The Complete Structure of the Mycobacterium smegmatis 70S Ribosome

Journal Article**Author(s):**

Hentschel, Jendrik; Burnside, Chloe; Mignot, Ingrid; Leibundgut, Marc; Boehringer, Daniel; Ban, Nenad

Publication date:

2017-07-05

Permanent link:

<https://doi.org/10.3929/ethz-b-000192333>

Rights / license:

[Creative Commons Attribution 4.0 International](#)

Originally published in:

Cell Reports 20(1), <https://doi.org/10.1016/j.celrep.2017.06.029>

Graphical Abstract

CellPress

The Complete Structure of the *Mycobacterium smegmatis* 70S Ribosome

Jendrik Hentschel,¹ Chloe Burnside,¹ Ingrid Mignot,¹ Marc Leibundgut,¹ Daniel Boehringer,¹ and Nenad Ban^{1,2,*}

¹Institute of Molecular Biology and Biophysics, Eidgenössische Technische Hochschule (ETH) Zürich, Otto-Stern-Weg 5, Zürich 8093, Switzerland

²Lead Contact

*Correspondence: ban@mol.biol.ethz.ch

<http://dx.doi.org/10.1016/j.celrep.2017.06.029>

SUMMARY

The ribosome carries out the synthesis of proteins in every living cell. It consequently represents a front-line target in anti-microbial therapy. Tuberculosis ranks among the leading causes of death worldwide, due in large part to the combination of difficult-to-treat latency and antibiotic resistance. Here, we present the 3.3-Å cryo-EM structure of the 70S ribosome of *Mycobacterium smegmatis*, a close relative to the human pathogen *Mycobacterium tuberculosis*. The structure reveals two additional ribosomal proteins and localizes them to the vicinity of drug-target sites in both the catalytic center and the decoding site of the ribosome. Furthermore, we visualized actinobacterium-specific rRNA and protein expansions that extensively remodel the ribosomal surface with implications for polysome organization. Our results provide a foundation for understanding the idiosyncrasies of mycobacterial translation and reveal atomic details of the structure that will facilitate the design of anti-tubercular therapeutics.

INTRODUCTION

The ribosome is responsible for protein synthesis throughout all three domains of life. A wealth of biochemical and structural data continues to emerge, revealing an ever more complete picture of this central cellular process. Structural insights into ribosomes from both Gram-negative and Gram-positive species have revealed that the structure of the bacterial ribosome is generally well conserved (Khusainov et al., 2016; Schmeing and Ramakrishnan, 2009; Sohmen et al., 2015). It consists of a small 30S subunit with 21 proteins and a 16S rRNA, as well as a large 50S subunit with 36 proteins, a 23S rRNA, and a 5S rRNA, with only subtle deviations between different species. Sequence analysis of ribosomes of the Gram-positive genus *Mycobacterium*, however, suggests that they exhibit more extensive structural differences compared to other species within the eubacterial domain. Several mycobacterial ribosomal proteins are extended by up to 70 residues when compared to the eubacterial core ribosome, while the mycobacterial 23S rRNA contains

several additional rRNA helices in domains I and II and, most strikingly, an insertion of more than 100 nt in size within domain III, termed “helix H54a” (Shasmlal and Sengupta, 2012).

The ribosome is successfully targeted by a variety of antibiotic classes, including the aminoglycosides, macrolides, lincosamides, and many others (Arenz and Wilson, 2016). The determination of high-resolution structures and the elucidation of the structural and functional differences between the prokaryotic and eukaryotic translation machinery are vital for the improvement and development of pathogen-specific drugs.

Mycobacterium tuberculosis is the etiological agent of human tuberculosis (TB), an illness that represents the leading cause of death caused by infectious diseases. Approximately one third of the world’s population is infected by the bacterium (World Health Organization [WHO], 2016); however, most infections are latent due to the ability of *M. tuberculosis* to persist in host cells in a dormant state, in which translation is downregulated and, hence, less susceptible to antibiotics (Kumar et al., 2012; Smith et al., 2013). In addition, the success of TB treatment is severely compromised by the high occurrence of multi-drug resistance. Since 2016, the WHO has sought to implement an “End TB Strategy,” with ambitious goals toward the reduction of the global number of TB-associated deaths, improvement of early diagnosis, and the development of better treatment options, including the development of new anti-tubercular compounds (Uplekar et al., 2015).

Oxazolidinones represent a novel class of antibiotics that target the peptidyl-transferase center within the 50S large ribosomal subunit (Shaw and Barbachyn, 2011). The oxazolidinone linezolid displays encouraging effects in the treatment of multi-drug-resistant bacteria such as *Staphylococcus aureus* (Livermore, 2003) but also *M. tuberculosis* (Alcalá et al., 2003). Other anti-tubercular antibiotics such as the aminoglycosides streptomycin and kanamycin or the peptide antibiotic capreomycin target the 30S small ribosomal subunit and interfere with decoding and translocation (Johansen et al., 2006; Magnet and Blanchard, 2005; Modolell and Vázquez, 1977).

Mycobacterium smegmatis represents a useful model organism for mycobacterial research in terms of safety, growth properties, and genetic tractability. It has been widely used to study antibiotic action and resistance biochemically (Sander et al., 2002). Low-resolution reconstructions of the *M. smegmatis* 70S ribosome to 12 Å (Shasmlal and Sengupta, 2012) and a stalled *M. tuberculosis* 50S ribosomal subunit to ~9 Å (Li et al., 2015) visualized some of the extended surface features of the

Figure 1. Structure of the *Mycobacterium smegmatis* 70S Ribosome

(A) Cryo-EM density map of the 70S ribosome filtered to local resolution (50S, blue; 30S, yellow). Mycobacterium-specific expansions are in red; the altered conformation of bL9 is shown in orange. (B) Atomic coordinates of the 70S ribosome colored as in (A). The location of ribosomal proteins bL37 and bS22, uncovered here, as well as the mRNA entrance and exit channels are indicated. The mRNA is indicated in green cartoon, and the P-site tRNA is indicated in pink surface.

mycobacterial ribosome. However, the reconstructions were of insufficient resolution to allow molecular interpretation or structure-based design of antibiotics.

Using electron cryo-microscopy (cryo-EM), we determined the near-atomic structure of the complete 70S ribosome from *Mycobacterium smegmatis*, in which we characterize additional ribosomal proteins and trace mycobacterial rRNA and protein expansions. These features are clustered around functional hotspots of the ribosome, revealing functional insights into the mechanism of translation, antibiotic binding, and drug resistance in *Mycobacteria*.

RESULTS AND DISCUSSION

The Structure of the *Mycobacterium smegmatis* 70S Ribosome

Samples of 70S ribosomes were isolated from *Mycobacterium smegmatis* strain mc² 155 and resolved by single-particle electron cryo-microscopy (cryo-EM) (Figure S1). We calculated and refined three-dimensional reconstructions of the 70S ribosome in the non-rotated P/P state, as indicated by the presence of a P-site tRNA in the active site of the 70S ribosome, to 3.3-Å resolution. Through focused alignment, we furthermore generated maps of the large and the small ribosomal subunits to 3.2-Å and 3.5-Å resolution, respectively (Figures 1, S1, and S2). The EM density maps enabled us to build and refine the structure of, essentially, the entire 70S ribosome (Figures S3 and S7; Tables S1 and S2). The more flexible ribosomal proteins uL10, uL11, and uS2 at the periphery of the 70S were resolved at lower local resolution (Figure S2). Therefore, we generated homology models of the relevant domains of these proteins, which were fitted as rigid bodies prior to refinement.

The structure reveals the substantial deviations between the architecture of the mycobacterial ribosome and the conserved features of the prototypical eubacterial ribosome (Figure 1A). Elaborate rRNA expansions reshape the surface of the mycobacterial ribosome at the lateral 50S side and the central protuberance (CP), whereas almost all alterations in the protein complement are located on the 30S solvent side and close to the mRNA entrance channel.

Several ribosomal proteins of *Mycobacteria* exist in multiple isoforms that are predicted to be variably expressed and incorporated into ribosomes depending on the presence or absence of zinc ions (Prisic et al., 2015). We observed and built the ribosomal proteins bL28, bL31, bL33, bL36, uS14, and bS18 in their zinc-bound form. Notably, we discovered two previously unknown ribosomal core proteins, which we named bL37 and bS22 in accordance with the new nomenclature of ribosomal proteins (Ban et al., 2014) (Figure 1B).

Unique rRNA Expansions Remodel the 50S Surface

Specific expansions on the ribosomal surface confer the potential for the ribosome to engage with other cellular processes and components in a species-specific manner. The 23S rRNA of *Mycobacteria* exhibits additional helical stem-loops in domain I (H15, H16a), domain II (H31a), and domain III (H54a) that are exposed on the 50S solvent side (Figures 1, 2A, and S12). With the exception of H31a, these expansions are conserved within the phylum Actinobacteria, which comprises the important genera *Mycobacterium*, *Corynebacterium*, and *Streptomyces* (Figures S4–S6).

In our structure, H31a establishes a contact to ribosomal protein bL27 at the base of the central protuberance (Figure 2B). This ribosomal protein is interesting, since its conserved N-terminal tail reaches into the ribosomal core to engage the essential 23S rRNA P loop and to interact directly with tRNAs in the P and A sites. Small N-terminal truncations of bL27 are known to negatively affect peptidyl transfer (Maguire et al., 2005). The interactions between the bL27 N terminus and tRNAs were visualized in crystal structures of *T. thermophilus* ribosomes; however, they are expected to be conserved also among other bacteria (Voorhees et al., 2009). In our structure, the N-terminal residues

Figure 2. Mycobacterial rRNA Expansions and Protein Rearrangements at the 50S Periphery

(A) The 70S ribosomal structure is shown in surface representation. 50S and 30S elements are in blue and yellow, respectively. Species-specific features cluster to a region around the L1-stalk base and the central protuberance (CP). The H54a expansion reaches toward the mRNA exit site. The mRNA is modeled from PDB: 4V7C (black).

(B) Interaction between the mycobacterium-specific H31a and bL27 at the CP. The contacts are established by flipped-out bases A758 and G759. The C terminus of bL27 is stabilized by H31a, while its N-terminal tail protrudes into the ribosomal core to engage the P-site tRNA (P-tRNA, purple). NTD, N-terminal domain.

(C) Additional intersubunit bridge formed between the H54a element and protein bS6. Arg17 points toward a conserved and base-paired stretch within H54a. Nucleotide ladder representation indicates regions of base-paired stretches.

(D) The actinobacterium-specific helices H15 (light red) and H16a (red) engage in a kissing-loop interaction. The *M. smegmatis* (Ms) bL9 C-terminal domain (orange) is accommodated in a previously unobserved position stabilized by H15 and H10. In *E. coli* (Ec), bL9 contacts bS6 (gray; PDB: 5AFI). See also Figure S9.

of bL27 (i.e., Ala2 to Ala7) were disordered, probably due to the absence of an A-site tRNA. Interestingly, H31a appears strictly conserved only within the *Mycobacterium tuberculosis* complex (MTC) of pathogens that is characterized by an extremely slow growth rate (Figure S4). *M. tuberculosis* H31a is slightly longer than its *M. smegmatis* counterpart and might provide the means by which Mycobacteria adjust the position of bL27, with potential alterations to the placement of the bL27 N-terminal tail.

The most prominent actinobacterium-specific feature is H54a (Figure 2A). In the *M. smegmatis* structure, the helical insertion of 105 nt could be resolved to a local resolution of 4 to 6 Å (Figure S2). The tip of the helical element is located in vicinity of the functionally important L1 stalk and the mRNA exit channel (Figure 2A). As L1-stalk movements are known to be coupled to ribosomal subunit rotation and mRNA threading during translocation (Fei et al., 2008), we analyzed the H54a interactions in this area in more detail.

We found that H54a establishes an additional intersubunit bridge to the small ribosomal subunit protein bS6 (Figure 2C). The contact appears to be mediated by a mycobacterium-specific arginine of bS6 (Arg17), the basic side chain of which protrudes into the major groove within a conserved helical stretch of H54a (Figures 2C, S5, and S7D). Based on structural comparison with the isolated 50S subunit of *M. tuberculosis*, where H54a abuts on the 50S core (Li et al., 2015), we observed that H54a undergoes considerable conformational changes upon subunit joining (Figure S8A), and it appears that bS6 is responsible and

sufficient for holding the tip of H54a in a detached position in our 70S structure.

Furthermore, the rotation of the small subunit relative to the large subunit during translocation can be expected to affect the conformation of H54a. Comparison to the structure of a viomycin-stalled *E. coli* 70S ribosome, which represents a fully rotated ribosomal state (Brilot et al., 2013), implies a displacement of bS6 by more than 10 Å from the position currently observed in our non-rotated ribosomal structure (Figure S8B). The corresponding motion during mycobacterial translocation would, therefore, require significant rearrangements of H54a to maintain the H54a-bS6 contact.

Furthermore, at the L1-stalk base of the 50S subunit, Mycobacteria exhibit two additional helices: H15 and H16a (Figures 2A and 2D). We were able to visualize both helices in our reconstructions at a local resolution of 4 to 5 Å, which allowed us to trace the complete rRNA protrusion extending off the ribosomal surface (Figures 2D and S2). Interestingly, while the stem of H16a exhibits a low degree of conservation among Actinobacteria, the apical loops of both H15 and H16a exhibit highly conserved complementary sequences and engage in an RNA kissing-loop interaction (Figures 2D, S6, and S7B), which leads to mutual stabilization of H15 and H16a. The conservation of this tertiary rRNA structure predicts that this ribosomal surface region is of general functional importance for this bacterial phylum. RNA kissing-loop interactions have previously been described in HIV particles in particular and are, hence, a focus of drug-development

Figure 3. Mycobacterial Protein Expansions at the 30S Solvent Side and the mRNA Entrance Channel

(A) View on the 30S solvent side of the 70S ribosomal structure shown in surface representation. Species-specific protein expansions are indicated in red, as detailed in (B)–(D). The mRNA entrance and exit sites as well as 30S landmarks are indicated. The mRNA is modeled from PDB: 4V7C (black).

(B) Protein uS5 is expanded at both termini near the mRNA entrance. The N-terminal domain (NTD; 35 amino-acid residues) was disordered (red dashes), while the C-terminal domain (CTD) reached toward uS4.

(C) The mycobacterium-specific CTD of uS16 contacts uS4 at the 30S shoulder. The model does not include the remaining 45 C-terminal residues, which were disordered. The 16S rRNA helix h17 is truncated in comparison to *E. coli* (gray; PDB: 4V7C).

(D) uS17 is expanded at its N terminus and stabilized between 16S rRNA helices h7 and h9. The mycobacterium-specific h7 nucleotide U200 is thereby flipped out and stacks with Tyr9 of uS17.

research, which has yielded co-crystal structures of kissing loops bound to various antibiotic compounds (Figure S6B) (Ennifar et al., 2006; Paillart et al., 2004). Thus, it will be interesting to investigate in more detail the functional role of this actinobacterium-specific rRNA structural motif and the effect of its destabilization, for example, by small inhibitory compounds.

One possible effect of the kissing-loop formation by H15 and H16a may be the accommodation of the ribosomal protein bL9, which adopts a previously unobserved conformation in our *M. smegmatis* 70S structure (Figure 2D, orange). The central linker helix in bL9 is distorted away from the 30S subunit and the bL9 C-terminal domain (CTD) is accommodated between helices H15 and H10 of the 23S rRNA. In the *E. coli* 70S ribosome, the CTD of bL9 is able to establish an intersubunit interaction with protein bS6 and approaches the 30S subunit of a trailing ribosome in a polysome context (Figures 2D and S9) (Fischer et al., 2015). Protein bL9 has, furthermore, been observed in an extended conformation, in which it mediates crystal contacts but might likewise engage trailing polysomal ribosomes at their elongation factor entrance site (Figure S9) (Fischer et al., 2015; Naganathan et al., 2015). Therefore, the conformation of bL9 in Mycobacteria might be an indication of a species-specific alteration of polysomal translation, as will be discussed later.

Species-Specific Protein Alterations Cluster on the 30S Solvent-Exposed Side beneath the mRNA Entrance Site

The mRNA entrance channel in the 30S subunit is enclosed by the 30S head, the 30S body protein uS5, and the 30S shoulder

thereby separates from uS5 and undergoes an inward rotation to participate in the stimulation of GTP hydrolysis within the ternary complex of elongation factor Tu (EF-Tu) and tRNA (Ogle et al., 2003; Voorhees et al., 2010).

Mycobacterial uS5 is significantly extended at both its termini (Figure 3B). We observed EM density for the uS5 CTD, while the N-terminal extension was disordered, indicating that it is flexible. As the N-terminal tail of uS5 has been implicated in remodeling the mRNA entrance upon mRNA binding in mitochondrial and chloroplast ribosomes (Bieri et al., 2017; Greber et al., 2015), it may play a similar role in Mycobacteria and become ordered in the actively translating ribosome.

In addition, in our structure, the shoulder protein uS4 is contacted by the C-terminal domain of protein uS16, which is extended by 74 amino-acid residues when compared to *E. coli* (Figure 3C). Given the dynamics that occur during decoding, the contact that we uncover between the 30S body and shoulder is noteworthy, as it may provide increased rigidity to the 30S subunit in this region. Adjacent to uS16, a species-specific N-terminal extension of uS17 is wedged between 16S rRNA helices h7 and h9 (Figure 3D), similarly resulting in additional stabilization of 30S surface-exposed elements.

Mycobacterial Peculiarities in Polysome Formation and Function

Translation of the mRNA code into protein occurs by means of polysomes, in which several ribosomes line up on a single

Figure 4. Implications of Mycobacterium-Specific rRNA and Protein Expansions for Polysome Formation

(A) Mycobacterial polysome model based on a rigid-body fit of two *M. smegmatis* 70S structures into an *E. coli* polysome map (EMDB: 1582). Lower panel: view on the respective polysome interface of the leading (left) and trailing (right) ribosomes. Outlines and filled areas indicate the position and interribosomal contacts within the polysome. Dashed circles show the location of the mRNA exit (left) and entrance (right) channels. Species-specific elements are indicated in red (50S rRNA) and violet (30S proteins). H54a engages uS4, while the uS5 and uS16 C-terminal domains (CTDs) approach H54a and bS6. The tip of H54a locates to the 30S head, the mRNA entrance, and both proteins uS5 and uS4, which undergo conformational changes in the process of mRNA decoding, rationalizing a functional role of H54a (as shown in B; see also [Results and Discussion](#)).

(B) At left, cross-sections through the mycobacterial polysome model. Viewing planes are along (1) the mRNA path and (2) the intersubunit bridge of H54a and bS6, respectively. (B1) The tip of H54a traverses the interribosomal path of mRNA and might be responsible for the stalling of trailing ribosomes by binding to the 30S head and the interface of uS5 and uS4. (B2) Subunit rotation and translocation might reposition H54a through its contact to bS6 and thereby release the downstream 30S subunit. The L1 stalk, the altered conformation of bL9 (orange), and the H15-16a loop-dimer are indicated.

(EMDB: 1582). This revealed an overlap between H54a and protein uS4 at the 30S shoulder, illustrating potential polysomal contacts between these two regions (Figure 4A).

The polysomal interface can be expected to be highly dynamic under the influence of intersubunit movements of both ribosomes, as well as intrasubunit movements involving the L1 stalk and the 30S head and shoulder of the trailing ribosome ([Achenbach and Nierhaus, 2015](#)).

Moreover, H54a likely undergoes positional rearrangements, particularly upon subunit rotation (Figure S8B). Remarkably, in the non-rotated *M. smegmatis* polysome model, the tip of H54a would be placed in front of the mRNA entrance site of the trailing ribosome, with conceivable access to the 30S head, shoulder, and body at the interface of uS5 and uS4 (Figure 4A). Given these observations, the large expansion H54a likely represents a major contact area between ribosomes in mycobacterial polysomes. The unique C-terminal domains of uS5 and uS16 on the 30S surface could thereby contact H54a (Figure 4A).

In *E. coli* polysomes, the conformationally variable protein bL9 has been proposed to engage trailing ribosomes at the 30S shoulder protein uS4 or at the elongation factor entrance site to

mRNA molecule (Figure 4A). This implies additional challenges with regard to reading-frame maintenance and translational fidelity, particularly when trailing ribosomes encounter leading ribosomes due to differential rates of translation or stalling events ([Naganathan et al., 2015](#)). EM tomographic reconstructions revealed the organization of a native polysome from *E. coli* ([Brandt et al., 2009](#)), and biochemical data indicate the existence of similar polysomes in Mycobacteria ([Seshadri et al., 2010](#)). In *E. coli*, parts of the 50S solvent-exposed side, as well as the mRNA exit channel of the leading ribosome, approach the mRNA entrance and the 30S solvent-exposed side of the trailing ribosome, delineating the inter-ribosomal path of the mRNA (Figure 4) ([Brandt et al., 2009](#)). In Mycobacteria, the corresponding ribosomal interfaces are extensively remodeled (Figures 2A, 3A, and 4A). To analyze specific polysomal contact areas in a mycobacterial context, we rigid-body docked two copies of the *M. smegmatis* 70S structure into the *E. coli* polysome map

Figure 5. Actinobacterium-Specific Ribosomal Proteins

(A) Schematic showing location and constituents of the protein binding sites.

(B and C) *De novo* built and refined structures of (B) bL37 and (C) bS22. Representative high-resolution EM density is shown as mesh.

(D) Binding site of bL37 near the peptidyl-transferase center (PTC). The 23S rRNA is in light blue, the 5S rRNA in light green. The A-site tRNA (A-tRNA, modeled from PDB: 4V5D) and P-site tRNA (our structure) are in green and purple, respectively. The complete bL27 N-terminal tail is modeled from PDB: 4V5D.

(E) Binding site of bS22 between h44 (gold) and h45 near the mRNA channel and the decoding center (DC). bS22 contacts 23S rRNA helix H70. 16S rRNA is indicated in yellow, 23S rRNA elements and tRNAs are indicated as in (D), and mRNA is shown as black cartoon.

Identification of Proteins bL37 and bS22 near Functional Hotspots in the Ribosomal Core

The regions of the ribosome that are involved in the decoding of mRNA on the 30S subunit, and peptidyl transfer and release on the 50S subunit, are highly conserved at the level of both rRNA and proteins. Therefore, any differences in ribosomal structure in the vicinity of these regions may have important implications for the species-specific characteristics of translation. The high quality of the density in our EM reconstruction allowed us to identify two additional ribosomal proteins with apparent conservation within the phylum Actinobacteria (Figures 5, S10G, and S10H). bL37 was found near the peptidyl-transferase center (PTC) in the large ribosomal subunit, whereas bS22 was identified in the vicinity of the decoding center (DC) of the small ribosomal subunit (Figures 5, S11, and S12).

control polysomal translation (Figure S9) (Fischer et al., 2015; Naganathan et al., 2015). Stalling trailing ribosomes is predicted to prevent miscoding and mRNA frameshifts until the leading ribosome moves on (Naganathan et al., 2015). In *M. smegmatis*, bL9 does not extend toward neighboring ribosomes in a polysome model and adopts a more compact conformation (Figures 2D, 4A, and S9A). In addition, the putative bL9-interaction site on uS4 is being occupied by the species-specific extension of the uS16 CTD in our structure (Figures 4A and S9B). However, in closely packed translating ribosomes of *M. smegmatis*, H54a might take over the role of bL9 in *E. coli* and interact with the trailing 30S subunit, which would cause stalling of translation by preventing conformational changes of the head and shoulder in the trailing 30S subunit (Figure 4B).

The first, bL37, represents a 24-amino-acid-residue polypeptide and adopts a short α -helical fold followed by a low-complexity tail (Figure 5, left panels). It establishes contacts with several parts of the 23S rRNA as well as with the tip of the 5S rRNA, which is significantly reduced in size compared to that in *E. coli* (Figures 5D and S10A–S10C). bL37 is sandwiched between helices H39 and H40 of 23S rRNA domain II, as well as between H72 and H89 of domain V, which contains the universally conserved PTC and harbors the essential tRNA binding loops (A and P loops) (Ban et al., 2000). Being surrounded by rRNA, bL37 appears to have evolved to stabilize the functionally important architecture in this critical part of the ribosome. A similar stabilization appears to be achieved in eukaryotes by an expansion in the 23S rRNA (Figure S10C).

On the opposite side of the intersubunit space, we found the second species-specific protein bS22 intruding into the 30S subunit between 16S rRNA helices h27, h44, and h45 beneath the mRNA channel (Figure 5, right panels). This short protein (33-amino-acid residues) folds into a kinked alpha-helix and closely resembles the N-terminal part of the mitochondria-specific ribosomal protein mS38 (Figure S10D) (Amunts et al., 2015; Greber et al., 2015), indicating a common evolutionary origin. bS22 also occupies a similar position as that observed for the eukaryotic ribosomal protein eL41, although this protein forms different interactions with the rRNA (Figure S10D) (Ben-Shem et al., 2011).

The sequence of *Ms* bS22 is identical to that of *Mt* bS22 (Figure S10H), which is listed as a protein of unknown function in the TubercuList (TubercuList: Rv0500B) and as a potential drug target in the WHO's tropical disease research (TDR, Special Programme for Research and Training in Tropical Diseases) database (Agüero et al., 2008; Lew et al., 2011). Protein bS22 is highly basic (pI = 12.9), due to its high content in arginines and lysines, and establishes numerous interactions with the surrounding rRNA. Therefore, bS22 has the potential for the stabilization of h44, which undergoes significant conformational changes during translocation of tRNAs (VanLoock et al., 2000). Furthermore, contacts of bS22 with h45 might influence the flexibility of elements that line the mRNA channel near its exit site immediately upstream of the anti-Shine-Dalgarno sequence.

bS22 establishes an additional intersubunit contact with 23S rRNA helix H70, thereby potentially providing increased stability to the assembled 70S complex (Figure 5E). Being exposed to the subunit interface close to the mRNA channel, bS22 might, moreover, act as a foothold for ribosome-associated proteins that bind the 30S subunit interface during translation initiation (such as IF3), biogenesis, or dormancy, providing the opportunity for species-specific modulation of these processes.

One of the biogenesis factors known to overlap with the respective binding site of bS22 at h45 is the 16S rRNA dimethyl-transferase KsgA (Boehringer et al., 2012), which modifies two adenine residues in the tip of h45 at a late stage of 30S subunit maturation prior to formation of the decoding site (Figures S10E and S10F) (Poldermans et al., 1979). By binding to the immature 30S subunit, bS22 might probe the conformation of h45 and subsequently assist in the recruitment of KsgA and the accommodation of h44.

Furthermore, dormancy-related factors engage the 30S subunit and block tRNA binding (Agafonov et al., 2001). Latent infections by *M. tuberculosis* are the most prominent and well-researched examples for dormant Mycobacteria, which likely use similar factors that target the 30S subunit to downregulate translation (Bunker et al., 2015; Kumar et al., 2012).

Implications of Mycobacterium-Specific Ribosomal Features for Antibiotic Binding and Resistance

The mycobacterial ribosome is the target for important anti-tubercular drugs. Numerous drug binding sites have been deciphered that cluster around conserved regions such as the peptidyl-transferase center of the large subunit or the decoding

site of the small subunit. They locate close to the bL37 and bS22 proteins, which are, hence, expected to influence the chemical environment of the compounds (Figures 6 and 7).

The oxazolidinone antibiotic linezolid (LZD) binds to an rRNA pocket established by the peptidyl-transferase loop of 23S rRNA domain V and impedes peptidyl transfer (Figure 6B) (Ippolito et al., 2008; Wilson et al., 2008). Despite the fact that the binding pocket of linezolid is universally conserved, resistance mutations are clustered within different regions in a species-specific manner, suggesting as-yet-uncharacterized structural alterations surrounding the linezolid binding site and the PTC of different species (Figure 6C) (Beckert et al., 2012; Long et al., 2010; Sander et al., 2002). Few of the residues that are mutated in resistance are directly involved in binding to linezolid but are, instead, likely inducing local rearrangements of rRNA elements or proteins, thereby interfering with antibiotic action in an indirect fashion.

The predominant linezolid-resistance mutation in *E. coli* (G2032A or G2032C; *E. coli* numbering throughout) affects nucleotide G2032 within H72 of the 23S rRNA (Figure 6C) (Xiong et al., 2000). In *M. smegmatis*, these mutations confer resistance to other PTC-acting antibiotics such as the pleuromutilin valnemulin or the lincosamide clindamycin, but not to linezolid (Long et al., 2010). However, double mutations of G2032A and U2504G synergistically confer high-level linezolid resistance also in *M. smegmatis* (Long et al., 2010). Given the presence of bL37, our structure provides a plausible explanation for the differential linezolid resistance mechanism among species. bL37 establishes contacts with the H72 loop of the 23S rRNA directly adjacent to nucleotide G2032 (Figure 6C, red dashes). Consequently, also mutations in bL37 itself have the potential to influence the orientation of G2032 and the surrounding bases and thereby potentially confer antibiotic resistance.

As opposed to G2032A, the mutation of G2447U in H74 was found to cause linezolid resistance in Mycobacteria but was lethal in *E. coli* (Figure 6C) (Sander et al., 2002). In our structure, we observed another species-specific alteration in the immediate surroundings of the PTC and the G2447 nucleotide, i.e., the absence of the otherwise universally conserved nucleotide U2068 in H74 of the *M. smegmatis* 23S rRNA domain V (Figure 6D). While the deletion of U2068 is conserved throughout Actinobacteria, in *E. coli* (as in every other known ribosomal structure), the U2068 base is flipped out and appears to engage in tertiary interactions with constituents of the E-tRNA binding pocket of the 50S subunit (Figure 6D) (Selmer et al., 2006). Due to the deletion of U2068 in Mycobacteria, the surrounding stretch of H74 is remodeled close to critical regions such as the tRNA-binding P loop, but also the linezolid binding site and the G2447 nucleotide in the PTC (Figures 6D and 6E). The lack of U2068 and its tertiary interactions implies a higher degree of flexibility of the 23S rRNA near the PTC, which could lead to tolerance of the G2447U mutation in Mycobacteria and, furthermore, cause alterations with regard to tRNA binding in the P and E sites of the 50S ribosomal subunit specific to Actinobacteria. The absence of U2068 presumably conferred beneficial properties in a species-specific environment, and a deletion of this residue was therefore retained throughout evolution.

Figure 6. Drug Binding Sites and Resistance Mutations near the PTC

(A–C) The linezolid (LZD) binding site at the peptidyl-transferase center (PTC). Peptidyl-transferase loop residues are indicated in orange, and resistance mutations are indicated in red. (B and C) Atomic detail of the *M. smegmatis* PTC and the linezolid binding site (linezolid modeled from PDB: 3DLL). Colors are as in (A). The bL27 N-terminal tail and the A-site tRNA (green) are modeled from PDB: 4V5D. The P-site tRNA is shown in purple. (C) bL37 contacts H72 near G2032 (red dashes), with possible impact on linezolid action. (D and E) Mycobacteria lack the highly conserved nucleotide U2068 (*E. coli* numbering) near (D) the E-tRNA binding pocket and near (E) the linezolid-resistance mutation of G2447. A G-rich stretch in 23S rRNA H74 is remodeled in *M. smegmatis*. Corresponding *E. coli* 23S rRNA residues (gray) and the E-site tRNA (E-tRNA, light red) are modeled from PDB: 4V7C.

respective methyltransferase TlyA (Maus et al., 2005). Remarkably, several contacts of the species-specific ribosomal protein bS22 to h44 and h45 are observed in *M. smegmatis* that are expected to contribute to the maintenance of the rRNA architecture, which forms the drug binding sites (Figures 7B–7D). A single-base substitution of U1406A in h44 has been shown not only to confer kanamycin resistance but also to attenuate the virulence of *M. tuberculosis* (Figure 7E) (Watanabe et al., 2016). The U1406 phosphate group is in contact with two basic side chains of bS22 (Lys16 and Lys19), underlining the importance of this critical region for antibiotic binding and encouraging the investigation of bS22 as a drug target (Figure 7E).

Conclusions

In light of the medical relevance of the mycobacterial genus, this study emphasizes both subtle and elaborate structural alterations in the mycobacterial ribosome, which we have resolved by cryo-EM. In the structure, we identified both bS22 and bL37 as actinobacterium-specific ribosomal proteins located at functionally important areas and drug target sites. Our results should, therefore, motivate researchers to include the genetic loci of bL37 and bS22 (*Mt* Rv0500B) in candidate screens for antibiotic resistance. The reorganization of rRNA and protein elements on the mycobacterial surface suggests unique implications for the geometry and function of polysomes. Translation is a key target for regulation in adaptation to certain environmental conditions, such as starvation; physical or chemical stress; and, notably, dormancy. In the future, understanding the degree to which the structural differences are involved in the characteristic life

In contrast to oxazolidinones, aminoglycosides and anti-tubercular peptide antibiotics target the upper part of the 16S rRNA helix h44 within the 30S ribosomal subunit (Figures 7A and 7B) (Carter et al., 2000; Stanley et al., 2010). The aminoglycoside antibiotics can be subdivided into the streptomycins (streptomycin) and the deoxystreptomycins (e.g., kanamycin and paromomycin) due to differences in their chemical structure. Consequently, they bind to two adjacent but distinct sites on the 16S rRNA in the vicinity of protein uS12 (Figures 7C and 7D) (Magnet and Blanchard, 2005; Shcherbakov et al., 2010; Suzuki et al., 1998). Binding of the cyclic peptide drug capreomycin critically depends on base-methylations in the 16S rRNA helix h44, as well as in the 23S rRNA helix H69 (Figure 7B, cyan), rationalizing capreomycin resistance through the inactivation of the

Figure 7. Anti-tubercular Compounds and Resistance Mutations near the DC

(A and B) The binding sites of cyclic-peptide antibiotics and aminoglycosides are in the vicinity of the decoding center (DC) and bS22. (B–E) Atomic details of drug binding sites near the DC of *M. smegmatis*, as established by superposition of structures with bound antibiotics. The mRNA is indicated with a black cartoon, the A-site tRNA is indicated in green (PDB: 4V5D), and the P-site tRNA is indicated in pink. (B) Binding of capreomycin (CPR, cyan; PDB: 4V7M) depends on the methylation of 16S rRNA (pale yellow) as well as 23S rRNA (light blue) residues (shown in stick representation, cyan). Streptomycin (STR, light green; PDB: 1FJG) and paromomycin (PAR, violet; PDB: 1FJG) compounds exemplify aminoglycoside binding sites at varying positions at h44. (C and D) Streptomycin- and kanamycin (KAN)-resistance mutations (red) are in proximity of bS22. Kanamycin is expected to bind in a similar fashion as that of paromomycin, for which structural data are available. (E) The h44 residue A1406 (*E. coli* numbering) is contacted by bS22 side chains Lys16 and Lys19 and constitutes a determinant for the virulence of *M. tuberculosis*.

MgCl₂, 1 mM DTT, 1 mg/mL lysozyme) using a Multiflex cell cracker at 50 psi. The lysate was cleared by centrifugation (20,000 rpm, 4°C, 40 min) in an SS34 rotor (Sorvall). The supernatant was loaded onto buffered sucrose cushions (20 mM HEPES-KOH [pH 7.5], 500 mM KCl, 20 mM MgCl₂, 1 mM DTT, 50% [w/v] sucrose) and centrifuged (35,000 rpm, 4°C, 18 hr) using a Ti70 rotor (Beckman Coulter). Ribosomal pellets were dissolved in lysis buffer (omitting lysozyme) by gentle shaking for 3 hr on ice. The suspension was layered onto buffered sucrose gradients (20 mM HEPES-KOH [pH 7.5], 60 mM KCl, 10 mM MgCl₂, 1 mM DTT, 10%–40% [w/v] sucrose) to resolve 70S ribosomes by ultracentrifugation (20,000 rpm, 4°C, 16 hr) in an SW32 rotor (Beckman Coulter). Ribosomal bands were visualized by light scattering and harvested manually using a syringe. The pooled sample was concentrated in a spin concentrator (100,000 MWCO [molecular weight cutoff], Vivaspin) and buffer exchanged to ribosome buffer (20 mM HEPES-KOH [pH 7.5], 60 mM KCl, 10 mM MgCl₂, 1 mM DTT) prior to flash freezing in liquid nitrogen.

Cryo-EM Data Acquisition and Processing

Quantifoil R2/2 holey carbon grids (Quantifoil Micro Tools) were washed in ethylene acetate and coated with carbon film prior to glow discharging (negative, 25 mA, 25 s) using an Emitech K100X (Quorum Technologies). The concentration of 70S ribosomes was adjusted to 100 nM in ribosome buffer. 5 μ L of sample were applied per grid, which were blotted and plunge-frozen in a 2:1 mixture of liquid ethane and propane using a Vitrobot (FEI).

Cryo-EM data were acquired on a Titan Krios cryo-transmission electron microscope (FEI) equipped with a Falcon II direct electron detector. The microscope was operated at 300 keV with a magnification of 100,719 $\times$ and a defocus range of -1.2 to -3.5 μ m. The EPU software was used to record seven frames per image (combined dose, 20 electrons per square angstrom) that were aligned with DOSEGPU DRIFT-CORR (Li et al., 2013). CTF parameters were estimated using CTFFIND (Mindell and Grigorieff, 2003), and

cycle of Mycobacteria, which includes slow growth and the ability to persist as an intracellular pathogen, will be of prime importance for mycobacterial research and antibiotic development. Our data provide a detailed structural framework to functionally address these questions and to allow further characterization of the mycobacterial ribosome as the target for anti-tubercular therapy.

EXPERIMENTAL PROCEDURES

Cultivation and Ribosome Preparations

Liquid cultures of *M. smegmatis* strain mc² 155 were grown in Terrific Broth medium, including 0.025% (v/v) Tween-80, in shaker flasks (110 rpm, 37°C) to an optical density at 600 nm (OD₆₀₀) of 2.0. Cells were harvested by centrifugation (7,200 rpm, 4°C, 15 min) in an SLC6000 rotor (Sorvall). Lysis was performed in lysis buffer (20 mM HEPES-KOH [pH 7.5], 200 mM KCl, 20 mM

micrographs were selected based on the quality of their power spectra. Particles were located using Batchboxer (EMAN software package) (Ludtke et al., 1999) and extracted at a 72-px (pixel) (binned, 5.56 Å/px) or 320 px (unbinned, 1.39 Å/px) frame size. Subsequent data processing was performed using RELION 1.4 (Scheres, 2012). Binned particle images were used for initial 2D and 3D classifications according to the scheme shown in Figure S1. Particle classes were selected based on ribosome integrity, intersubunit movements, and tRNA occupation. High-resolution 3D reconstructions were calculated from structurally homogeneous classes using unbinned images. Focused refinements were carried out using masks for the 50S and the 30S subunits, respectively, which were created using UCSF Chimera (Pettersen et al., 2004). The focused refinements resulted in final maps at an overall resolution of 3.2 Å for the 50S subunit and 3.5 Å for the 30S subunit according to the gold-standard FSC (Fourier shell correlation) = 0.143 (Scheres, 2012). For structural interpretation and manual atomic model building, B-factor sharpening in RELION was applied. Local resolution plots were calculated by ResMap (Kucukelbir et al., 2014). Where indicated, cryo-EM maps were filtered according to local resolution for visualization purposes (Dr. C.H.S. Aylett, personal communication).

Structure Building and Refinement

Starting from the subunits of a recent high-resolution structure of the *E. coli* ribosome (PDB: 4YBB) (Noeske et al., 2015), which were individually docked into the high-resolution EM density maps of the *M. smegmatis* 50S and 30S obtained by focused classification, almost complete atomic models of both ribosomal subunits could be built using the software programs O (Jones, 2004) and COOT (Emsley et al., 2010) (Table S2). Protein bL31, located at the central protuberance, was rebuilt using the docked *T. thermophilus* homolog as a start (PDB: 2WH4) (Weixlbaumer et al., 2008). Two unassigned areas of well-resolved protein density in the EM map allowed direct and unambiguous identification of previously unknown *M. smegmatis* ribosomal proteins bL37 and bS22 (Figures 5B and 5C). For the interpretation of certain peripheral proteins (uS2, uL10, and uL11), which were resolved to lower local resolution, PHYRE models were generated and docked as rigid bodies (Kelley et al., 2015). Similarly, for the less well-resolved areas representing the peripheral rRNA elements H15, H16a, and H54a, the crystal structure of an RNA kissing loop (PDB: 2CFY) (between H15 and H16a; Figures S6B and S7) and segments of standard RNA helices representing the predicted base pairing pattern (H54a; Figures S5 and S7) were docked as rigid bodies, followed by manual readjustment and connection of the segments with each other and to the body of the rRNA in a sterically favorable manner. Remaining stereochemical outliers were corrected using PHENIX.ERRASER (Chou et al., 2016) as well as reciprocal and real-space algorithms implemented in PHENIX (Afonine et al., 2012), as described later.

The 50S and 30S structures were subjected to multiple cycles of phase-restrained reciprocal space refinement against amplitudes back-calculated from the EM maps and the maximum-likelihood target using Hendrickson-Lattman coefficients (MLHL) with PHENIX.REFINE as previously described (Greber et al., 2014). To stabilize the coordinate refinement in the less well-ordered areas of the EM map, protein secondary structure and RNA base-pair restraints were applied. Discrepancies between the EM maps and the models were detected by inspection of the $F_{\text{obs}} - F_{\text{calc}}$ difference Fourier maps (in which F_{obs} represents the observed, and F_{calc} represents the calculated structure factors), and the models were locally readjusted using O and COOT. Numerous isolated map peaks representing hexa-coordinated magnesium ions were modeled as individual magnesium atoms. The final atomic coordinates of the 50S and 30S subunits were validated using PHENIX (Afonine et al., 2012) and the MOLPROBITY server (Table S1) (Chen et al., 2010). During the geometry minimization steps of reciprocal space refinement, an optimal weighting of the model geometry versus the structure factors was obtained at a wxc value of 1.2, which resulted in both good model geometry and map fit. The estimated resolutions of the models versus the map FSCs according to the FSC = 0.5 criterion coincided well with the resolutions of the maps established from the Fourier half-set FSC correlation at the gold-standard criterion of 0.143 (Figure S3).

To obtain a complete *M. smegmatis* 70S model, the refined atomic coordinates of the 50S and 30S subunits were docked as rigid bodies into an 3.3-Å

map of a cryo-EM reconstruction encompassing the entire 70S (Figure S1). After readjustment of the intersubunit contacts and positioning of an optimized canonical *E. coli* tRNA-Phe (derived from PDB: 4V5D) (Voorhees et al., 2009), together with a short segment of mRNA (based on PDB: 1FJG) (Carter et al., 2000) into the P site to account for additional density that represents a mixture of tRNAs bound to the anticodon, the model was subjected to three cycles of reciprocal space coordinate and B-factor refinement, as outlined earlier, using PHENIX.REFINE with an optimal geometry weighting value of wxc = 1.2. The temperature factors were then refined to convergence by an additional five cycles of individual B-factor refinement (Table S1).

Creation of Figures

Graphical representations of molecular models and cryo-EM density maps were generated using PyMOL (The PyMOL Molecular Graphics System, v1.7, Schrödinger) and UCSF Chimera (Pettersen et al., 2004).

ACCESSION NUMBERS

The accession numbers for the 3.3-Å cryo-EM map of the *M. smegmatis* 70S ribosome, as well as for the 3.2-Å and 3.5-Å cryo-EM maps of the *M. smegmatis* 50S and 30S ribosomal subunits are EMD: 3751, 3750, and 3748, respectively. The accession numbers for the refined coordinates of the atomic structures of the 70S ribosome and the 50S and 30S subunits are PDB: 5O61, 5O60, and 5O5J, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes 12 figures and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2017.06.029>.

AUTHOR CONTRIBUTIONS

J.H., C.B., and I.M. purified ribosomes and prepared cryo-EM samples. J.H. and D.B. acquired the cryo-EM data. J.H. calculated the cryo-EM reconstructions. J.H., C.B., and M.L. built and refined the structure. J.H. interpreted the structure and wrote the manuscript. N.B. supervised the project. All authors contributed to the final version of the paper.

ACKNOWLEDGMENTS

Cryo-EM data were collected at the electron microscopy facility of ETH Zurich (ScopeM). We thank P. Tittmann for technical support. We acknowledge J. Läderach and Prof. Dr. E. Weber-Ban (ETH Zurich) for providing the *M. smegmatis* strain mc² 155 and for support. We thank Dr. D. Scherbakov and Prof. Dr. E. Böttger (University of Zurich) for discussions on experimental procedures. We thank Dr. C.H.S. Aylett and Dr. A. Jomaa for support with cryo-EM data processing and critical reading of the manuscript. This work was supported by the Boehringer Ingelheim Fonds PhD Fellowship (to J.H.) and the Swiss National Science Foundation (SNSF; grant number 310030B_163478), and the National Center of Excellence in Research (NCCR) RNA & Disease Program of the SNSF (grant number 51NF40_141735) (to N.B.).

Received: April 24, 2017

Revised: June 3, 2017

Accepted: June 10, 2017

Published: July 5, 2017

REFERENCES

- Achenbach, J., and Nierhaus, K.H. (2015). The mechanics of ribosomal translocation. *Biochimie* 114, 80–89.
- Afonine, P.V., Grosse-Kunstleve, R.W., Echols, N., Headd, J.J., Moriarty, N.W., Mustyakimov, M., Terwilliger, T.C., Urzhumtsev, A., Zwart, P.H., and Adams, P.D. (2012). Towards automated crystallographic structure refinement with phenix.refine. *Acta Crystallogr. D Biol. Crystallogr.* 68, 352–367.

- Agafonov, D.E., Kolb, V.A., and Spirin, A.S. (2001). A novel stress-response protein that binds at the ribosomal subunit interface and arrests translation. *Cold Spring Harb. Symp. Quant. Biol.* **66**, 509–514.
- Agüero, F., Al-Lazikani, B., Aslett, M., Berriman, M., Buckner, F.S., Campbell, R.K., Carmona, S., Carruthers, I.M., Chan, A.W., Chen, F., et al. (2008). Genomic-scale prioritization of drug targets: the TDR Targets database. *Nat. Rev. Drug Discov.* **7**, 900–907.
- Alcalá, L., Ruiz-Serrano, M.J., Pérez-Fernández Turégano, C., García De Viedma, D., Díaz-Infantes, M., Marín-Arriaza, M., and Bouza, E. (2003). In vitro activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* that are susceptible or resistant to first-line antituberculous drugs. *Antimicrob. Agents Chemother.* **47**, 416–417.
- Amunts, A., Brown, A., Toots, J., Scheres, S.H., and Ramakrishnan, V. (2015). Ribosome. The structure of the human mitochondrial ribosome. *Science* **348**, 95–98.
- Arenz, S., and Wilson, D.N. (2016). Bacterial protein synthesis as a target for antibiotic inhibition. *Cold Spring Harb. Perspect. Med.* **6**, a025361.
- Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905–920.
- Ban, N., Beckmann, R., Cate, J.H., Dinman, J.D., Dragon, F., Ellis, S.R., Lafontaine, D.L., Lindahl, L., Liljas, A., Lipton, J.M., et al. (2014). A new system for naming ribosomal proteins. *Curr. Opin. Struct. Biol.* **24**, 165–169.
- Beckert, P., Hillemann, D., Kohl, T.A., Kalinowski, J., Richter, E., Niemann, S., and Feuerriegel, S. (2012). rplC T460C identified as a dominant mutation in linezolid-resistant *Mycobacterium tuberculosis* strains. *Antimicrob. Agents Chemother.* **56**, 2743–2745.
- Ben-Shem, A., Garreau de Loubresse, N., Melnikov, S., Jenner, L., Yusupova, G., and Yusupov, M. (2011). The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science* **334**, 1524–1529.
- Bieri, P., Leibundgut, M., Saurer, M., Boehringer, D., and Ban, N. (2017). The complete structure of the chloroplast 70S ribosome in complex with translation factor pY. *EMBO J.* **36**, 475–486.
- Boehringer, D., O'Farrell, H.C., Rife, J.P., and Ban, N. (2012). Structural insights into methyltransferase KsgA function in 30S ribosomal subunit biogenesis. *J. Biol. Chem.* **287**, 10453–10459.
- Brandt, F., Etchells, S.A., Ortiz, J.O., Elcock, A.H., Hartl, F.U., and Baumeister, W. (2009). The native 3D organization of bacterial polysomes. *Cell* **136**, 261–271.
- Briolot, A.F., Korostelev, A.A., Ermolenko, D.N., and Grigorieff, N. (2013). Structure of the ribosome with elongation factor G trapped in the pretranslocation state. *Proc. Natl. Acad. Sci. USA* **110**, 20994–20999.
- Bunker, R.D., Mandal, K., Bashiri, G., Chaston, J.J., Pentelute, B.L., Lott, J.S., Kent, S.B., and Baker, E.N. (2015). A functional role of Rv1738 in *Mycobacterium tuberculosis* persistence suggested by racemic protein crystallography. *Proc. Natl. Acad. Sci. USA* **112**, 4310–4315.
- Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Wimberly, B.T., and Ramakrishnan, V. (2000). Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* **407**, 340–348.
- Chen, V.B., Arendall, W.B., 3rd, Headd, J.J., Keedy, D.A., Immormino, R.M., Kapral, G.J., Murray, L.W., Richardson, J.S., and Richardson, D.C. (2010). MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 12–21.
- Chou, F.C., Echols, N., Terwilliger, T.C., and Das, R. (2016). RNA structure refinement using the ERRASER-Phenix pipeline. *Methods Mol. Biol.* **1320**, 269–282.
- Emsley, P., Lohkamp, B., Scott, W.G., and Cowtan, K. (2010). Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486–501.
- Ennifar, E., Paillart, J.C., Bodlénner, A., Walter, P., Weibel, J.M., Aubertin, A.M., Pale, P., Dumas, P., and Marquet, R. (2006). Targeting the dimerization initiation site of HIV-1 RNA with aminoglycosides: from crystal to cell. *Nucleic Acids Res.* **34**, 2328–2339.
- Fei, J., Kosuri, P., MacDougall, D.D., and Gonzalez, R.L., Jr. (2008). Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation. *Mol. Cell* **30**, 348–359.
- Fischer, N., Neumann, P., Konevega, A.L., Bock, L.V., Ficner, R., Rodnina, M.V., and Stark, H. (2015). Structure of the *E. coli* ribosome-EF-Tu complex at <3 Å resolution by Cs-corrected cryo-EM. *Nature* **520**, 567–570.
- Greber, B.J., Boehringer, D., Leibundgut, M., Bieri, P., Leitner, A., Schmitz, N., Aebersold, R., and Ban, N. (2014). The complete structure of the large subunit of the mammalian mitochondrial ribosome. *Nature* **515**, 283–286.
- Greber, B.J., Bieri, P., Leibundgut, M., Leitner, A., Aebersold, R., Boehringer, D., and Ban, N. (2015). Ribosome. The complete structure of the 55S mammalian mitochondrial ribosome. *Science* **348**, 303–308.
- Ippolito, J.A., Kanyo, Z.F., Wang, D., Franceschi, F.J., Moore, P.B., Steitz, T.A., and Duffy, E.M. (2008). Crystal structure of the oxazolidinone antibiotic linezolid bound to the 50S ribosomal subunit. *J. Med. Chem.* **51**, 3353–3356.
- Johansen, S.K., Maus, C.E., Plikaytis, B.B., and Douthwaite, S. (2006). Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2'-O-methylations in 16S and 23S rRNAs. *Mol. Cell* **23**, 173–182.
- Jones, T.A. (2004). Interactive electron-density map interpretation: from INTER to O. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2115–2125.
- Kelley, L.A., Mezulis, S., Yates, C.M., Wass, M.N., and Sternberg, M.J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* **10**, 845–858.
- Khusainov, I., Vicens, Q., Bochler, A., Grosse, F., Myasnikov, A., Ménétret, J.F., Chicher, J., Marzi, S., Romby, P., Yusupova, G., et al. (2016). Structure of the 70S ribosome from human pathogen *Staphylococcus aureus*. *Nucleic Acids Res.* **44**, 10491–10504.
- Kucukelbir, A., Sigworth, F.J., and Tagare, H.D. (2014). Quantifying the local resolution of cryo-EM density maps. *Nat. Methods* **11**, 63–65.
- Kumar, A., Majid, M., Kunisch, R., Rani, P.S., Qureshi, I.A., Lewin, A., Hasnain, S.E., and Ahmed, N. (2012). *Mycobacterium tuberculosis* DosR regulon gene Rv0079 encodes a putative, 'dormancy associated translation inhibitor (DATIN)'. *PLoS ONE* **7**, e38709.
- Lew, J.M., Kapopoulou, A., Jones, L.M., and Cole, S.T. (2011). TubercuList–10 years after. *Tuberculosis (Edinb.)* **91**, 1–7.
- Li, X., Zheng, S.Q., Egami, K., Agard, D.A., and Cheng, Y. (2013). Influence of electron dose rate on electron counting images recorded with the K2 camera. *J. Struct. Biol.* **184**, 251–260.
- Li, X., Sun, Q., Jiang, C., Yang, K., Hung, L.W., Zhang, J., and Sacchettini, J.C. (2015). Structure of ribosomal silencing factor bound to *Mycobacterium tuberculosis* ribosome. *Structure* **23**, 1858–1865.
- Livemore, D.M. (2003). Linezolid in vitro: mechanism and antibacterial spectrum. *J. Antimicrob. Chemother.* **51** (Suppl 2), ii9–ii16.
- Long, K.S., Munck, C., Andersen, T.M., Schaub, M.A., Hobbie, S.N., Böttger, E.C., and Vester, B. (2010). Mutations in 23S rRNA at the peptidyl transferase center and their relationship to linezolid binding and cross-resistance. *Antimicrob. Agents Chemother.* **54**, 4705–4713.
- Ludtke, S.J., Baldwin, P.R., and Chiu, W. (1999). EMAN: semiautomated software for high-resolution single-particle reconstructions. *J. Struct. Biol.* **128**, 82–97.
- Magnet, S., and Blanchard, J.S. (2005). Molecular insights into aminoglycoside action and resistance. *Chem. Rev.* **105**, 477–498.
- Maguire, B.A., Beniaminov, A.D., Ramu, H., Mankin, A.S., and Zimmermann, R.A. (2005). A protein component at the heart of an RNA machine: the importance of protein I27 for the function of the bacterial ribosome. *Mol. Cell* **20**, 427–435.
- Maus, C.E., Plikaytis, B.B., and Shinnick, T.M. (2005). Mutation of tlyA confers capreomycin resistance in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **49**, 571–577.
- Mindell, J.A., and Grigorieff, N. (2003). Accurate determination of local defocus and specimen tilt in electron microscopy. *J. Struct. Biol.* **142**, 334–347.

- Modolell, J., and Vázquez. (1977). The inhibition of ribosomal translocation by viomycin. *Eur. J. Biochem.* **81**, 491–497.
- Naganathan, A., Wood, M.P., and Moore, S.D. (2015). The large ribosomal subunit protein L9 enables the growth of EF-P deficient cells and enhances small subunit maturation. *PLoS ONE* **10**, e0120060.
- Noeske, J., Wasserman, M.R., Terry, D.S., Altman, R.B., Blanchard, S.C., and Cate, J.H. (2015). High-resolution structure of the *Escherichia coli* ribosome. *Nat. Struct. Mol. Biol.* **22**, 336–341.
- Ogle, J.M., Carter, A.P., and Ramakrishnan, V. (2003). Insights into the decoding mechanism from recent ribosome structures. *Trends Biochem. Sci.* **28**, 259–266.
- Paillart, J.C., Shehu-Xhilaga, M., Marquet, R., and Mak, J. (2004). Dimerization of retroviral RNA genomes: an inseparable pair. *Nat. Rev. Microbiol.* **2**, 461–472.
- Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605–1612.
- Poldermans, B., Roza, L., and Van Knippenberg, P.H. (1979). Studies on the function of two adjacent N6,N6-dimethyladenosines near the 3' end of 16 S ribosomal RNA of *Escherichia coli*. III. Purification and properties of the methylating enzyme and methylase-30 S interactions. *J. Biol. Chem.* **254**, 9094–9100.
- Prisic, S., Hwang, H., Dow, A., Barnaby, O., Pan, T.S., Lonzanida, J.A., Chazin, W.J., Steen, H., and Husson, R.N. (2015). Zinc regulates a switch between primary and alternative S18 ribosomal proteins in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **97**, 263–280.
- Sander, P., Belova, L., Kidan, Y.G., Pfister, P., Mankin, A.S., and Böttger, E.C. (2002). Ribosomal and non-ribosomal resistance to oxazolidinones: species-specific idiosyncrasy of ribosomal alterations. *Mol. Microbiol.* **46**, 1295–1304.
- Scheres, S.H. (2012). RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J. Struct. Biol.* **180**, 519–530.
- Schmeing, T.M., and Ramakrishnan, V. (2009). What recent ribosome structures have revealed about the mechanism of translation. *Nature* **461**, 1234–1242.
- Selmer, M., Dunham, C.M., Murphy, F.V., 4th, Weixlbaumer, A., Petry, S., Kelley, A.C., Weir, J.R., and Ramakrishnan, V. (2006). Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* **313**, 1935–1942.
- Seshadri, A., Singh, N.S., and Varshney, U. (2010). Recycling of the posttermination complexes of *Mycobacterium smegmatis* and *Escherichia coli* ribosomes using heterologous factors. *J. Mol. Biol.* **401**, 854–865.
- Shasmal, M., and Sengupta, J. (2012). Structural diversity in bacterial ribosomes: mycobacterial 70S ribosome structure reveals novel features. *PLoS ONE* **7**, e31742.
- Shaw, K.J., and Barbachyn, M.R. (2011). The oxazolidinones: past, present, and future. *Ann. N Y Acad. Sci.* **1241**, 48–70.
- Shcherbakov, D., Akbergenov, R., Matt, T., Sander, P., Andersson, D.I., and Böttger, E.C. (2010). Directed mutagenesis of *Mycobacterium smegmatis* 16S rRNA to reconstruct the *in vivo* evolution of aminoglycoside resistance in *Mycobacterium tuberculosis*. *Mol. Microbiol.* **77**, 830–840.
- Smith, T., Wolff, K.A., and Nguyen, L. (2013). Molecular biology of drug resistance in *Mycobacterium tuberculosis*. *Curr. Top. Microbiol. Immunol.* **374**, 53–80.
- Sohmen, D., Chiba, S., Shimokawa-Chiba, N., Innis, C.A., Berninghausen, O., Beckmann, R., Ito, K., and Wilson, D.N. (2015). Structure of the *Bacillus subtilis* 70S ribosome reveals the basis for species-specific stalling. *Nat. Commun.* **6**, 6941.
- Stanley, R.E., Blaha, G., Grodzicki, R.L., Strickler, M.D., and Steitz, T.A. (2010). The structures of the anti-tuberculosis antibiotics viomycin and capreomycin bound to the 70S ribosome. *Nat. Struct. Mol. Biol.* **17**, 289–293.
- Suzuki, Y., Katsukawa, C., Tamaru, A., Abe, C., Makino, M., Mizuguchi, Y., and Taniguchi, H. (1998). Detection of kanamycin-resistant *Mycobacterium tuberculosis* by identifying mutations in the 16S rRNA gene. *J. Clin. Microbiol.* **36**, 1220–1225.
- Takay, S., Hickerson, R.P., and Noller, H.F. (2005). mRNA helicase activity of the ribosome. *Cell* **120**, 49–58.
- Uplekar, M., Weil, D., Lonnroth, K., Jaramillo, E., Lienhardt, C., Dias, H.M., Falzon, D., Floyd, K., Gargioni, G., Getahun, H., et al.; WHO's Global TB Programme (2015). WHO's new end TB strategy. *Lancet* **385**, 1799–1801.
- VanLoock, M.S., Agrawal, R.K., Gabashvili, I.S., Qi, L., Frank, J., and Harvey, S.C. (2000). Movement of the decoding region of the 16 S ribosomal RNA accompanies tRNA translocation. *J. Mol. Biol.* **304**, 507–515.
- Voorhees, R.M., Weixlbaumer, A., Loakes, D., Kelley, A.C., and Ramakrishnan, V. (2009). Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. *Nat. Struct. Mol. Biol.* **16**, 528–533.
- Voorhees, R.M., Schmeing, T.M., Kelley, A.C., and Ramakrishnan, V. (2010). The mechanism for activation of GTP hydrolysis on the ribosome. *Science* **330**, 835–838.
- Watanabe, S., Matsumura, K., Iwai, H., Funatogawa, K., Haishima, Y., Fukui, C., Okumura, K., Kato-Miyazawa, M., Hashimoto, M., Teramoto, K., et al. (2016). A mutation in the 16S rRNA decoding region attenuates the virulence of *Mycobacterium tuberculosis*. *Infect. Immun.* **84**, 2264–2273.
- Weixlbaumer, A., Jin, H., Neubauer, C., Voorhees, R.M., Petry, S., Kelley, A.C., and Ramakrishnan, V. (2008). Insights into translational termination from the structure of RF2 bound to the ribosome. *Science* **322**, 953–956.
- Wilson, D.N., Schlutzen, F., Harms, J.M., Starosta, A.L., Connell, S.R., and Fucini, P. (2008). The oxazolidinone antibiotics perturb the ribosomal peptidyl-transferase center and effect tRNA positioning. *Proc. Natl. Acad. Sci. USA* **105**, 13339–13344.
- World Health Organization (2016). Global Tuberculosis Report 2016. <http://apps.who.int/medicinedocs/documents/s23098en/s23098en.pdf>.
- Xiong, L., Kloss, P., Douthwaite, S., Andersen, N.M., Swaney, S., Shinabarger, D.L., and Mankin, A.S. (2000). Oxazolidinone resistance mutations in 23S rRNA of *Escherichia coli* reveal the central region of domain V as the primary site of drug action. *J. Bacteriol.* **182**, 5325–5331.