Correlating ribosome function with high-resolution structures

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Ribosome research has undergone astonishing progress in recent years. Crystal structures have shed light on the functional properties of the translation machinery and revealed how the striking architecture of the ribosome is ingeniously designed as the framework for its unique capabilities: precise decoding, substrate-mediated peptide-bond formation and efficient polymerase activity. New findings include the two concerted elements of tRNA translocation: sideways shift and a ribosomal-navigated rotatory motion; the dynamics of the nascent-chain exit tunnel and the shelter formed by the ribosome-bound trigger-factor, which acts as a chaperone to prevent nascent-chain aggregation and misfolding. The availability of these structures has also illuminated the action, selectivity, resistance and synergism of antibiotics that target ribosomes.

An overview of recent progress

Translation of the genetic code into proteins is the second stage of protein biosynthesis. It is performed by a complex apparatus comprising ribosomes, mRNA, tRNAs and accessory protein factors. The ribosome (Box 1), a universal dynamic cellular ribonucleoprotein complex, is the key player in this process.

Remarkable accomplishments in characterizing the machinery of protein biosynthesis have been made at the turn of the millennium. Following two decades of preparative efforts [1], structures of ribosomal particles have been determined. These include the large ribosomal subunit of the archaeon *Haloarcula marismortui*, H50S [2], the large ribosomal subunit of the eubacterium *Deinococcus radiodurans*, D50S [3], the small subunit from the eubacteria *Thermus thermophilus*, T30S [3,4], and the entire ribosome from the same source, T70S [5]. These earlier studies are reviewed extensively (e.g. Refs [6–8]). More recent structures include a vacant ribosome [9], functional complexes of ribosomes with mRNA and tRNAs [10–14] and/or with recycling [15,16] and release factors [17]. Additional crystal structures are of: functional complexes of small subunits with mRNA [18] and modified tRNAs [19,20]; large subunits with substrate analogs extending from the initial (e.g. Ref. [21]) to more sophisticated complexes [22,23]; large subunits with non-ribosomal auxiliary factors such as the first chaperone to encounter the emerging nascent protein, the trigger factor [24–26] and the ribosomal recycling factor [27]. Most of the currently available structures are of ribosomes from

organisms that have adapted to extreme environments because these are more suitable for crystallization. Yet, owing to the high level of conservation (Box 1) of the ribosomal functionally relevant domains, the extremophile ribosomes and their genetically modified phenotypes can represent ribosomes from non-extremophile species [28].

Stimulated by the emerging structures, ribosome research has undergone a quantum jump, yielding exciting findings concerning various aspects of protein biosynthesis in prokaryotes (e.g. Refs [29–52]), which could be extended and/or paralleled with corresponding events in eukaryotes (e.g. Refs [53,54]). Likewise, the structural basis for clinical relevance of antibiotics targeting ribosomes despite their high conservation has progressed greatly (Box 2). Crystal structures of complexes of ribosomal particles with their antibiotics obtained up to 2005 have been reviewed elsewhere (e.g. Refs [55–59]). More recent findings are reported in Refs [60,61] or presented here. Still emerging are elaborate analyses of results that have led to plausible [62] or controversial biological implications. An example of the latter is the finding that a mutation of the nucleotide-determining macrolide antibiotic binding to eubacterial ribosomes (at position 2058) from guanine (in eukaryotes) to adenine (as in pathogens) [63] results in antibiotic binding, but does not confer antibiotic sensitivity [64] as originally expected [63].

Time is ripe, therefore, for assessing the crystallographic data and highlighting the issues that remain unresolved, thus hinting at possible future directions in ribosome research. In this review, we summarize briefly the functional implications of the recent structures of bacterial ribosomes because of their immense contributions to understanding the universality of protein biosynthesis and the divergence from it. Thus, although the translation apparatus in eukaryotes is larger and more complicated than in bacteria, the research on the bacterial ribosome has led to imperative insights into key issues concerning ribosomes of the eukaryotic kingdom. Owing to space constraints, the brief account of high-resolution structures presented here is accompanied by only a few (out of many) of the recently published numerous biochemical, genetic and cryo-electron microscopy (EM) studies that expand ribosome research beyond the crystal structures.

A bird's eye view of the ribosome roles in translation

Ribosomes comprise two ribonucleoprotein subunits (Figure 1a) that associate to form the functional ribosome (Box 1). While elongation proceeds, both subunits operate

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Box 1. What is the ribosome?

An adult human body has $\sim10^{14}$ cells, each containing approximately a billion proteins. Proteins are constantly being degraded, and simultaneous production of proteins is therefore required. Hence, typical mammalian cells can contain more than a million ribosomes (the 'factories' that translate the genetic code into proteins). Even bacterial cells contain $\sim$100 000 ribosomes. Ribosomes function as polymerases, synthesizing proteins by one-at-a-time addition of amino acids to a growing peptide chain while translocating the mRNA chain. In bacteria, ribosomes produce proteins on a continuous basis at an incredible speed of >15 peptide bonds per second.

Ribosomes are composed of two subunits that comprise long chains of ribosomal RNA (rRNA), within which many ribosomal proteins (r-proteins) are entangled (Table I). The ratio of 2:1 for rRNA:r-proteins is maintained throughout evolution, with the exception of the mammalian mitochondrial ribosome, in which almost half of the bacterial rRNA is replaced by r-proteins (with a ratio of r-protein:rRNA $\sim$2:1).

Despite the size difference, ribosomes from all kingdoms of life are functionally conserved; the highest level of sequence conservation is in the functional domains. Comparisons of rRNA sequences of widely diverged species and extrapolation of structures from eubacteria via archaea to eukaryotes indicate that the largest structural differences are at the periphery, away from the central core. This core contains the ribosomal active sites within a highly conserved symmetrical region [38,39] in which 98% of the nucleotides are 'frequent' (found in >95% of sequences from 930 different species from the three domains of life), whereas only 36% of all *E. coli* nucleotides, excluding the symmetrical region, can be categorized as such. Importantly, 75% of the 27 nucleotides lying within 10 Å from the symmetry axis are highly conserved. Among them, seven are completely conserved [39].

Table I. The composition of ribosomes

Prokaryotic ribosomes	Eukaryotic ribosomes
Sedimentation coefficient, 70S ($\sim$2.4 MDa)	Sedimentation coefficient: 80S ($\sim$4 MDa)
Small subunit, 30S; large subunit, 50S	Small subunit, 40S; large subunit, 60S
One rRNA molecule (16S with $\sim$1500 nucleotides)	One rRNA molecule (18S with 1900 nucleotides)
$\sim$21 different proteins (S1–S21)	$\sim$33 different proteins (S1–S33)
Two rRNA molecules (5S and 23S, with $\sim$120 and $\sim$2900 nucleotides, respectively)	Three rRNA molecules (5S, 5.8S and 28S, with 120, 156 and 4700 nucleotides, respectively)
$\sim$31 different proteins (L1–L31), among which only L12 is present in more than a single copy	$\sim$50 different proteins (L1–L50)

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cooperatively. The small subunit provides (i) the mRNA-binding machinery (Figure 1b), (ii) the path along which the mRNA progresses, (iii) the decoding center, and (iv) most of the components that control translation fidelity. The large subunit performs the main ribosomal catalytic function, namely amino acid polymerization, and provides the protein-exit tunnel. tRNAs, the molecules that decode the genetic information and carry the amino acids to be incorporated in the growing protein, are the non-ribosomal entities that join the two subunits at each of their three binding sites, A (aminoacyl), P (peptidyl) and E (exit), which reside on both subunits (Figure 1a). The initial tRNA binds to the first codon of the mRNA at the P-site and the next tRNA, which enters the ribosome via the dynamic L7/L12 stalk (Figure 1a), attaches to the next codon at the A-site. While a peptide bond is formed, the A-site tRNA is translocated to the P-site and the deacylated tRNA moves from the P-site to the E-site on its way out of the ribosome through the mobile L1 stalk (Figure 1a). At each elongation cycle, both subunits participate dynamically in translocating the mRNA and the tRNA molecules by a single codon.

The surface of the inter-subunit interface is composed predominantly of rRNA and, in the assembled ribosome, all functional sites are located close to this interface. Hence, unlike typical polymerases, which are protein enzymes,

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Box 2. Insights into antibiotics targeting ribosomes

Antibiotics that target ribosomes exploit divergent strategies with common denominators. All antibiotics bind to functionally relevant regions, and each prevents a crucial step in the biosynthetic cycle. These include causing miscoding, minimizing essential functional mobility, inhibiting translation initiation, interfering with tRNA substrate binding at the decoding center, hindering tRNA substrate accommodations at the peptidyl transferase center (PTC), preventing interactions of the ribosomal recycling factor and blocking the protein exit tunnel.

Elucidation of the various modes of action of antibiotics that target ribosomes and a careful analysis of the ribosomal components comprising the binding pockets confirms that the imperative distinction between eubacterial pathogens and mammalian ribosomes hinges on subtle structural differences within the antibiotic-binding pockets [55,57]. Furthermore, comparisons of the different crystal structures of ribosomal particles in complexes with antibiotics indicate that minute variations in the chemical entities of the antibiotics can lead to markedly different binding modes, and that the mere binding of an antibiotic is not sufficient for therapeutic effectiveness.

Among the ribosomal antibiotics, the pleuromutilins are of special interest because they bind to the almost fully conserved PTC, yet they discriminate between eubacterial and mammalian ribosomes. To circumvent the high conservation of the PTC, pleuromutilins exploit the inherent functional mobility of the PTC and trigger a novel induced-fit mechanism that involves a network of remote interactions between flexible PTC nucleotides and less conserved nucleotides residing in the PTC vicinity. These interactions reshape the PTC contour and trigger its closure on the bound drug [69]. The uniqueness of pleuromutilins mode of binding led to new insights into ribosomal functional flexibility because it indicated the existence of an allosteric network around the ribosomal active site. Indeed, the value of these findings is far beyond their perspective clinical usage because they highlight basic issues such as the possibility of remote reshaping of binding pockets and the ability of ribosome inhibitors to benefit from the ribosome functional flexibility.

The elucidation of common principles of the mode of action of antibiotics that target the ribosome, combined with variability in binding modes, the revelation of diverse mechanisms acquiring antibiotic resistance and the discovery that remote interactions can govern induced-fit mechanisms that enable species discrimination even within highly conserved regions justify expectations for structural-based improved properties of existing antibiotics in addition to the development of novel drugs.
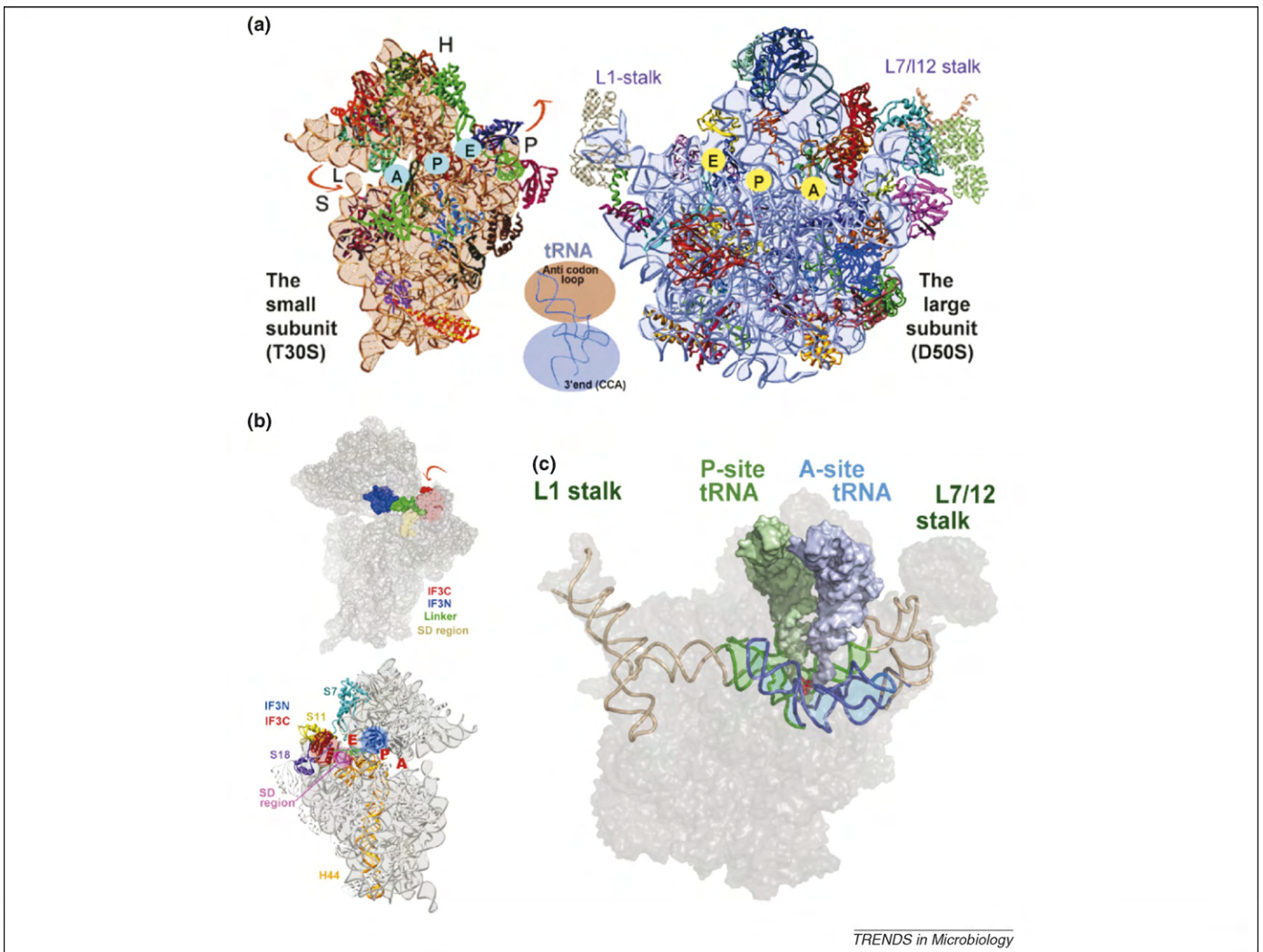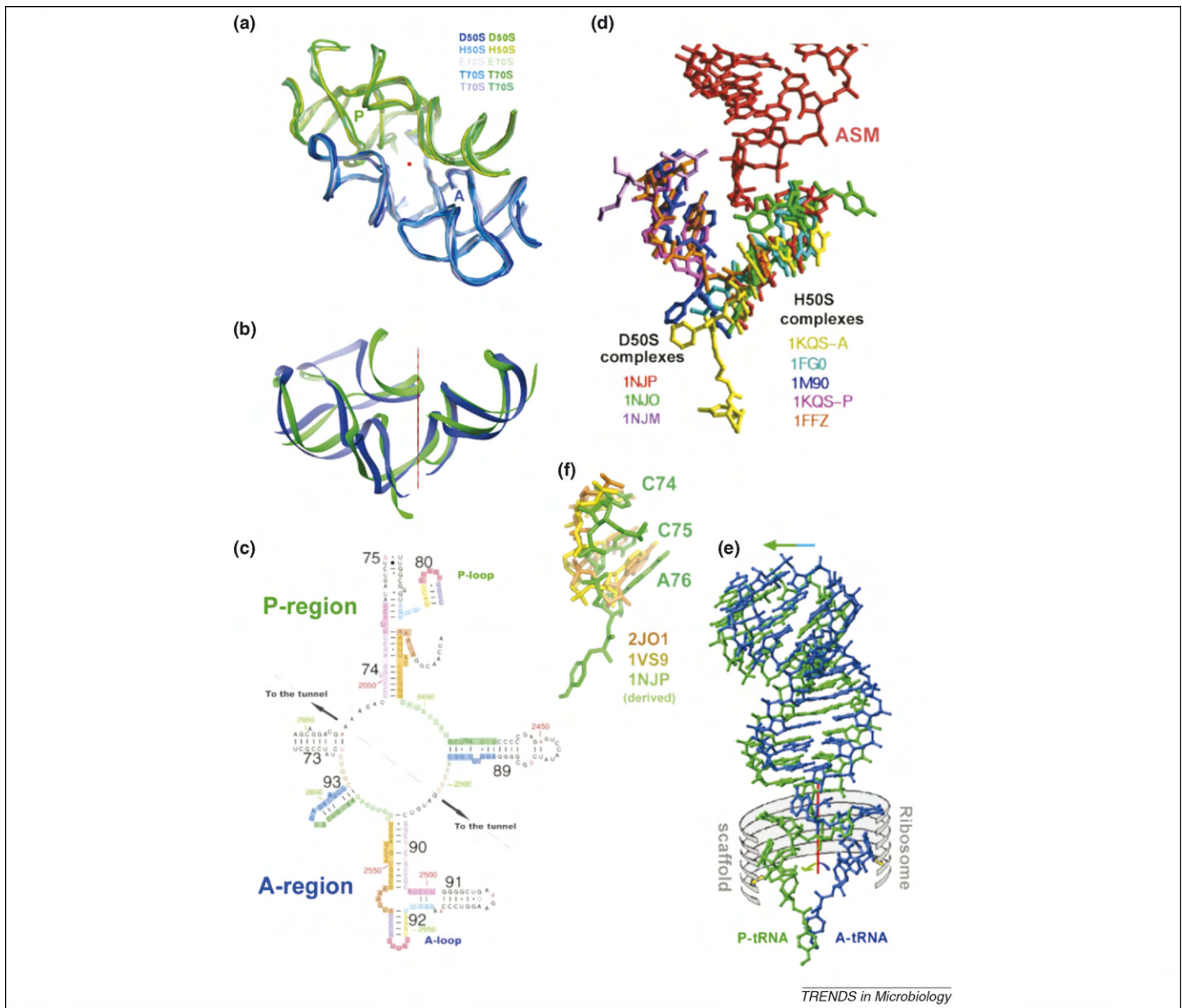
Figure 1. The ribosome functional centers. **(a)** The two ribosomal subunits. Left: the small ribosomal subunit (T30S) [4]. The approximate positions of codon–anticodon interactions of A-, P- and E-tRNAs are shown and the main functional domains are indicated: H, head; L, latch; P, platform; S, shoulder. The arrows designate the approximate directions of the coordinated motions associated with mRNA binding and translocation. The orange arrow on the left of the subunit indicates the creation of the mRNA pore, that is, the latch motion [4]. Right: The large ribosomal subunit (D50S) [2]. Regions that are involved in amino acid polymerization are indicated. These include the two stalks controlling the A-site tRNA entrance (L7/L12) and the E-site tRNA exit (L1), which are known to undergo a coordinated lateral movement during elongation; the positions where the acceptor stems of the three (A-, P- and E-) tRNA molecules interact with this subunit. Insert: a tRNA molecule on which its two functional domains (the anticodon loop and CCA 3′ end, which binds the incoming amino acid or the newly born protein) are marked. The brown oval indicates the portion of the tRNA molecule interacting with the small subunit, and the blue oval shows the portion bound to the large subunit. **(b)** The positions of initiation factor 3 (IF3) and Shine-Dalgarno (SD) region on the small subunit. The small ribosomal subunit is shown in gray. The arrow indicates the possible motion of IF3 C-terminal domain (IF3C). Top: a space-filled view similar to that shown in (a). Bottom: a more detailed representation of the opposite view. Marked are the IF3 domains (C terminus, N terminus and the linker between them), the SD region, the anticodon loops of the three tRNAs (A, P and E), and the proteins involved in IF3 binding. **(c)** The central location of the symmetrical region in the large ribosomal subunit from D50S (gray), with A- and P-site tRNAs (docked according to Ref. [5]) and the symmetrical region (blue and green) with its extensions (gold). The symmetrical region is shown in blue and green (for A- and P-sites, respectively) with the pseudo twofold imaginary axis in red. Note that the symmetrical region connects directly or through its extensions (gold) to all the large subunit functional regions, including the bridge, between it and the decoding site on the small subunit [38,39].

RNA is the major player in ribosome activities. The site of peptide-bond formation, the peptidyl transferase center (PTC), is positioned within a universal pseudo twofold 'symmetrical region' (Figure 1c), which is composed of highly conserved nucleotides. The 90 nucleotides that comprise one-half of the symmetrical region are related by a rotation of 180° around an imaginary axis located at the middle of the PTC to the other half of the symmetrical region, which is also composed of 90 nucleotides. In addition to the rRNA fold, this internal symmetry relates the orientation of the nucleotides (Figure 1d and Figure 2a–c), but not nucleotide sequences. The central location of the symmetrical region and its link to all ribosomal features involved in amino acid polymerization

[6,22,38,39] (Figure 1c) indicates that it can serve as the element signaling between remote ribosomal locations (up to 200-Å away from each other) and thus can coordinate translation processes. This is consistent with the observed relationship between PTC occupation and the binding of mRNA to the small subunit [47].

The ribosome is a dynamic molecular machine that involves structural rearrangements as an integral part of the translation machinery. Various motions have been detected by investigating the reasons for disorder (multiple simultaneous conformations) in functionally relevant regions in crystals grown under far from physiological conditions [2,21] or by cryo-EM (e.g. Ref. [65]) and single-particle methods [47]. In addition, interpolation

Figure 2. The symmetrical region and peptide-bond formation. **(a–c)** The universal symmetrical region backbone fold. In all structures, the A- and P-sub-regions are shown in blue and green, respectively. The imaginary symmetry axis is shown in red. (a) Superposition of fold of the 180 nucleotides comprising the symmetry region in all known structures, shown as ribbons. The center of the PTC lies approximately on this axis. (b) Superposition of the backbones of the rRNA comprising the A- and P-sub-regions of the symmetrical region, as obtained by a 180° rotation around the imaginary symmetrical axis, indicating the level of the ribosomal internal symmetry. (c) 2D representation of the 23S rRNA segment that belongs to the symmetrical region. Symmetrical features are shown in identical colors. **(d)** Superposition of the locations of short substrate analogs used in crystallographic studies together with H50S and D50S. The PDB accession codes are indicated. **(e)** The tRNA-translocation motion, comprising a synchronized sideways shift, performed as part of the overall mRNA–tRNA sideways translocation (in the direction of the horizontal arrow), and the rotatory motion of the A-tRNA 3′ end along a path confined by the PTC (gray) walls (shown here as ribs). The A-site tRNA and the derived 3′ end of the P-site tRNA are shown in blue and green, respectively. The direction of the rotatory motion is indicated by a blue–green curved arrow, the imaginary twofold symmetry axis is red, and the approximated positions of the symmetrical base pairs [22,31,38,39] are shown in yellow. **(f)** Superposition of the derived P-site CCA (from ASM 3′ end by the rotatory motion) on the crystallographically determined locations of the P-site CCA in crystals of 70S complexes [10,11]. The PDB accession codes are indicated.

between the structure of the unbound large subunit, D50S [66] and that of the entire ribosome, T70S, with three tRNAs [5] identified several fundamental motions. Among them is the coordinated movement of the two large subunit stalks [33,48,66] (Figure 1a) that are involved in the entrance and release of the A- and E-tRNAs. Also detected are the head–shoulder movement [7] and the head–platform correlated motions (Figure 1a) that provide guidance to mRNA progression in the small subunit [4,9,12] together with elongation factor EF-G [67] as part of ratchet-like inter-subunit reorganization [68]. Additional motions were correlated with tunnel gating [62], possible trafficking of nascent-chain progression [24], rearrangements caused by

elongation factor EF-Tu ternary complex binding that are linked to fidelity control [7], motions within the PTC that could be correlated with activation or deactivation [29], inhibitory action of antibiotics [69] and the rotatory module of the translocation of the substrate [6,22,38].

Over the years, views on the contribution of the ribosomal proteins (r-proteins) to ribosome function have changed dramatically. Originally, r-proteins were thought to carry out the ribosomal catalytic tasks [70], but later it was shown that rRNA performs most of the ribosome functions. The high-resolution crystal structures show that, in addition to their peripheral globular domains, almost all r-proteins possess elongated loops or terminus extensions,

penetrating into the rRNA core, and thus seem to serve as entities stabilizing the rRNA conformation. However, alongside their stabilization roles, some r-proteins can facilitate functions that require mobility (reviewed in Ref. [6]). For example, protein L22 seems to cause transient tunnel blockage [62], and proteins L1 and L12 (Figure 1a) seem to be involved in tRNA translocation (reviewed in Refs [6–8]). In addition, proteins situated in proximity to functional regions were proposed to support specific activities. Thus, proteins S5, S6 and S12 assist mRNA-binding fidelity [7], and it has been suggested that proteins L27 [10,34] (which does not exist in the archaeon H50S) and L2 [71] affect peptidyl transferase activity. S12 and L2 are among the few proteins that reside partially on the inter-subunit interface and can support the biosynthetic process. Importantly, results of computational methods indicated that S12 and L2 are among the most ancient ribosomal proteins [72].

Initiation, subunit association, decoding and translocation

A prerequisite for correct translation is accurate positioning of mRNA on the ribosome. This step is of utmost importance, hence any divergence can destabilize tRNA binding and inhibit canonical translation initiation [60,73]. In prokaryotes, mRNA placement is assisted by a pyrimidine-rich region ('anti Shine-Dalgarno') located at the 3′ end of the 16S RNA. This region anchors the complementary purine-rich sequence at the 5′ end of mRNA ('Shine-Dalgarno') by numerous interactions [12] (Figure 1b) and creates a chamber for transient stabilization of this otherwise labile double helix [18]. In eukaryotes, mRNA placement requires highly sophisticated machinery [53,54] and throughout evolution it involves various non-ribosomal factors (Box 3).

Crystal structures of prokaryotic ribosomes imply that the entry of mRNA into its groove on the small subunit involves a latch-like closing–opening mechanism [4,5,14]. These structures also indicate that the mRNA kinks between the A- and P-sites at the decoding region [4,5], and that this conformation seems to be stabilized by a metal ion, which delineates the border between the two sites and prevents uncontrolled mRNA sliding [10]. Once the mRNA and the initiator P-site tRNA bind to the small subunit, the two subunits associate to form the functional ribosome. The surface complementarily is stabilized by more than a dozen inter-subunit bridges formed by conformational changes of the interface components [5,10,11,66]. Several bridges seem to have roles beyond merely guaranteeing correct subunit interactions. Among them, bridge B2a is particularly important because it connects the immediate environments of the PTC with the decoding center. Furthermore, this bridge has the ability to adopt several conformations, depending on the functional state of the ribosome [2,22].

The elongation cycle consists of decoding, peptide-bond formation, amino acid polymerization, detachment of the P-site tRNA from the growing polypeptide chain and release of the deacylated tRNA. These processes are facilitated by translocation, which is a successive coordinated movement of the mRNA and its associated tRNAs through

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Box 3. Non-ribosomal compounds involved in initiation and elongation

tRNA molecules decode the genetic information by matching the complementary bases of their anticodon loop with the codon on the mRNA. All tRNAs are double helical L-shaped molecules, except for their anticodon loop and the single-stranded 3′ end (almost universally CCA) to which the cognate amino acid or the growing peptidyl chain is bound (see Figure 1a in main text).

Three non-ribosomal protein factors are involved in the initiation. Initiation factor (IF)-2 is a GTPase that binds preferentially to initiator tRNA. IF2 functions in a cooperative manner with IF1, which occludes the ribosomal A-site at the small subunit (see Figure 1b) and flips out two functionally important bases (A1492 and A1493). These localized changes lead to global alterations in the 30S conformation [7], which seem to be essential for the next steps in translation. IF3 interferes with subunit association and promotes the ribosome fidelity at the initial phase by assisting the selection of the initial P-site codon–anticodon interactions. The crystal structure of the C-terminal domain of IF3 (IF3-c) in complex with T30S indicates that it binds to a region proximal to the mRNA channel [7] in a mode suggestive of exploiting its inherent flexibility for an over-the-platform swing to a location suitable for facilitating subunit dissociation [55] (see Figure 1b). Interestingly, IF1 and IF2 (a/eIF1A and a/eIF5B in eukaryotes) are conserved across all three kingdoms of life and cryo-EM studies indicate that they interact with the small subunit in a similar manner, although initiation in eukaryotes and archaea requires additional factors.

In prokaryotes, the elongation cycle is driven by GTPase activity of elongation factors. Elongation factor (EF)-Tu delivers the cognate aminoacylated-tRNA to the ribosomal A-site as a ternary complex with GTP, induces long- and short-range conformational alterations, and dissociates after GTP hydrolysis. EF-G contributes to bias the translocation in the forward direction [67]. It binds preferentially to the ribosome at its ratcheted conformation, which is created by a rotation of the small subunit relative to the large subunit in the direction of the mRNA movement [68], thus facilitating GTP hydrolysis. Both EF-Tu and EF-G bind to the mobile L7/L12 entrance stalk (see Figure 1a) via a conserved region of the L12 C-terminal domain [48]. In concert with these motions, the deacylated tRNA at the E-site moves towards protein L1 on the other side of the ribosome (see Figure 1a) and, consequently, this protein undergoes a marked conformational alteration to release it [2,5,9–11].

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the ribosome from A-site to the P-site and then to the E-site, one codon at a time (in a 3′→5′ direction). Decoding fidelity, namely avoiding disparity between the mRNA codons and the tRNA anticodons, is vital for guaranteeing translation accuracy. The incoming aminoacylated tRNAs are selected for forming the codon–anticodon base pairing with an error rate of 10^{-3}–10^{-4} at the highly conserved RNA-rich decoding center of the small ribosomal subunit. The ribosome has a major role in this selection, exploiting the inherent flexibility of the decoding center for strictly monitoring the base pairing at the first two positions of each codon, but tolerating non-canonical base pairs at the third position [7]. Furthermore, it seems that normal triplet pairing is not an absolute constraint of the decoding center. For example, flexible expanded anticodon loops of frameshift promoting tRNAs can adopt conformations that enable three bases of the anticodon to span four mRNA bases [19].

The current integrated model for decoding proposes that tRNA selection hinges on discrimination based on the interactions between the ribosomal rRNA and the minor groove of the codon–anticodon duplexes, with a potential to lead to domain closure. Cognate tRNA binding induces

global structural rearrangements by domain movements, and these modify the conformation of the universally conserved decoding regions so that bases residing in it can interact with the first two base-pairs of the codon–anticodon helix.

The ribosome is a polymerase

Although tRNA molecules are the natural substrates of the ribosome, the past three decades, the preferred substrate analogs used for determining ribosomal functional activity have been 'minimal substrates', namely puromycin derivatives capable of creating a single peptide bond. Using similar compounds, which were believed to function as substrate and transition-state analogs for complexes with H50S, it was proposed that four universally conserved rRNA nucleotides catalyze peptide-bond formation by a general acid–base mechanism [21]. This proposition was soon challenged by various biochemical and mutational studies (e.g. Refs [29,30,42]). Additional crystallographic studies on complexes of H50S with similar, albeit more sophisticated, substrate analogs (e.g. Ref. [23]) illuminated several aspects of peptide-bond formation, such as local conformational rearrangements, but did not lead to a feasible consensus mechanism. This could be linked to the finding that in all structures of H50S and its complexes with substrate analogs almost all regions involved in ribosome function (e.g. inter-subunit bridges and the stalks involved in tRNA entrance and exit) are disordered (i.e. posses multiple conformations simultaneously), presumably because they were constructed under far from physiological conditions [2,21]. Thus, although these structures did not yield the mechanism of peptide-bond formation, they did illuminate an important aspect in cellular regulation of ribosome function: disorder of functionally relevant ribosome regions might represent a common ribosomal strategy for avoiding non-productive protein biosynthesis.

Structures of a complex of D50S with an A-site tRNA-acceptor-stem mimic (composed of 35 nucleotides and including an aminoacylated 3′ end called ASM) [22] obtained under conditions close to those optimized for protein biosynthesis revealed that the acceptor stem of A-site tRNA interacts extensively with the cavity leading to the PTC, and the bond between it and the tRNA 3′ end overlaps the pseudo-symmetry axis (Figure 2d). The high conservation of the components of the symmetrical region, the linkage between the elaborate PTC architecture and the position of the A-site tRNA indicates that the translocation of the tRNA is performed by a combination of two independent, albeit synchronized, motions: a sideways shift, performed by the overall mRNA–tRNA translocation at a straight or hybrid-state conformation (e.g. as in Ref. [15]), and a rotatory motion of the A-tRNA 3′ end (Box 3) along a path confined by the PTC walls (Figure 2e). Navigated and guided by the ribosomal architecture, this rotatory motion provides all of the structural elements for ribosome function as an amino acid polymerase, including the formation of two symmetrical universal base pairs between the tRNAs and the PTC [22,38,39]. This formation of symmetrical base pairs is a prerequisite for substrate-mediated acceleration, rather than acid–base catalysis

[31,32,42,50], and for directing the nascent protein into the exit tunnel.

Remarkably, the position of the 3′end of P-site, derived by the rotatory motion that was suggested based on the mode of binding of a tRNA mimic to an unbound large ribosomal subunit (D50S), overlaps the positions of full-size tRNAs bound to the entire 70S ribosome [10,11] (Figure 2f). Furthermore, all nucleotides involved in this rotatory motion of the tRNA 3′ end have been shown to be essential by a comprehensive genetic selection analysis [44]. Consistently, quantum mechanical calculations based on D50S structural data have indicated that the transition state (TS) for this reaction is formed during the rotatory motion, and is stabilized by hydrogen bonds formed between the rotating moiety and the same rRNA nucleotides [45]. The location of the computed TS is similar to that observed crystallographically for a chemically designed TS analog in the large subunit from a different ribosome, H50S [23].

Differences between full-size tRNAs and 'minimal substrates' were also observed by biochemical, mutagenesis, kinetics and computational studies (e.g. Refs [29–32,35,36,41–43,49,50,52]). These studies showed that the mechanism of peptide-bond formation by full-size tRNAs involves substrate-mediated catalysis [31] and requires the stereochemistry obtained by the rotatory motion [38]. Consistently, this motion depends on accurate positioning of the tRNAs, achieved by the interactions of the acceptor-stem nucleotides with proximal ribosomal nucleotides [6,22]. It is important to note, however, that a symmetrical relationship between the reactants of peptide-bond formation has been observed in all known structures of ribosomal complexes (Figure 2d), including 'minimal substrates' requiring additional rearrangements. In principle, suitable systems for studying this machinery should include a full-length A-site tRNA bound to the ribosome. However, although 70S ribosomes complexed with full-length aminoacylated-tRNA were crystallized, a full-size A-site tRNA 3′ ends could not be detected in any of the electron-density maps [10–12]. Hence, the only relevant crystallographic information currently available originates from the structure of the complex of D50S with ASM [22].

The correlation between the rotatory motion and amino acid polymerization revealed the exact location of the growing protein chain, which could not be observed by the minimal substrate analogs designed for producing a single peptide bond that do not undergo A- to P-site translocation. Furthermore, the difference between the formation of single peptide bond by minimal substrates and amino acid polymerization highlights the PTC ability to rearrange itself upon substrate binding [6,29,57].

The conservation of the symmetrical region is consistent with its vital functions in intra-ribosomal signaling, peptide-bond formation and amino acid polymerization. The preservation of the 3D structure of the two halves of the ribosomal frame, regardless of the sequence, demonstrates the rigorous requirements of accurate substrate positioning in the stereochemistry that supports peptide-bond formation. This, and the universality of the symmetrical region, led to the assumption that the ancient ribosome

was made of a pocket confined by RNA chains and that the ribosome evolved by gene fusion or duplication [39].

In short, the intricate ribosomal architecture positions its substrates in an orientation that promotes peptide-bond formation [22,38,39] and provides the machinery required for enabling the repetition of peptide-bond formation, which results in amino acid polymerization. The current consensus is that the ribosome contributes positional catalysis to peptide-bond formation and provides the path along which A-to-P-site translocation occurs, whereas the proximal 2′-hydroxyl of P-site tRNA A76 provides the catalysis [31,50]. This view solves most of the issues associated with this function. Nevertheless, further studies are clearly required to shed more light on the still unresolved issues such as the possible involvement of protein L27 in this step [34].

Termination
The hydrolytic cleavage of the ester bond in peptidyl-tRNA during the termination step is also catalyzed by the ribosome. In addition to the participation of ribosomal components such as A2602 ribose [56], peptide release requires auxiliary release factors that recognize the termination codons and promote the P-site peptidyl-tRNA hydrolysis and seem to induce ribosome conformational changes [17]. The disassembly of the ribosome at the end of translation is facilitated in bacteria by the ribosome recycling factor (RRF) in a manner yet to be elucidated. Thus, motions in inter-subunit bridges that have been suggested based on a crystal structure of RRF bound to the large ribosomal subunit [27] and to the vacant ribosome [9], were not seen in the crystal structure of T70S in a complex containing a tRNA in the P-site, an E-site tRNA and RRF [15]. The

mode of E-site tRNA release, its possible involvement in codon–anticodon interactions and the biological meanings of the different conformations of vacant ribosomes, remain open questions.

The voyage of the nascent protein
Nascent polypeptides progress through their exit tunnel (Figure 3). This universal feature of the large ribosomal subunit lies adjacent to the PTC [2,66] and is lined primarily by rRNA with a few r-proteins reaching its walls from its exterior (Figure 3a). This tunnel (~120 Å in length, with a varying diameter of 10–25 Å) possesses the dynamics required for interacting with the nascent protein. Thus, it seems to have an active role in the sequence-specific arrest of nascent chains and in response to cellular signals [62], namely in gating and discriminating, as well as in controlling the operational mode of the translocon at the ER membrane [46]. Elements of the tunnel wall that seem to sense nascent-peptide-specific sequences include, in addition to the rRNA, r-proteins L22 [6,62] and L4 that form the constricted region of the tunnel, L23 that in eubacteria extends into the tunnel [24], and a crevice adjacent to the tunnel wall that can provide space for co-translational transient folding, which was indicated by results obtained by non-crystallographic methods including fluorescence resonance energy transfer (FRET) measurements [40] and computational analyses [37].

While being translated, nascent proteins emerge from their protective exit tunnel into the crowded cellular environment before gaining sufficient length to acquire the final fold. Molecular chaperones support correct folding within the crowded cells. In eubacteria, the first chaperone encountered by the emerging nascent chain, called trigger
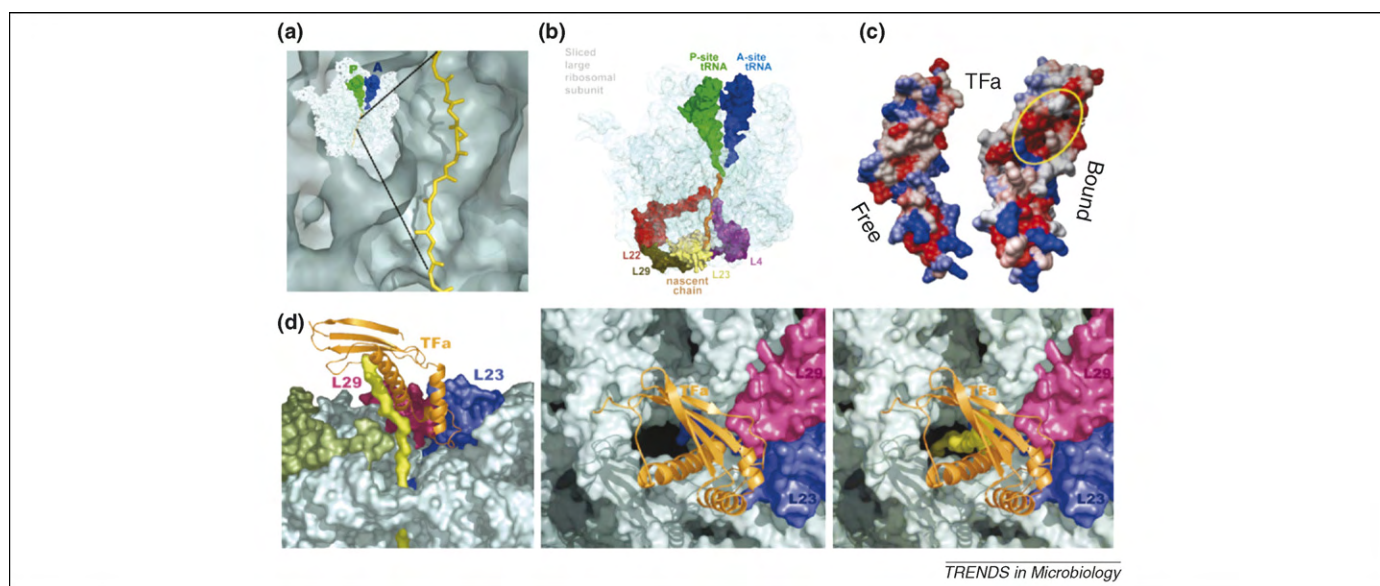

Figure 3. The nascent protein-exit tunnel and chaperoning the emerging proteins. **(a)** The position, curvature and varying diameter of the protein-exit tunnel within the large ribosomal subunit are indicated by a modeled polyalanine (yellow). **(b)** Proteins reaching the walls of the tunnel from the large subunit exterior. The tunnel interior is marked by a modeled nascent chain (orange). The large subunit is shown in blue–gray. **(c)** Conformational differences between free and ribosome bound trigger-factor-binding domain (TFa), based the structure of the homologous complex of TFa and the large ribosomal subunit from *Deinococcus radiodurans* [24] and on the very high level of homology between TF molecules in *Escherichia coli* and in *D. radiodurans*. The yellow ellipse delineates the sizable hydrophobic region that becomes exposed upon its binding to the ribosome. The coordinates of *E. coli* free TFa were taken from Ref. [26]. **(d)** Space-filling representation of rRNA (in gray) and r-proteins (blue, dark red and dark green) at the tunnel opening. TFa is shown as gold ribbons, and a modeled nascent chain as yellow ribbons. Left: the emerging protein (modeled polyalanine) enters the shelter provided by the TFa. The proteins associated with the trigger factor (TF), L23 and L29, are shown. Note L23 extension reaching the tunnel wall [as shown also in (b)]. Middle and right: a view perpendicular to the view shown on the left, of the tunnel opening. Middle: empty tunnel. Right: a modeled polyalanine chain is emerging from the tunnel. Note that, in this crystal structure, the tunnel was empty.

factor (TF), binds to the translating ribosome at ~1:1 stoichiometry by interacting with ribosomal proteins L23 and L29 [24–26]. Protein L23 belongs to the small group of ribosomal proteins that display significant evolutionary divergence. Whereas its globular domain is conserved [24], only in eubacteria does it possess a sizable elongated loop, which extends from the ribosome exterior all the way into the tunnel walls (Figure 3). At this position, the L23 extended loop can undergo allosteric conformational changes that, in turn, can modulate the shape of the tunnel, which implies trafficking of the nascent protein [24,25]. Modeling of full-length TF and the signal-recognition particle (SRP) onto the TFa–50S complex indicates simultaneous cohabitation [25] in a manner that, presumably, enables screening for hydrophobic signal sequences on the emerging nascent chains [74]. Hence, interplay between TF, SRP and the trafficked nascent chain while progressing through the tunnel is plausible.

Based on the structure of unbound TF from *Escherichia coli* [9], the homology between TF from *E. coli* and *D. radiodurans*, and analyses of crystal structures of physiologically meaningful complexes of D50S with a TF-binding domain (called TFa) from the same source [24,25], it was found that TFa undergoes conformational rearrangements that expose a sizable hydrophobic region (Figure 3), thus acquiring a configuration that is suitable for adherence to hydrophobic patches on the nascent chain. Consistent with dynamic studies [40], it seems that TFa prevents the aggregation of the emerging nascent chain by providing a hydrophobic surface that can transiently mask exposed hydrophobic regions of the elongating polypeptide chains until they become buried in the interior of the mature protein.

Antibiotics targeting ribosomes

Despite ribosome conservation (Box 1) many of antibiotics that target ribosomes are clinically relevant (reviewed in Refs [55–64]) (Box 2). Unsuccessful attempts have been made to crystallize ribosomes from pathogenic organisms, but structural information from the crystallizable eubacterial ribosome has shown to be relevant for determining antibiotic targets of pathogens. Alongside rationalizing many genetic, biochemical and medical observations, the available structures have revealed unexpected inhibitory modes. An example is the exploitation of the inherent flexibility of ribosomes for antibiotic synergism [55]. Similarly, ribosome mobility is used for triggering an induced-fit mechanism by remote interactions that reshape the antibiotic-binding pocket [69]. These motions mean that an antibiotic family that binds to conserved functional regions, and so are not expected to be clinically relevant, are in fact therapeutically useful.

The previously described and the more recent [75–78] structures have also helped to identify factors that discriminate between pathogenic bacteria and non-pathogenic eukaryotes, which are of crucial clinical importance because most ribosomal antibiotics target highly conserved functional sites. Thus, comparisons between the antibiotic-binding sites in ribosomes from eubacteria (e.g. from *D. Radiodurans*) and those from the archaeon *H. marismortui,* which shares properties

with eukaryotes, highlighted the distinction between binding and inhibitory activity. Specifically, this comparison indicated that the identity of a single nucleotide determines antibiotic binding, whereas proximal stereochemistry governs the antibiotic orientation within the binding pocket [55,57] and, consequently, its therapeutic effectiveness. This is in accord with recent mutagenesis studies showing that mutation from guanine to adenine in rRNA at the position equivalent to *E. coli* A2058 does not confer erythromycin sensitivity in *Saccharomyces cerevisiae* [64].

Concluding remarks and future perspectives

The high-resolution structures have shown that all ribosomal tasks are governed by the ribosome architecture and have stimulated an unpredictable expansion of ribosome research, resulting in new insights into the translation process. Among the new, less expected, findings are the intricate mode of decoding, the mobility of most of the ribosomal functional features, the symmetrical region, the dynamic properties of the ribosomal tunnel and its interactions with the progressing nascent chains, the possible signaling between the ribosome and cellular components, and the way the TF prevents misfolding. In addition, unique structural tools for improving antibiotic targets are now available and key issues associated with the structural bases involved in antibiotic resistance, synergism and selectivity can now be addressed. However, despite the extensive research and the immense progress, several key issues are still unresolved, some of which are described here. Thus, it is clear that the future of ribosome research and its applicative aspects hold more scientific excitement.

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