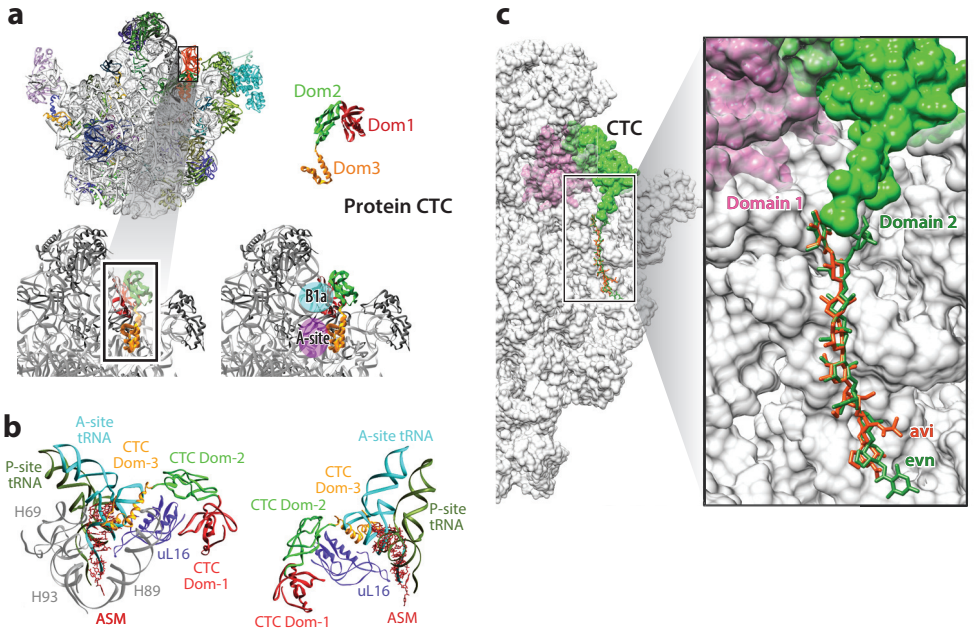

Figure 5

Avilamycin (avi) and evernimicin (evn) binding sites to the 50S ribosomal subunit of *Deinococcus radiodurans* and their interactions with protein CTC. (*a*) The domain structures of the three domains of CTC and their binding sites are shown at the entrance to the A-site transfer RNA (tRNA) accommodation corridor. (*b*) The detailed three-dimensional structure of the environment of CTC is shown. (*c*) An enlarged view showing the actual interactions of avi (*red*) and evn (*green*) with CTC domain 2.

showed a similar binding location at higher details, thus revealing a network of additional, crucial interactions with a ribosomal component, namely the CTC middle domain. This domain is a major component in many gram-positive pathogenic bacteria [e.g., *S. aureus* (47), *Enterococcus faecium* (120), *Enterococcus faecalis* (121), and *Streptococcus pneumoniae* (122)] but does not exist in *E. coli*.

Protein CTC is of particular interest because of its various molecular versions (see below and in **Figure 5**) and because it fills a gap between the central protuberance (CP) and the uL1 stalk, which is located adjacent to the A-site tRNA entrance corridor (12, 123). The molecular compositions of the CTC protein (named after a general shock protein), as in *D. radiodurans*, exhibit evolutionary adaptation to environmental conditions. In its full-size version, 253 amino acids, it comprises three globular domains (12) (**Figure 5**). The C-terminal domain (also known as domain 1) is homologous to protein L25 in *E. coli*, namely the 5S rRNA-binding protein. Domains 1 + 2 (namely the C-terminal + the middle domains) are homologous to protein TL5 in the *T. thermophilus* ribosome (124). Domain 3 (the N-terminal domain) is bound to the middle domain (domain 2) by a single strand, presumably facilitating the extreme (unusual) flexibility in its position within the ribosome, as indicated by the lower quality of its electron density map. This flexibility allows it to swing out of the ribosome upon binding of A-site tRNA (17) or of the orthosomycins antibiotics that occupy its space. Thus, the relative orientation of the N-terminal and the middle CTC domains differs from that determined for the two domains of TL5 in isolation. The third domain of CTC, the C-terminal domain, bears some resemblance to the structural motif seen in some ribosomal proteins.

Domain 1 of CTC is located on the solvent side, at the rim of the large ribosomal subunit, and thus may protect the intersubunit bridge B1a. The middle domain fills the space between the 5S rRNA and the uL11 arm and interacts with H38, the helix that forms the intersubunit bridge B1a. These interactions and the partial wrapping of the large subunit CP are likely to provide the additional stability that pathogens, as well as thermopiles, require to function well at higher temperatures (**Figure 5**). The inherent flexibility of domain 3 may indicate that it serves as an A-site regulator and also somewhat influences the progression of the mRNA. Its interactions with the A-finger (A-site tRNA corridor), its capability to manipulate the binding of the A-site tRNA corridor, and the enhanced stability of the CP seem to provide a mechanism for survival under extremely stressful conditions, including hunger or minimal resources (123).

The middle domain, which frequently exists in pathogens and other gram-positive bacteria but not in common eubacteria, is extensively interacting with antibiotics from the orthosomycins family, namely avi and evn, which block the A-site tRNA-binding corridor and prevent IF2 from binding to the large ribosomal subunit. The various unique and pathogen-specific interactions of this feature (namely, with bridge B1a, the CP, IF2, and A-site tRNA-binding sites) may provide a basis for future targets for innovative species-specific antibiotics. Thus, the pathogenic-specific structural motifs of protein CTC seem to be potential antibiotic-binding sites in several pathogens.

4. CONCLUSIONS AND THOUGHTS ABOUT THE FUTURE

As can be concluded from the above observations, resistance to antibiotics, which is currently a severe global medical problem, may be partially dealt with by the implication of new concepts, such as biodegradable compounds; by exploring unconventional research paths; and by searching for compounds (e.g., advanced antisense) that can paralyze the ribosome by binding to peripheral ribosomal target sites.

Among the directions that may yield new strategies are (*a*) focusing on species-specific antibiotics (despite the economic limitations of this narrow-spectrum suggestion) and (*b*) benefiting from presently known or future discoveries of multiple binding sites that should lead to the creation of new relevant sites by chemical extension of seemingly insignificant, partially occupied binding sites. As no genes that may modify the above-mentioned unique regions have currently been identified, resistance is expected to appear very slowly.

Additional and less conventional, albeit rather promising, approaches that may yield some relief in the seemingly hopeless resistance scene are based on exploiting biofilms (125); on data emerging from genomic studies, like genome-wide ribosome profiling (126); on whole-genome analysis of mutations leading to resistance to specific antibiotics (127); on whole-genome sequencing in search for indications of in vivo evolution of multidrug resistance (128); and on the finding of abundant ribo-regulation switches of antibiotic-responsive features that control resistance genes in pathogens and in the microbiome (129).

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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