ACS chemical biology

Reviews

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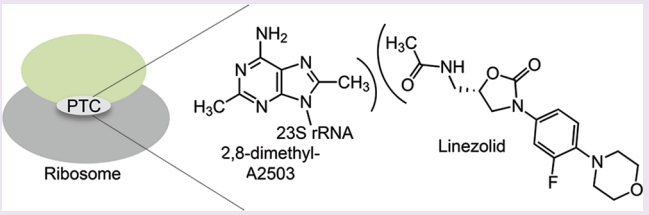
The Chemistry of Peptidyltransferase Center-Targeted Antibiotics: Enzymatic Resistance and Approaches to Countering Resistance

Kevin P. McCusker[†] and Danica Galonić Fujimori[†,‡,*]

[†]Department of Cellular and Molecular Pharmacology and [‡]Department of Pharmaceutical Chemistry, University of California, San Francisco, 600 16th St, MC2280, San Francisco, California 94158, United States

ABSTRACT: The continued ability to treat bacterial infections requires effective antibiotics. The development of new therapeutics is guided by knowledge of the mechanisms of action of and resistance to these antibiotics. Continued efforts to understand and counteract antibiotic resistance mechanisms at a molecular level have the potential to direct development of new therapeutic strategies in addition to providing insight into the underlying biochemical functions impacted by antibiotics. The interaction of antibiotics with the peptidyltransferase center and adjacent exit tunnel within the bacterial ribosome is the predominant mechanism by which antibiotics impede translation, thus stalling growth. Resistance enzymes catalyze the chemical modification of the RNA that composes these functional regions, leading to diminished binding of antibiotics. This review discusses recent advances in the elucidation of chemical mechanisms underlying resistance and driving the development of new antibiotics.



The advent of antibiotics in the early twentieth century, followed by further development of these compounds, played a critical role in the treatment of infectious diseases and the corresponding decrease in mortality and morbidity from those causes.[1−3] Among these early antibiotics was the macrolide erythromycin, a member of a large and chemically diverse group of antibiotic compounds that exert their action by interacting with the ribosomal RNA (rRNA) component of critical functional sites within the ribosome. Perhaps the most commonly targeted of these sites are the peptidyltransferase center (PTC) and the adjacent exit tunnel. Antibiotic binding to either of these sites interferes with the process of translation, which is the basis of the antibiotic activities of these compounds.[4,5] The acquisition of resistance by pathogenic microbes jeopardizes the continued clinical utility of antibiotic compounds.[1,6] Of the common resistance mechanisms,[7−9] target mutations and modifications that impact the binding of PTC-targeted antibiotics have a dominant role. Relatively subtle changes to the rRNA architecture, *i.e.*, methylation of a single nucleotide, yield significant resistance phenotypes.[7,10] When combined, these target modifications and mutations can lead to unpredicted and more severe resistance phenotypes; however, even a single modification can be sufficient to elicit a multidrug resistance (MDR) phenotype[10] owing to the overlapping binding sites of antibiotics within the PTC and its immediate vicinity.[7,11−13] The enzymatic mechanisms of these resistance modifications, their structural consequences within the PTC and chemical approaches to countering this resistance are the focus of this review.

The antibiotic families that interact with the PTC include macrolides and their derivatives ketolides, lincosamides, streptogramins, oxazolidinones, phenicols, and pleuromutilins (Figure 1).[4,7,14] The overlapping nature of the binding sites of these compounds is evidenced by multiple, distinct multidrug resistance phenotypes involving combinations of the aforementioned drugs, each arising from a single modification.[7] Compounding this is the rapid pace of genetic changes and facility of horizontal gene transfer in prokaryotic pathogens. Shortly after the introduction of the natural product erythromycin into clinical use in 1953, resistance arose in *Staphylococci*, presumably due to the acquisition of the indigenous resistance mechanism of the producing strain.[15,16] As the indigenous resistance mechanism to the natural product penicillins, β-lactamase activity, was known *prior* to the introduction of penicillin to the clinic, this indigenous resistance to erythromycin should not have come as a great surprise.[1,17] However, when microbes exhibited resistance to the entirely synthetic oxazolidinone linezolid fifty-some years later,[18] acquisition of an indigenous resistance mechanism seemed an unlikely explanation. At that juncture, it was evident that microbes could become resistant to virtually every PTC-targeted antibiotic—natural product, semisynthetic, or fully synthetic—that was currently available[19] and that a more complete understanding of the interrelated mechanisms of resistance was needed.

CHEMISTRY OF RRNA MODIFICATIONS AND RESISTANCE

The indigenous resistance mechanism to erythromycin and related macrolides mentioned above involves the post-transcriptional modification of the 23S rRNA (rRNA) by a specific

Received: October 14, 2011
Accepted: December 8, 2011
Published: December 30, 2011

 © 2011 American Chemical Society dx.doi.org/10.1021/cb200418f | *ACS Chem. Biol.* 2012, 7, 64−72

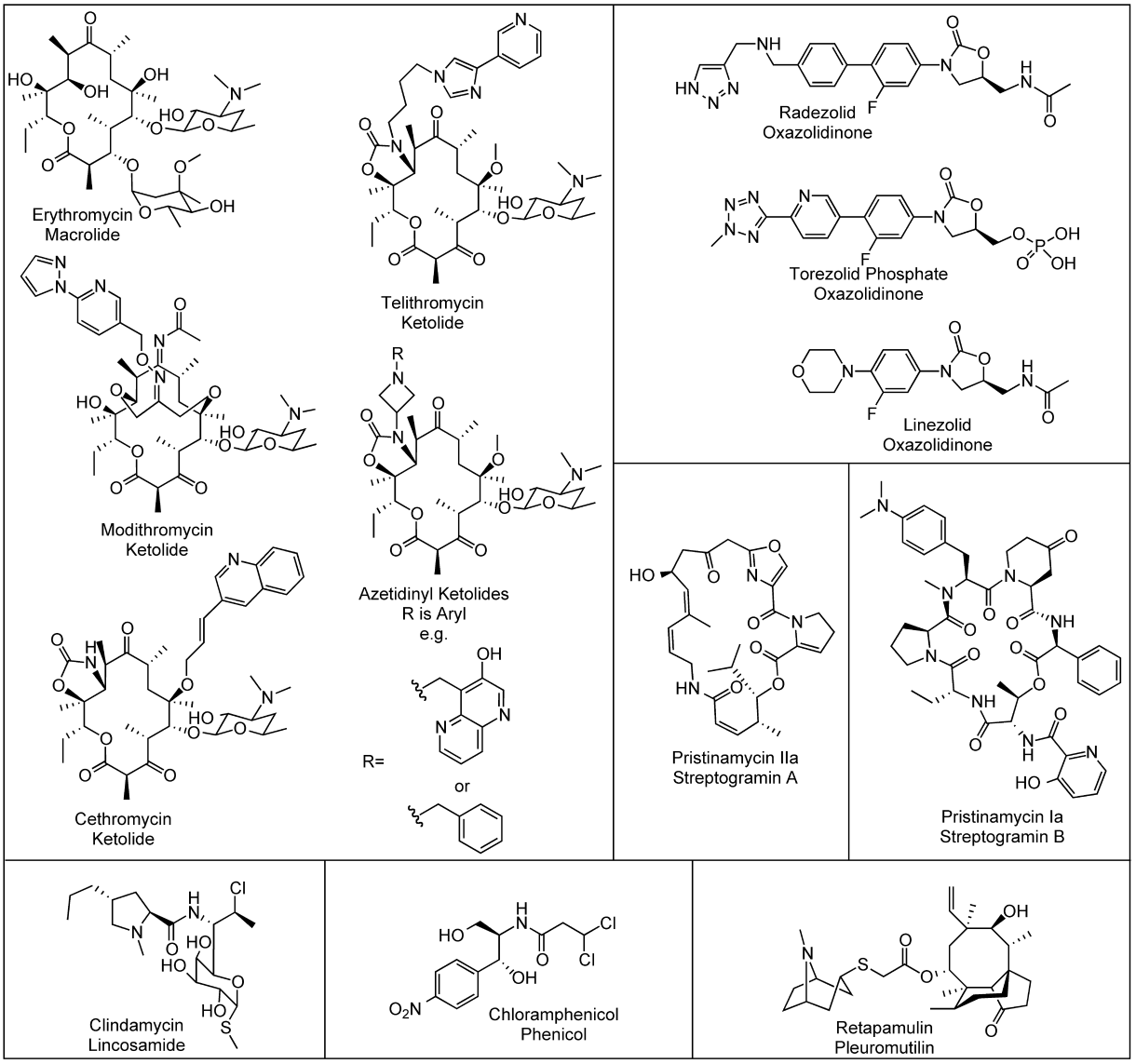


Figure 1. PTC-targeting antibiotics.

N-methyltransferase enzyme encoded by an *erm* (erythromycin ribosome methylation) gene[8] (Figure 2a). This modification still plays a significant role in resistant bacteria.[7] There are currently 34 annotated *erm* genes and corresponding enzymes,[7] which lead to the mono- or di-methylation of N6 of A2058 and occasionally A2509 (*E. coli* numbering will be employed throughout).[4,20] By itself, this modification usually results in resistance to macrolides, lincosamides, and streptogramins B.[7] However, an *erm* gene has recently been found in an operon with *cfr* (chloramphenicol florfenicol resistance) leading to resistance against all of the previously noted classes of PTC-targeted antibiotics.[21] The *cfr* gene was initially identified on plasmids in methicillin-resistant *Staphylococci* isolated from animals exhibiting antibiotic-resistant infections[22] but has since been found in the chromosome of clinical isolates of MRSA (methicillin-resistant *Staphylococcus aureus*).[23] The enzyme encoded by *cfr* is responsible for the installation of a methyl group at the C8 position of A2503 within 23S rRNA.[24] This single modification confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramins A.[18] Despite the superficial similarity of rRNA methylation, the products of the *erm* and *cfr* genes catalyze distinct chemical transformations, differing in their sites of action (heteroatom *vs* carbon) and mechanisms (Figure 2).

The canonical reaction, catalyzed by methyltransferases, in which the electrophilic *S*-adenosyl-L-methionine (SAM) donates a methyl group to a suitable nucleophilic site on RNA *via* a polar mechanism, has long been known. There are traditionally two types of sites at which this nucleophilic methylation occurs: (1) inherently nucleophilic heteroatoms[25] and (2) carbon centers rendered nucleophilic by a conjugate-addition mechanism[26] (see Figure 2 for mechanisms and modified bases). Heteroatom methylation by this mechanism accounts for a significant proportion of the modified bases in *E. coli* rRNA,[27−29] and the enzymes responsible for carrying out these reactions have largely been identified.[27] The functional impacts of these methylations and other rRNA modifications have been reviewed recently.[29] The polar reactions occurring at carbon centers require a more complicated mechanism than direct reaction with nucleophilic heteroatoms. These reactions modify the C5 position of the pyrimidine bases cytosine and uridine, but this position is only sufficiently nucleophilic to

dx.doi.org/10.1021/cb200418f | *ACS Chem. Biol.* 2012, 7, 64−72

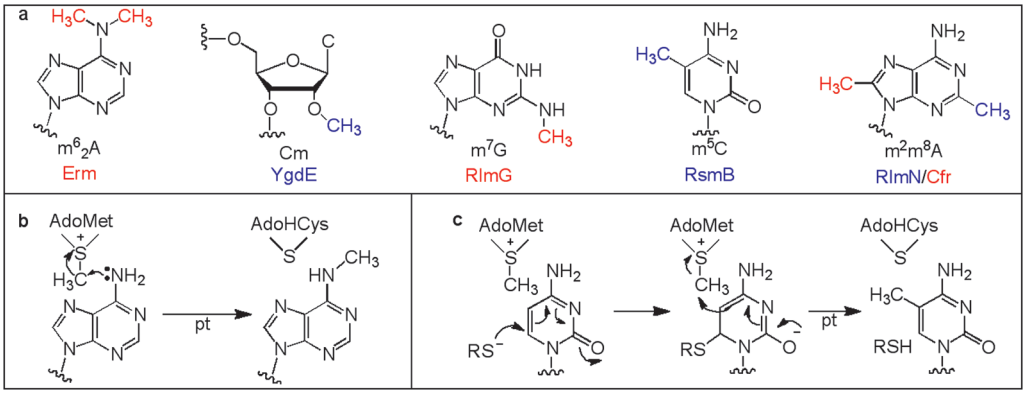


Figure 2. RNA modifications and polar methylation mechanisms. (a) Methylated bases, labeled with the modification and representative enzymes responsible for the transformation. Superscripts denote the position of modification on an RNA base; subscripts indicate the stoichiometry of methylation; 2′-*O* methylation is indicated by an m following the base that is ribose methylated. (b) Mechanism of N-methylation by a SAM-dependent methyltransferase, *e.g.*, Erm. (c) Mechanism of C5 methylation by a SAM-dependent methyltransferase, *e.g.*, RsmB. In both panels b and c, pt is used to indicate a proton transfer has taken place but is not shown explicitly.

participate in the polar reaction subsequent to the addition of an enzyme-derived cysteine to C6 (Figure 2c).[26] While resulting in the addition of a methyl group to a carbon atom, these methyltransferase reactions are distinct from the installation of a methyl group on an electrophilic position such as the C8 of adenosine, as catalyzed by Cfr employing a significantly different mechanism.

Radical SAM Methyl Synthases. The chemical demands of installing a methyl group onto the electrophilic C8 or C2 of adenosine are unique among RNA modification reactions. Cfr activity results in the methylation of the C8 of A2503,[24] and the related enzyme, RlmN, catalyzes the analogous installation of a methyl group at C2 of the same adenosine[30] (Figure 2). The recent identification of the enzymes responsible for these modifications in 23S rRNA has drawn attention to both the role that C8 methylation plays in a multidrug resistance phenotype[18] and the novel catalytic mechanism employed by these enzymes.[31−37] The electrophilic, rather than nucleophilic, character of the C2 and C8 amidine positions of adenosine, coupled with the observation of the cysteine-rich motif (CX_3CX_2C), characteristic of the radical SAM superfamily, in Cfr and RlmN implicate a radical mechanism.[24]

To assess this unique mechanism, enzymes, purified anaerobically due to the presence of oxygen-sensitive iron−sulfur clusters, were assayed with intact ribosomes and individual ribosomal components to determine likely substrates. Both Cfr and RlmN were shown to act only on A2503 within naked 23S rRNA, using either full-length rRNA or truncated substrates.[32] These *in vitro* experiments confirmed the prior *in vivo* observations of enzymatic activities.[24,30] Interestingly, in the *in vitro* experiments, Cfr was shown to modify both the C8 and C2 of A2503, *i.e.*, 2,8-dimethyladenosine was seen as a product when rRNA with no prior modification at A2503 was used as a substrate for Cfr.[32] This implies a significant degree of flexibility in the base orientation about the glycosidic linkage in A2503. In fact, both *syn* and *anti* conformations of A2503 in intact ribosomes have been observed crystallographically;[38−40] however, the relevance to the naked RNA substrate bound to Cfr remains unclear. It seems probable that the activity of the housekeeping RlmN was expanded in Cfr to include the second amidine carbon. The transition between the ancestral *rlmN*, through duplications and horizontal gene transfers, to the current *cfr* is incompletely defined, and it is unclear when *cfr*'s current role as an antibiotic resistance determinant arose.[41] There is no evidence that *cfr* is an indigenous resistance mechanism in an organism that produces an antibiotic to which *cfr* confers resistance.

Further experiments were carried out to assess the role(s) of SAM in these reactions, which apparently require SAM as both a radical initiator and as a source of newly introduced carbon. Consistent with radical SAM enzymology,[42,43] 5′-deoxyadenosine (5′-dA) and methionine were produced from the reductive cleavage of SAM.[32] Methylated bases and *S*-adenosyl homocysteine (SAH) were also observed,[32] as expected from the use of SAM as a source of the newly introduced carbon.[44] The canonical radical SAM mechanism predicts that the 5′-deoxyadenosyl radical (5′-dA$^{\bullet}$) generated by reductive cleavage of SAM will subsequently be used to abstract a hydrogen atom from the prime substrate,[45] in this case from one of the amidine positions on the adenosine base, in order to initiate the subsequent radical transformation. Exceptions to this direct substrate activation mechanism have been noted.[46−48] However, the energetics of the abstraction of an amidine hydrogen atom (BDE $\geq$ 105 kcal mol^{-1})[49,50] would appear to exceed the capacity of even as potent an oxidant as 5′-dA$^{\bullet}$.

Subsequent deuterium labeling studies revealed additional unique aspects of the reaction catalyzed by these enzymes. When truncated RNA substrates bearing 2-^{2}H adenosine (2-D A) at all positions normally occupied by adenosine were employed, the resulting methyladenosine products bore -CH_2D groups, indicating the amidine hydrogen was retained in the product. Furthermore, the 5′-dA product from these reactions bore no deuterium, demonstrating that the 5′-dA$^{\bullet}$ was not being employed to abstract a hydrogen atom from the RNA substrate. Reciprocal studies using unlabeled RNA and [*methyl*-$^2H_3$]-SAM (CD_3-SAM) yielded CD_2H methyl groups in the methyladenosine products and monodeutero 5′-dA.[31] This outcome indicated that 5′-dA$^{\bullet}$ activates a methyl group derived from SAM for addition into the RNA substrate, rather than activating the RNA substrate directly. Together, these observations led to the notion that these enzymes do not act as methyltransferases but rather as methyl synthases, which assemble a methyl group from a methylene (ultimately derived from SAM) and the hydrogen atom from the substrate amidine carbon[31] (Figure 3a).

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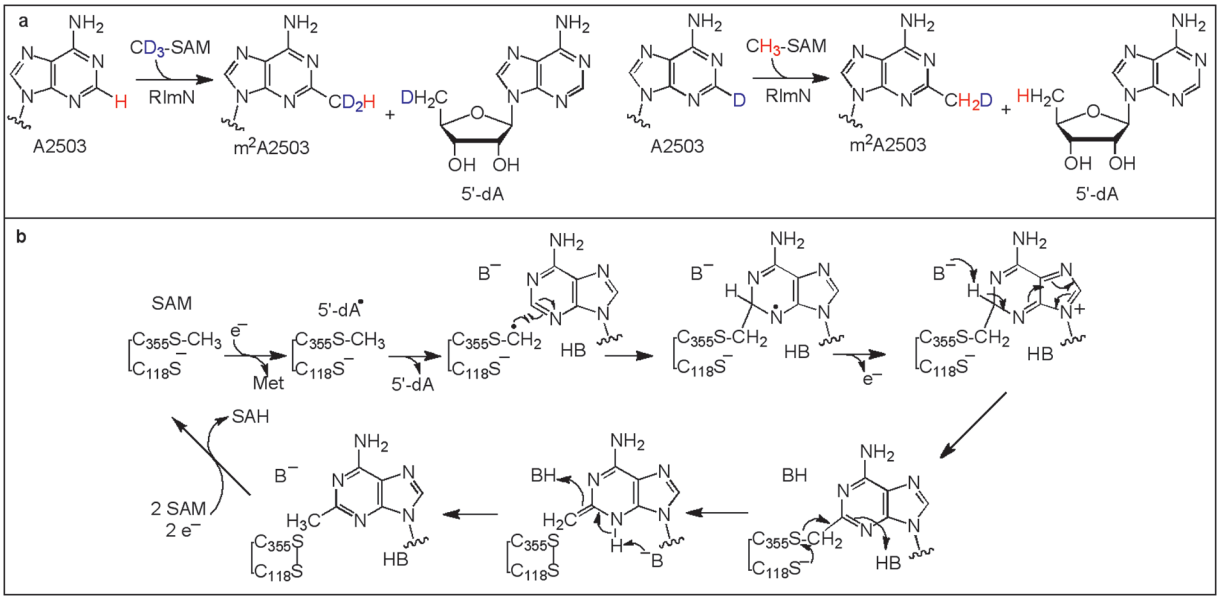

Figure 3. Deuterium labeling patterns observed in RlmN and the proposed RlmN mechanism. (a) The observed incorporation and retention of deuterium from various labeling experiments carried out with RlmN. (b) The mechanism of catalysis by RlmN proposed by Grove *et al.* (modified from ref 34).

Labeling studies carried out by a second group yielded additional evidence supporting the methyl synthase activity of Cfr and RlmN.[34] When reactions were carried out using a significantly truncated RNA substrate under single turnover conditions, it was noted that the methyl group installed did not directly reflect the isotopic composition of the SAM added to the reaction (*i.e.*, the methylated base contained a -CH_3 whether CD_3-SAM or unlabeled SAM was used). However, when the enzymes were produced in a methionine auxotroph supplemented with [*methyl*-$^2H_3$]-methionine (resulting in CD_3 labeling of all methionine residues and positions methylated by SAM-dependent reactions *in vivo*), the RlmN methylated products bore CD_2H groups.[34]

Parallel experiments in Cfr revealed CH_3, CDH_2, and CD_2H groups in the methylated product, implying significant proton exchange of an intermediate and requiring further mechanistic evaluation.[34] These results are consistent with the incorporation of a methylene fragment, rather than an intact methyl group, but they also implied that the methylene fragment was protein-derived. This was further evaluated by mass spectrometric analysis of RlmN peptides, revealing an *S*-methylated cysteine residue at position 355,[34] which was observed subsequently by crystallography.[33]

Recently, it has been demonstrated that enzymes purified without intact iron sulfur clusters are devoid of the *S*-methyl group. Upon reconstitution of the clusters, the *S*-methyl group is formed in a SAM-dependent reaction concomitantly with SAH production, implying SAM binding at the cluster is required for the typical polar reaction of SAM with the enzyme-derived cysteine.[35] This combined evidence for the methylated cysteine has led to the proposed mechanism (Figure 3b) in which Cys355 is premethylated by SAM. The cluster-generated 5′-dA• then reacts with this *S*-methyl to produce the methylene fragment, which is then added to the substrate.[34] This mechanism is likely to be more energetically favorable than abstracting a hydrogen atom directly from the methyl group of SAM, due to the stabilizing effects of the sulfur lone pair, which is diminished in SAM, due to the presence of the positive charge on sulfur.[51] While a subsequent general base abstraction of the amidine hydrogen is proposed, the observed complete retention of this hydrogen requires that this general base be fully protected from solvent. The proposed mechanism also includes roles for two cysteine residues, unassociated with the iron sulfur cluster, that were previously noted to be required to confer antibiotic resistance *in vivo*.[41] Cumulatively, these labeling data indicate that nature evolved a new chemical strategy to incorporate a methyl group at an electrophilic center, one where the methyl group assembly is initiated *via* addition of a thiomethylene into the substrate.

The rapid expansion of our mechanistic understanding of these enzymes has generated ample questions for immediate study.[31−37] Multiple experiments have indicated that the amidine hydrogen is retained in these reactions,[31] yet the flexibility implied by Cfr's dual specificity[32] would seem contrary to the controlled active site environment or total solvent exclusion required to achieve this retention. The reactions catalyzed by Cfr and RlmN require the input of 2 electrons; however, the timing of electron injection, whether critical microscopic steps are oxidative or reductive, and the identity of the physiological reductant all remain unresolved. Further characterization of proposed intermediate species, particularly adducts or radicals, would seem the most informative in terms of understanding the critical microscopic steps involving electron transfer.

While enzymologists are well situated to continue elucidating the mechanistic aspects of this novel radical SAM methyl synthase activity, the physiological and functional roles underlying the initial evolution of C2 and C8 methylation remain elusive. The impact of C2 methylation on overall genetic fitness is minimal, and any antibiotic resistance is modest.[52] However, this modification may play a role in ribosome stalling during the translation of regulatory genes and subsequent activation of inducible resistance genes.[38] The C8 modification is more complicated, as the duplication and mutation leading to C8 reactivity may have occurred in plants, where its activity and biological role are entirely undefined.[41]

dx.doi.org/10.1021/cb200418f | *ACS Chem. Biol.* 2012, 7, 64−72

This gives few clues as to why bacteria obtaining the gene by horizontal gene transfer would have maintained it outside of an antibiotic-selecting environment, despite its low fitness cost.[53] Perhaps the most overarching question is whether there are additional sites modified by this mechanism, as it seems unlikely that this novel mechanism would have evolved exclusively to modify a single position.

STRUCTURAL BASIS OF RESISTANCE TO PTC-TARGETED ANTIBIOTICS

The emergence of pathogens with multiple resistance phenotypes such as those carrying the *mlr* (modification of large ribosomal subunit) operon (containing both *cfr* and *erm*), which confers resistance to 7 classes of PTC targeting antibiotics,[21] is certainly a cause for alarm from a public health perspective. Further, the relatively rapid emergence of resistance phenotypes in clinical strains[15,16] highlights the need for drug development strategies that can counter these resistance modifications.[1,19] Recent work with ancient bacteria has indicated that the selective pressure exerted by clinical overuse of antibiotics may not be directly responsible for the evolution of resistance mechanisms; however, misuse of antibiotics may still hasten the spread of these resistance determinants and diminish the utility of the corresponding antibiotics.[54] High-resolution structures of the bacterial ribosome with antibiotic compounds bound to the PTC has provided both a molecular-level understanding of the interactions between antibiotics and PTC residues as well as a basis for modifications to PTC-targeted drugs that may help evade resistance modifications.[11−13,55]

Two varieties of structural changes (and their combination) can be envisioned to counteract perturbations introduced by target modification: elaboration of the compound to gain additional favorable interactions and removal of moieties predicted to clash with target modifications. Both of these design strategies have yielded positive results,[56,57] due in part to the availability of high-quality structural models.[11,13,55] Despite this, species-dependent idiosyncratic interactions of antibiotics with target ribosomes[11] and incomplete understanding of PTC-targeted antibiotic mechanisms of action[4,58,59] still present challenges to drug development efforts. The most recent structural information regarding PTC-targeting antibiotics and the bacterial ribosome has served to clarify the interactions of four classes of antibiotics with the bacterial PTC, including those of a human pathogen (*E. coli*).[11] While largely affirming the conservation of the binding sites and interactions of these drugs with bacterial PTCs in general,[11,13] these structures also illustrated that species-dependent interactions can contribute significantly to the overall affinity and specificity of these drugs.[11]

The critical interaction of macrolides with A2058, as indicated by Erm-dependent resistance mechanisms and the frequency of mutation of A2058 in resistant strains,[58,60−63] has led to many attempts to counteract this resistance by introducing changes to the macrolide scaffold.[64] The success of ketolides at inhibiting the growth of strains rendered resistant to erythromycin by the action of Erm was attributed to additional favorable interactions afforded by the alkyl-aryl substituent of these drugs. The combined evidence of telithromycin resistance mutations, chemical footprinting experiments, and structural data now reveal the nature of the interaction between the *E. coli* ribosome and the alkyl-aryl arm of telithromycin.[11,65−69] A π-stacking interaction of the aryl group with the A752:U2609 base pair, which forms an interdomain bridge not present in ribosome structures from other organisms (Figure 4), confirmed that a species-dependent

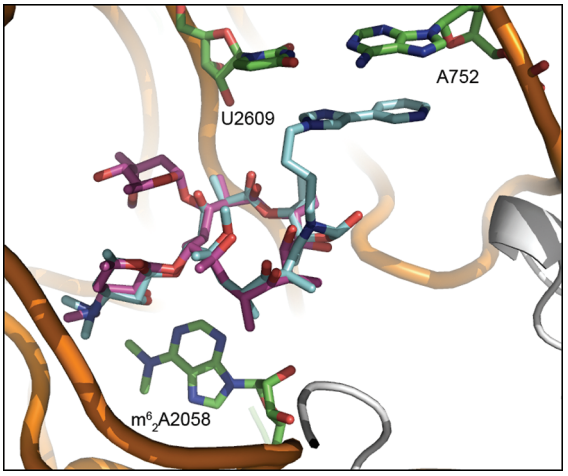

Figure 4. Erythromycin and telithromycin bound to *E. coli* ribosomes. Critical residues are indicated. The RNA backbone is shown as an orange ribbon; the protein chain is shown as a gray ribbon. Erythromycin is colored fuschia, and telithromycin is colored light blue. The figure was generated using Pymol from PDB files 3OAT and 3OFR.

interaction can indeed be responsible for increasing the affinity of these compounds several hundred-fold.[13,56] However, an alkyl-aryl arm cannot be relied upon to function identically to that of telithromycin in all cases. When a similar compound was made with the pendant group at C6 (Cethromycin, Figure 1), it exhibited encouraging preliminary results but failed during phase III trials due to lack of efficacy.[64,70−72]

Beyond the elaboration of the alkyl-aryl substituent on ketolides, the point of attachment and, to some extent, the macrolide scaffold have been modified to recover some of the interaction lost due to resistance mechanisms.[64] The so-called bicyclolides introduce a second heterocycle between the 6 and 11 or 6 and 3 positions.[73,74] The 6,11-bicyclolide modithromycin (Figure 1) exhibits improved *in vitro* efficacy against *erm*$^+$ strains of *S. pyogenes* showing idiosyncratic resistance to telithromycin.[75] Another area of development unrelated to resistance is the alteration of the alkyl-arm to one containing an azetidine (azetidinyl ketolides in Figure 1). Addition of this moiety diminishes the hepatotoxicity of telithromycin.[76] This improvement would allow application of the drug to a wider range of infections and a more expanded patient pool, both critical criteria for broad-spectrum agents.

An indirect approach to mitigate Erm-mediated resistance and restore clinical utility of impacted macrolides would be to inhibit the Erm enzymes. Previous inhibitors of Erm enzymes were found to bind to the conserved SAM binding site.[77−79] However, employing the conserved binding site of a common metabolite makes selective inhibition challenging. New approaches have been used to probe the sequence and structural elements of RNA substrates critical for methylation by Erm enzymes. This work has elucidated the minimal RNA substrate for Erm enzymes, which could guide the design of RNA analogs as Erm-specific inhibitors.[80] These compounds would then be coadministered with macrolides, similar to the

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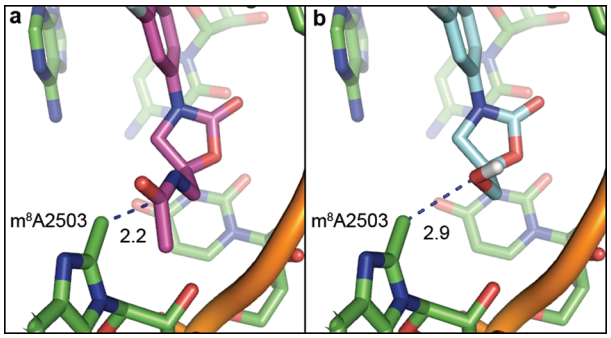

Figure 5. Linezolid and torezolid in *Deinococcus radiodurans* ribosomes. The 8-methyl group on A2503 was modeled using Pymol. (a) Linezolid is shown in fuschia (from PDB file 3DLL). (b) Torezolid is shown in light blue; the acetamidomethyl pendant group of linezolid was replaced by the hydroxymethyl of torezolid in Pymol. The distance, in angstroms, demarcated by a dashed blue line, between the C-8 methyl on A2503 and the nearest heavy atom of the antibiotic is indicated in both a and b.

coadministration of β-lactams with β-lactamase inhibitors (*e.g.*, Augmentin).

Substantial development of PTC-targeted antibiotics can also be found in pleuromutilins and oxazolidinones.[64] The first pleuromutilin for human clinical use (Retapamulin) was FDA-approved in 2007, although there had been extensive (greater than 30 years) prior veterinary usage.[64,81,82] Significant development of this underrepresented class was aimed at addressing macrolide resistance, as Erm-mediated mechanisms do not generally confer resistance to pleuromutilins. As a fully synthetic (as opposed to the predominant semisynthetic and natural product-derived) scaffold, the oxazolidinones held significant promise as a method to evade existing resistance mechanisms while avoiding new ones as there were no antibiotic producing strains serving as pools of resistance genes.[83]

The emergence of Cfr-related resistance to multiple antibiotic classes including the oxazolidinones proved that a novel, fully synthetic scaffold was not a late twentieth-century magic bullet, as the oxazolidinones bind to a site already exploited by other antibiotics.[84−86] However, in the case of Linezolid, both the modification of the aryl pendant group to gain further favorable binding interactions and the "de-elaboration" of the acetamide to an alcohol (administered as a prodrug phosphate) led to the development of radezolid and torezolid (phosphate), respectively.[87,88] Both of these compounds exhibit activity against linezolid resistant strains, although radezolid is not as effective against Cfr-mediated resistance as it is against mutations in the 23S rRNA or ribosomal proteins L3 or L4.[57] Torezolid is active against *cfr*$^+$ strains at least in part due to the removal of the bulk of the acetamide that clashes with the 8-methyl group on A2503, the site of *cfr*-mediated methylation (Figure 5).

Currently, among the PTC-targeted antibiotics, the clinical candidate pool is skewed toward oxazolidinones and pleuromutilins due both to the novelty of the scaffolds to human clinical use and the ability of both families to evade (at least partially) widespread Erm-based resistance. Clearly the spread of *cfr*$^+$ strains poses a challenge for the continued clinical utility of oxazolidinones and pleuromutilins. It is probable that new resistance mechanisms will continue to surface, requiring the elaboration of existing scaffolds, the development of novel scaffolds as well as efforts to rescue some drugs from obsolescence by inhibition of resistance-causing enzymes.

CONCLUSIONS

The critical function of the PTC in translation has led to the large number of fine-tuning modifications of the proximal rRNA; it has also made it a common target for natural product antibiotics and subsequent resistance modifications. The continuing structural work on the ribosome provides an ever more refined picture of both the critical sites within the ribosome as well as the interactions between the ribosome and its small molecule binding partners, particularly PTC-targeting antibiotics.[11,13,55] This progress is crucial, as even small refinements can yield significant insights into biological function and drug design when interrogating a system as finely tuned and critically important as the ribosome.[12] Our growing appreciation of the mechanisms (including the newly discovered methyl synthase activity of Cfr and RlmN[31−35]), the timing of action, and the specificity of rRNA modifying enzymes that lead to antibiotic resistance may allow inhibition of these enzymes and the rescue of antibiotics rendered ineffective by rRNA modifying resistance mechanisms.[8,9,19,80] Multiple drug design approaches are yielding some ability to counter resistance mechanisms, but the overlapping binding sites of most PTC-targeted antibiotics and the relatively minor variations that can lead to resistance present significant challenges. Multidrug resistant phenotypes are unlikely to disappear, and thus continued work on these fronts is critical to ensure a supply of safe, broad-spectrum compounds to safeguard the gains in public health enabled by the use of antibiotics.

Meanwhile, innovative techniques have allowed researchers to compete with microbes on their terms. Recent efforts in bacterial phenotypic profiling have uncovered novel interactions of gene and drug function, raising the possibility of drug−drug synergies of potential clinical value.[89] Thorough validation of antibiotic targets in multiple genetic backgrounds in a cell-based assay has proven to correlate a drug to its target while revealing its method of entry, efflux sensitivity, and resistance mechanism(s). Possessing this information from the outset of drug development, while also starting from compounds with empirical biological activity, would seem to provide a significant advantage.[90] Animal (*C. elegans*) models have been employed to screen for compounds that would otherwise fail during *in vitro* screening, such as those that act as prodrugs, target virulence factors, or influence host immune response.[91] Given the wide range of subtle factors employed by microbes to fine-tune ribosomal function and evade antibiotic activity, sustained, innovative, and cooperative efforts must be made in research and drug development to counteract resistance and maintain the efficacy of antibiotics. We should also bear in mind that the public health utility of antibiotic compounds is enhanced by the increased comprehension of biological complexity and underlying bacterial biochemical function afforded by ongoing investigation of modes of antibiotic action and resistance mechanisms.[92]

AUTHOR INFORMATION

Corresponding Author

*E-mail: fujimori@cmp.ucsf.edu.

■ ACKNOWLEDGMENTS

We would like to thank C. Fitzsimmons and F. Yan for critical comments on this manuscript. Our work on RNA modification and antibiotic resistance is supported by NIAID (R01AI095393-01) and NSF (CAREER 1056143).

■ KEYWORDS

Methyltransferase: an enzyme that catalyzes the transfer of an intact methyl group from the biological methyl donor, *S*-adenosyl-L-methionine, to a nucleophilic site on a target molecule *via* a polar mechanism; Methyl synthase: an enzyme that catalyzes the installation of a methyl group at a specific site through the assembly of the methyl from constituent fragments, such as a methylene and a hydride; Radical SAM superfamily: a family of enzymes that catalyze diverse reactions initiated by a 5′-deoxyadenosyl radical formed by the reductive cleavage of *S*-adenosyl-L-methionine at a [4Fe-4S] cluster ligated by a conserved CX_3CX_2C motif; Peptidyltransferase center: the active site of the ribosome, where peptide linkages are formed and from which the peptide is released. This is also a common site of interaction for antibiotic compounds; Ribosome: the protein biosynthetic machinery of the cell, composed of rRNA and protein components, commonly subdivided into the large and small subunits; Antibiotic: a chemotherapeutic agent employed to inhibit the growth of microbes; this term is often used interchangeably with antibacterial; Antibiotic resistance: a means by which bacteria evade the activity of an antibiotic compound; commonly employed resistance mechanisms include active drug efflux, drug modification or inactivation, and modification of the target of the antibiotic compound

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