A structural understanding of the dynamic ribosome machine

Thomas A. Steitz

Abstract | Ribosomes, which are central to protein synthesis and convert transcribed mRNAs into polypeptide chains, have been the focus of structural and biochemical studies for more than 50 years. The structure of its larger subunit revealed that the ribosome is a ribozyme with RNA at the heart of its enzymatic activity that catalyses peptide bond formation. Numerous initiation, elongation and release factors ensure that protein synthesis occurs progressively and with high specificity. In the past few years, high-resolution structures have provided molecular snapshots of different intermediates in ribosome-mediated translation in atomic detail. Together, these studies have revolutionized our understanding of the mechanism of protein synthesis.

Anticodon
A unit that is composed of three nucleotides in the tRNA that are complementary to and recognize the three bases of the codon on the mRNA.

A site
The site on the ribosome that binds aminoacyl-tRNA.

Aminoacyl-tRNA
A tRNA attached to an amino acid, which is ester linked to the sugar of the 3′ nucleotide.

P site
The site on the ribosome that binds peptidyl-tRNA.

Peptidyl-tRNA
A tRNA with the peptide being synthesized linked to the 3′ nucleotide.

Department of Molecular Biophysics and Biochemistry, Department of Chemistry, Yale University, and the Howard Hughes Medical Institute, New Haven, Connecticut 06520-8114, USA.
e-mail:
peggy.eatherton@yale.edu

Structural studies of the ribosome exemplify the evolution of structural studies in cell biology from the early negatively stained images of macromolecular assemblies in whole cells, to a detailed atomic understanding of the mechanism of action of a large assembly. The earliest electron microscopy (EM) images that captured ribosomes in a cell were obtained by George Palade, and were initially called Palade particles[1].

Biochemical studies in the 1960s showed that the larger subunit of this 2.3-MDa RNA–protein assembly from *Escherichia coli* catalysed peptide bond formation, whereas interactions of the anticodon of tRNA with mRNA bound to the small subunit effected translation of the message. At the same time, specialized binding sites were identified on the large ribosomal subunit: the A site that binds aminoacyl-tRNAs and a P site that binds peptidyl-tRNAs. It was hypothesized that translocation from the A site to the P site occurs following peptidyl transfer, thereby suggesting how protein synthesis may progress along the mRNA[2].

Our understanding of ribosomal structure has proceeded from the early reconstructions of the shapes of the two interacting subunits from negatively stained images by Jim Lake[3], to the current atomic-resolution structures of the prokaryotic 70S ribosome[4–6] and of its large and small subunits captured in various functional states[7–11]. In prokaryotes, the larger of the two subunits sediments at 50S, is ~1.5 MDa in molecular weight and contains ~3,000 nucleotides of ribosomal RNA (rRNA) and 34 proteins. The smaller subunit sediments at 30S, has a molecular weight of 0.8 MDa and comprises 1,500 nucleotides of rRNA and 21 proteins.

An increasingly detailed view of the mechanisms by which this large RNA–protein machine can accurately translate the information that is encoded in the mRNA into specific proteins is now emerging from high-resolution crystal structures of each of its two subunits and of the whole ribosome, as well as from cryo-EM-derived models that capture several states of the amino-acid incorporation cycle. The mechanistic level of structural insights into ribosome function now exceeds that achieved in the early structural studies of lysozyme, carboxypeptidase and ribonuclease. After a short overview of the mechanism of ribosome-mediated translation, the process as it is currently understood will be discussed step by step as it occurs in more detail.

Introducing ribosome-mediated translation

The ribosome consists of two ribonucleoprotein subunits, one of which is about twice the size of the other, and these two subunits perform different roles in protein synthesis[12]. The small subunit mediates the correct interactions between the anticodons of the tRNAs and the codons in the mRNA that they are translating in order to determine the order of the amino acids in the protein being synthesized. The large subunit contains the peptidyl-transferase centre (PTC), which catalyses the formation of peptide bonds in the growing polypeptide.

Both subunits contain three binding sites for tRNA molecules that are in three different functional states (FIG. 1). The A site binds the aminoacyl-tRNAs that are about to be incorporated into the growing polypeptide chain, the P site positions the peptidyl-tRNA and the
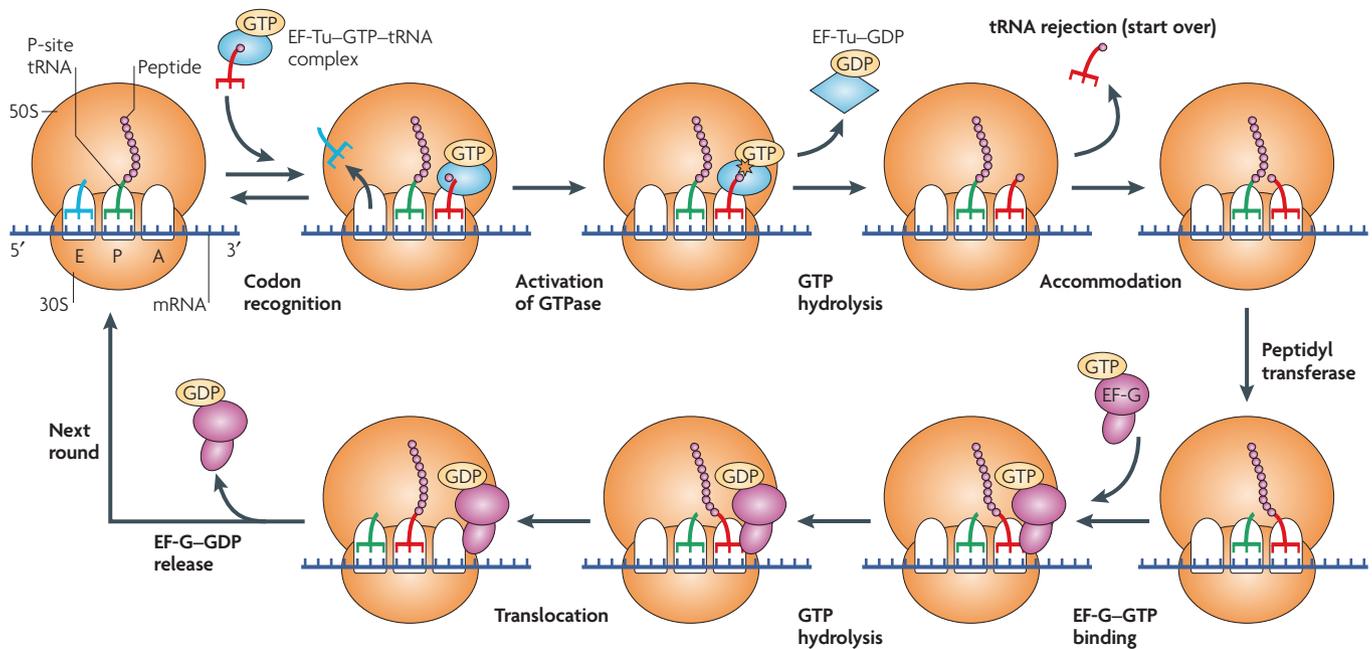
Figure 1 | **An overview of ribosomal structure and mRNA translation.** mRNA translation is initiated with the binding of tRNA[fmet] to the P site (not shown). An incoming tRNA is delivered to the A site in complex with elongation factor (EF)-Tu–GTP. Correct codon–anticodon pairing activates the GTPase centre of the ribosome, which causes hydrolysis of GTP and release of the aminoacyl end of the tRNA from EF-Tu. Binding of tRNA also induces conformational changes in ribosomal (r)RNA that optimally orientates the peptidyl-tRNA and aminoacyl-tRNA for the peptidyl-transferase reaction to occur, which involves the transfer of the peptide chain onto the A-site tRNA. The ribosome must then shift in the 3′ mRNA direction so that it can decode the next mRNA codon. Translocation of the tRNAs and mRNA is facilitated by binding of the GTPase EF-G, which causes the deacylated tRNA at the P site to move to the E site and the peptidyl-tRNA at the A site to move to the P site upon GTP hydrolysis. The ribosome is then ready for the next round of elongation. The deacylated tRNA in the E site is released on binding of the next aminoacyl-tRNA to the A site. Elongation ends when a stop codon is reached, which initiates the termination reaction that releases the polypeptide (FIG. 7). Figure adapted from REF. 61.

70S ribosome
The complete prokaryotic ribosome particle, which is composed of the small (30S) subunit and large (50S) subunit.

Ribosomal RNA
(rRNA). A type of RNA that is synthesized in the nucleolus by RNA polymerase I. Approximately 65% of a ribosome is composed of rRNA.

Peptidyl-transferase centre
(PTC). The active site of the ribosome where peptide bond formation occurs.

E site
The site on the ribosome that binds the deacylated tRNA before it leaves the ribosome.

EF-Tu
Elongation factor Tu (temperature unstable), known as EF-1 in other kingdoms, delivers the aminoacyl tRNA to the ribosome in a codon-specific manner.

E site is occupied by all deacylated tRNAs before they dissociate from the ribosome.

Of central interest are the mechanisms of peptide bond formation and mRNA decoding, which are crucial processes in the elongation phase of protein synthesis by the ribosome. During this phase of protein synthesis, nascent polypeptides are elongated from the N to the C terminus by the addition of one amino acid at a time. This process is facilitated by two protein factors: elongation factor Tu (EF-Tu), which facilitates the delivery of aminoacyl-tRNA to the A site of the ribosome, and elongation factor G (EF-G), which promotes the translocation of the tRNAs and associated mRNA from their positions in the A site and P site to the P site and E sites, respectively, and dissociates the previously bound E-site tRNA.

At the end of the elongation cycle when the stop codon has been positioned in the A site, one of two protein release factors (RFs), RFI or RFII, binds to the A site and promotes the deacylation of the peptidyl-tRNA. A recycling factor, with the help of EF-G, then leads to the dissociation of the release factor and the two ribosomal subunits.

Delivery of aminoacyl-tRNAs to the A site
The accurate delivery of the correct aminoacyl-tRNA to the A site is facilitated by EF-Tu and involves at least two distinct steps. First, an interaction is made between the anticodon base triplet in the tRNA and the corresponding codon of the mRNA that resides in the A site of the ribosome. Second, the correct delivery of the appropriate anticodon to a messenger RNA is somehow communicated to the GTPase centre located in the large subunit, which results in the hydrolysis of GTP bound to EF-Tu. This causes the release of EF-Tu from the tRNA and ribosome and the subsequent accommodation of the aminoacyl end of the tRNA into the PTC, which is followed rapidly by peptide bond formation. Although the structural basis of recognition of the correct anticodon by the small ribosomal subunit is now well understood[7], the way in which the GTPase centre is stimulated by this event and how accommodation is achieved has not been completely elucidated.

Determinants of codon–anticodon specificity. The structural basis by which the ribosome facilitates and detects the specificity of codon–anticodon interaction in the A site has been derived from structures of the small subunit of *Thermus thermophilus* in complex with mRNA and a cognate anticodon stem-loop mimic bound in the A site[7], as well as a more recent structure of the 70S ribosome with tRNAs bound in all three sites[5]. These structures show that when cognate bases of the anticodon of the tRNA form a Watson–Crick base-paired complex with the mRNA codon bound to the A site, three 16S rRNA bases (A1492, A1493 and G530) — which previous
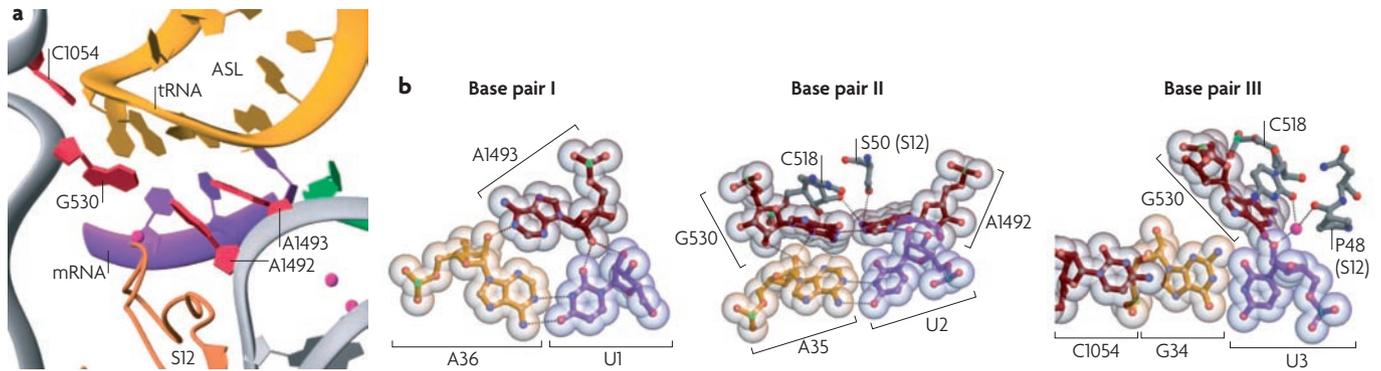
Figure 2 | **Recognition of codon–anticodon interactions by the ribosome. a** | Cartoon of the decoding site of the 30S subunit, showing the codon of mRNA in the A site (purple) and the tRNA anticodon stem-loop (ASL; gold). Crucial bases of the 16S RNA (grey) that bind to and stabilize the tRNA–mRNA complex are shown in red. Protein S12 (orange) is in the area. The magenta spheres are thought to be magnesium ions. **b** | Details of minor groove recognition at the first (I), second (II) and third (III) base pairs between codon and anticodon. In base pair I, A1493 is shown making a type I A–minor interaction with A36 from the tRNA and U1 from the mRNA. In base pair II, A1492 makes a type II A–minor interaction with A35 and U2 is stabilized by G530. In base pair III, no restrictive interactions are seen with G34 and U3. Figure reproduced with permission from REF. 7 © (2001) American Association for the Advancement of Science.

EF-G

Elongation factor G (GTPase), known as EF-2 in other kingdoms, binds to the ribosome and promotes tRNA and mRNA translocation powered by GTP hydrolysis.

Stop codon

A codon that codes for the end of the message that is recognized by the release factor.

Release factor

(RF). A protein factor that recognizes a stop codon in mRNA and catalyses the deacylation of the peptidyl-tRNA.

GTPase centre

The region of the ribosome 50S subunit that includes the sarcin–ricin RNA and stimulates the GTPase activity of elongation factors.

Stem-loop

The structure that is formed when a self-complementary nucleic acid sequence forms a duplex joined by a loop.

Type I A–minor interaction

A specific hydrogen-bonding interaction between an A base and a G–C base pair through the minor groove of duplex RNA.

Watson–Crick base pairs

The complementary hydrogen bond between bases A and T (or U) and G and C that form duplex nucleic acids.

genetic and biochemical experiments[12] had implicated in decoding — alter their conformations to interact with the anticodon[7] (FIG. 2). In the absence of the A site mRNA and anticodon ligands, these three bases point away from the codon–anticodon binding site. However, when the cognate complex between tRNA and mRNA binds to the A site, these three bases reorientate to interact within the minor groove of the three-base-pair helix that is formed by the codons and anticodons.

On codon–anticodon recognition, A1493 makes a type I A–minor interaction[13] with a first base pair made by the anticodon with the codon. This interaction consists of three hydrogen bonds between A1493 and the minor groove base pair that is identical for all four Watson–Crick base pairs and is not possible for any non-cognate base pairs. Hence, this interaction in the minor groove not only stabilizes the complex of a Watson–Crick base pair, but this conformational change in A1493 also appears to occur only in the presence of Watson–Crick base pairing[7]. The orientation and interactions between the anticodon stem-loop of a tRNA bound to the 70S ribosome and the mRNA codon are observed to be identical to what was seen in the previous complex with the 30S subunit alone[5].

The formation of a correct Watson–Crick base pair at the second position of the anticodon is stabilized and detected by a change of conformation (from *syn* to *anti*) in G530. This allows G530 to interact with A1492, which in turn forms a type II A–minor interaction with the second codon–anticodon base pair in the minor groove[7]. As with the type I A–minor interaction, the type II A–minor interaction will work with any of the four Watson–Crick base pairs but not with a non-Watson–Crick base pair. This conformational change in the 16S rRNA and the conformational change that results from the recognition of the first codon–anticodon recognition constitute the first step in a signal that the correct base pairs have been delivered. By contrast, the interactions between a small

subunit with a third base pair in the anticodon helix is significantly less specific, consistent with the observation that wobble base pairs (for example, GU base pairs) can occupy this position.

The importance of the conformational changes that these three bases induce by cognate codon–anticodon interactions is reinforced by the mechanism of action of the antibiotic paromomycin. It induces miscoding errors through binding to the 30S ribosomal subunit and by inducing the same conformational changes in the three bases that are produced by formation of Watson–Crick base pairs at codon positions 1 and 2, even in the presence of mismatches or no bound substrate[14].

How the delivery of a correct anticodon is communicated to the GTPase centre[15] is partly answered by the observation that global changes in the overall conformation of the small subunit accompany the local conformational changes that occur in the A site when the small subunit interacts with the cognate codon–anticodon complex[14]. The 'head' domain of the small subunit rotates towards the large subunit when cognate interactions occur at the A site, which results in it clamping down on the tRNA-binding site. Although these conformational changes do not occur with non-cognate codon–anticodon complexes in the A site, they do occur with non-cognate complexes in the presence of paromomycin, again suggesting that these conformational changes are functionally significant.

Signalling correct codon–anticodon interactions. The recognition of correct codon–anticodon interactions is communicated to the GTPase centre in the large ribosomal subunit ~70 Å away. This causes the hydrolysis of GTP on the EF-Tu and its subsequent release, followed by accommodation of tRNA in the A site. The question arises as to how the conformational changes in the rRNA that were induced by correct codon–anticodon interactions are transmitted. Some tantalizing clues have

been provided from a cryo-EM reconstruction of the 70S ribosome in complex with mRNA and EF-Tu–aminoacyl-tRNA that has been stalled by the antibiotic kirromycin[16]. In this model, EF-Tu in complex with aminoacyl-tRNA is observed to bind in a wide, large RNA cleft that lies between the 30S and 50S subunits. The aminoacylated CCA end of the tRNA lies >70 Å from the PTC in what is called the pre-accommodation state, and the GTPase active site of EF-Tu lies close to the sarcin–ricin RNA loop of the 50S subunit. (The sarcin–ricin loop appears to be at the centre of this activity because its cleavage by the toxins sarcin or ricin kills the GTPase activity.) When the anticodon arm of the tRNA is fitted onto the cryo-EM map, it shows a new conformation of tRNA that appears to be necessary to facilitate the initial codon–anticodon interaction. There is a significant kink or bend between the anticodon stem-loop and the D stem. Furthermore, the elbow region of the tRNA appears to contact the GTPase centre of the 50S subunit of the ribosome. Frank and colleagues[16] hypothesized that the tRNA has an active role in the transmission of the signal between codon–anticodon interaction and the GTPase hydrolysis on codon recognition. It remains to be established how a non-cognate complex differs from a cognate complex and therefore does not produce hydrolysis, and exactly how contacts between tRNA and the GTPase centre stimulate hydrolysis. Another cryo-EM reconstruction of the same complex at a similar resolution from van Heel and colleagues was interpreted differently[17] and concluded that the structural deviation of the aminoacyl-tRNA from that of the crystal structure results from a distortion in the anticodon loop; however, a subsequent cryo-EM study at a resolution of 8 Å confirmed that the distortion in the tRNA is indeed a kink[18].

The anticodon stem-loop in the pre-accommodation complex with EF-Tu on the ribosome exhibits the same orientation relative to the small subunit as the stem-loop bound to the isolated 30S subunit[7,16] and a deacylated tRNA bound to the 70S ribosome[5]. The rest of the tRNA, however, is significantly bent from the accommodated structure at the junction between the anticodon stem and D stem, which results in the aminoacyl end of the tRNA that is bound to EF-Tu being positioned ~70 Å away from the peptidyl-transferase centre. Whichever way the signal is transduced, GTP hydrolysis causes conformation changes in EF-Tu that result in its dissociation from the aminoacyl-tRNA. Presumably, the departure of EF-Tu allows the tRNA to adapt the more stable, unkinked tRNA structure, which places the aminoacyl end into the PTC.

Interactions of tRNA at the P site

Several recent structures have provided insights into the interaction between the P-site tRNA anticodon and mRNA[5,6,14,19]. Watson–Crick base pairs are formed between the mRNA and the P-site tRNA but, unlike the A site, the rRNA does not make interactions with the codon–anticodon base-paired triplets that 'check' the correctness of this interaction (FIG. 3). Instead, extensive interactions between the P-site tRNA and both ribosomal proteins and RNA are made that bury 2,200 Å[2] of tRNA surface and assure its tight and stable binding.

A special scenario is proposed to exist during the initiation of translation, whereby the initiating formyl-methionine tRNA binds directly to the P site, rather than being transferred from the A site. The extra stable binding of initiator tRNA to the P site can be explained, at least in part, by the observation that A1339 and G1338 of
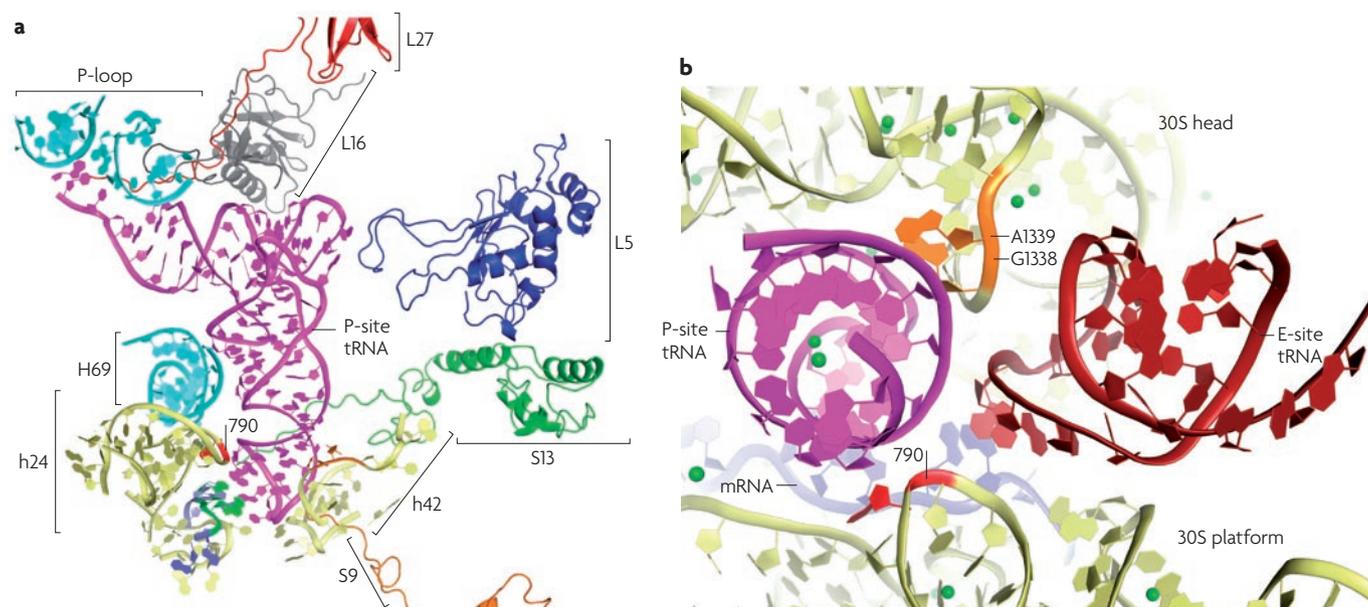
Figure 3 | **P-site–tRNA interactions in the ribosome. a** | Overview of both ribosomal RNA and protein interactions with P-site tRNA. P-site tRNA interacts with many ribosomal protein tails, such as L27, L16, L5, S13 and S9, and with 16S and 23S RNA. These extensive interactions ensure tight and stable binding to the P site. **b** | 16S RNA bases 790, 1338 and 1339 interact with the anticodon stem, thereby acting as a gate between the P and E sites. Presumably, during translocation they move out of the way as the head domain of the 30S subunit rotates.

Box 1 | **Do the two subunits function alone as in the full ribosome?**

For many decades, the biochemistry of the peptidyl-transferase reaction has been studied using small analogues of the tRNA substrates and the isolated large ribosomal subunit, in what is known as the fragment assay. The most detailed structural analysis of peptide bond formation has likewise been achieved using the *Haloarcula marismortui* (Hma) large subunit. The possibility that the conformation of the peptidyl-transferase centre (PTC) in the 70S ribosome might differ slightly from that in the isolated 50S subunit and affect the rate of catalysis was suggested by Schuwirth *et al.*[4] on examination of their model of the 70S apo-ribosome PTC at 3.5-Å resolution. Likewise, Korostelev *et al.*[6] concluded that the 3.7-Å resolution structure of the 70S ribosome in complex with tRNAs bound at the P and E sites exhibited significant differences in the CCA end of the P-site tRNA and in several PTC bases compared with the structures of complexes with the 50S subunit at 2.4–2.8-Å resolution. By contrast, Selmer *et al.*[5] found no difference between the structures of the Hma 50S subunit complexes with CCA analogues bound to the P site and their 2.8-Å resolution structure of the 70S ribosome bound to a P-site tRNA substrate. Because the only obvious difference between the two recent 70S complex structures is the tRNA in the P site (tRNA[phe] versus tRNA[fmet]), it appears that the significantly lower resolution of the Korostelev *et al.* study[6] fails to provide convincing evidence for the veracity of the proposed differences from the other structures. Indeed, further analysis using data from both recent 70S crystal forms leads to the conclusion that both exhibit the same structure of the PTC as the 50S subunit[59]. Furthermore, recent kinetic studies by Rodnina and co-workers show no major differences in the rate of peptide bond formation by the large subunit alone or by the 70S ribosome if appropriate substrates are used[60]. Thus, it appears that there are no significant structural differences between the PTCs of the 50S subunit and the 70S ribosome that are relevant to catalysis.

16S RNA form type I and type II A–minor interactions[13] with G–C base pairs 30–40 and 29–41 of the P-site tRNA[5,6,19]. Other tRNAs may contain AU base sequences in these regions and are unable to make these interactions, which may explain the greater stability. It has also been suggested that G1338 and A1339, which lie between the P-site and E-site tRNAs, may prevent inappropriate movement from the P to E sites and may act as a switch during both initiation and translocation[5].

The structure of a tRNA bound to the P site shows that the tRNA is distorted compared with the crystal structure of yeast tRNA[Phe]. The main distortion is a deformation of the anticodon stem, which exhibits an opening up of its narrow major groove around the base pair that is formed between tRNA residues 26 and 44 (REF. 5). The authors of this study suggest that if these constraints are released after peptidyl transfer, the relaxation of the deformation may assist in drawing the tRNA towards the E site, where it assumes the straight and, presumably, energetically more stable conformation. The movement of the peptidyl-CCA of the A-site tRNA to the P site — thereby forming the hybrid state — might be favoured by the additional base pair with the 23S rRNA P loop that is formed by C74 as well as the 180° rotated conformation of the CCA relative to the acceptor stem[8].

Interestingly, some interactions that stabilize tRNA binding to the P site may also have a catalytic role in peptide bond formation. Selmer *et al.*[5] found that a polypeptide tail of protein L27 interacts with the acceptor stem of the P-site tRNA and extends down to interactions with A76. Presumably, this interaction with L27 stabilizes the positioning of the CCA end of P-site tRNA and thereby contributes to a modest increase in catalysis[5]. Consistent with this, the removal of the first three residues of L27 reduces peptidyl-transferase activity[20]. A disordered loop of L10e in the *Haloarcula marismortui* (Hma) 50S subunit (which is a homologue of eubacterial L16) occupies a similar location; a model of it in a fully extended conformation is consistent with the possibility that it might also interact with the acceptor stem, but might not participate directly in peptide bond formation[21].

Perhaps most striking is the close similarity of the structures of the PTC interacting with the CCA of P-site tRNA in the *T. thermophilus* 70S ribosome[5] and with the CCA complex in the Hma 50S subunit[9,22]. The interactions that are made are, within error, identical in both ribosomal subunits, which is consistent with the conclusion that the structures of the 50S subunit in complex with substrates, products or intermediates accurately reflect the structures of full tRNA substrates bound to the complete ribosome. However, at this point, it cannot be excluded that the different interpretation of a considerably lower resolution (3.7 Å) map of a similar complex with the 70S ribosome[6] is a crystallographic artefact of the lower resolution and weaker data (BOX 1).

Ribosome-catalysed translation

The emerging structural studies of the ribosome are not only providing a detailed look at the process of protein synthesis, but also demonstrate that the rRNA, which is at the heart of all aspects of the processes that make the ribosome a ribozyme[8], undergoes an A-site substrate-induced conformational change that prevents nonspecific hydrolysis of the peptidyl-tRNA[9].

Substrate-induced conformational changes. The catalytic mechanism of many enzymes, including that of the ribosome, involves the activation of a nucleophilic attack by one substrate on a second substrate; therefore, enzymes must guard against erroneously activating the hydrolytic attack of an omnipresent water molecule on the second substrate when the first substrate is absent. How does the ribosome guard against activating a bound water molecule (which would hydrolyse the peptidyl-tRNA) when the A site is empty, which is the case for most of the time? The answer is substrate-induced fit — a mechanism that Koshland proposed nearly 50 years ago to explain why hexokinase does not hydrolyse ATP in the absence of glucose[23]. In this case, the active site of the enzyme is not properly configured to perform catalysis until the proper attacking substrate (for example,

---

Ribozyme
A ribosome is classified as a ribozyme (ribonucleic acid enzyme) because its active site is composed of RNA.

Nucleophilic attack
A reaction whereby an electron-rich atom (such as nitrogen) attacks an electropositive group.
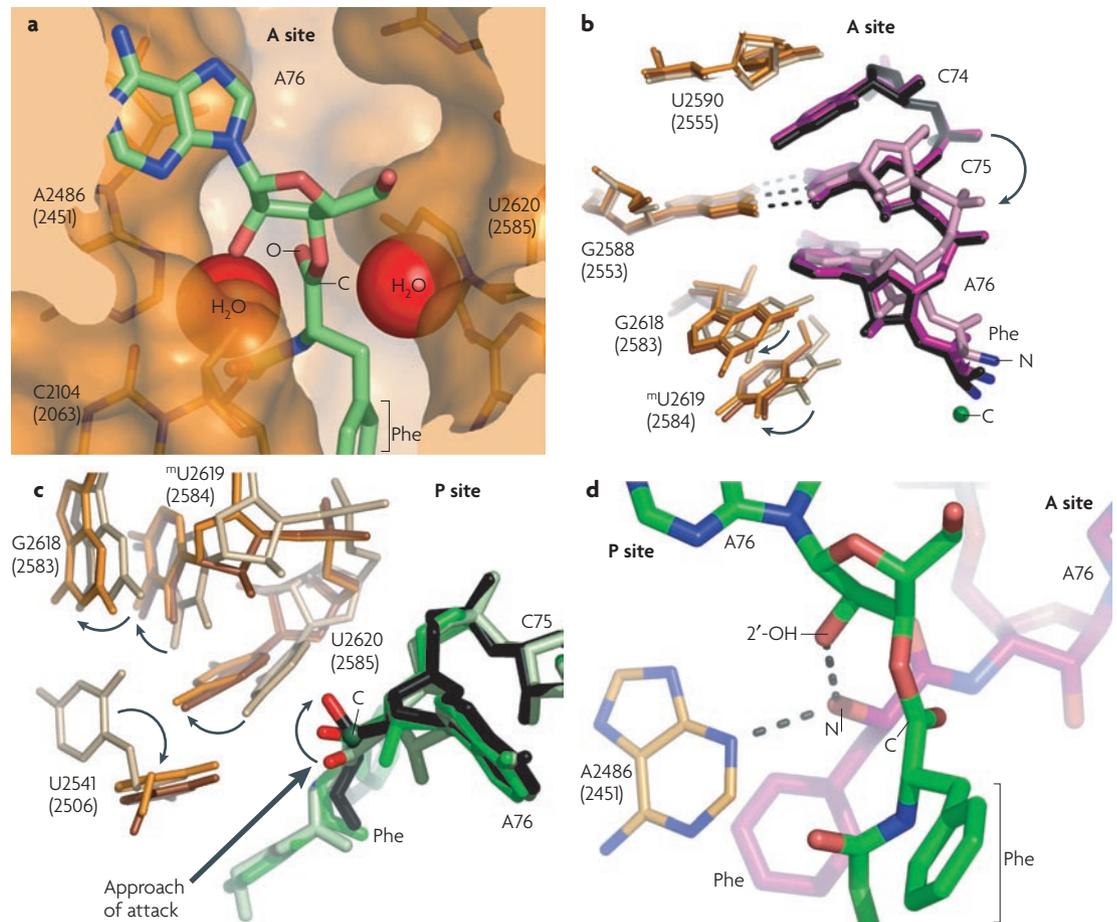
Figure 4 | **Substrate-induced rearrangements in the peptidyl-transferase centre promote peptide chain formation and suppress hydrolysis. a** | Conformation of the peptidyl-transferase centre when only the P site is occupied. The ester-linked carbonyl-carbon (labelled C) of the peptidyl-tRNA (shown in green) is sterically protected from nucleophilic attack by water (red spheres) by bases A2486, C2104 and U2620 in *Haloarcula marismortui*. A76 is the 3′ nucleotide of the P-site tRNA, O is the carbonyl oxygen and Phe is the side chain of the linked peptide. **b** | Binding of an aminoacyl-CCA analogue of aminoacyl-tRNA to the A site induces conformational changes in the ribosome, and repositions the peptidyl-tRNA to facilitate peptide bond formation. With an A-site substrate that lacks C74 (cytidine-hydroxypuromycin (ChPmn); shown in light pink with the ribosomal (r)RNA in beige) and mimics an empty A site, the substrate is positioned higher in the A site. In substrates that contain C74 and more closely mimic the tRNA occupied A-site state (cytidine-cytidine-hydroxypuromycin (CChPmn); shown in magenta with orange rRNA) or in a transition-state analogue (TSA; black, with brown rRNA) that mimics the transition-state intermediate conformation, C74 stacks with U2590 of the ribosome. This shifts the substrate down and moves the α-amino group closer to the ester carbon of the P-site substrate. To maintain interactions between the rRNA and the substrate occupying the A site, G2618 is shifted, which also causes methyl-U2619 to move. **c** | A view of the same three complexes described in part **b**, but from the P site. The movement that is induced in G2618 by A-site substrate binding breaks its G–U wobble pair with U2541, which swings through 90°. This allows a rearrangement of methyl-U2619 and U2620, which previously protected the carbonyl-carbon from nucleophilic attack. This then allows a rotation of the carbonyl-carbon (compare the position of the light-green P-site substrate (when ChPmn occupies the A site) with the dark-green substrate (with CChPmn bound)), which makes it accessible to the A-site substrate. The post-attack, transition-state position is also shown (TSA; black). **d** | The α-amino group is stabilized in the pre-attack conformation (CChPmn) through hydrogen bonding to N3 of the ribosomal base A2486 and to the 2′ hydroxyl group of the P-site substrate. Although mutation of A2486 has no effect on the rate of peptide bond formation, removal of the 2′ hydroxyl group of A76 greatly reduces this rate. In this ground-state structure, the reactive groups are 3.7 Å apart. The nucleotide numbers in parentheses are the numbers in the *E. coli* rRNA sequence.

glucose) is bound[24], whereupon it induces a conformational change in the enzyme that correctly configures the active site and substrates.

When the A site is unoccupied during protein synthesis on the ribosome, the conformation of the P-site rRNA sequesters the ester-linked carbonyl-carbon of the peptidyl-CCA (which is the carbon atom through which

the peptide bond will be formed) from a nucleophilic attack by water molecules (FIG. 4a). However, when the CCA of the aminoacyl-tRNA binds to the A site, it induces a conformational change in the rRNA of the PTC. This change optimally positions the α-amino group of the aminoacyl-tRNA for its nucleophilic attack, and exposes and reorientates the target carbonyl-carbon[9] (FIG. 4b).

Evidence for the induced-fit mechanism comes from the structures of the Hma 50S subunit in complex with various substrate and intermediate analogues[9–11] and is consistent with biochemical data[25,26]. When the analogue of peptidyl-tRNA, CCA-Phe-caproic acid-biotin (CCApcb), is bound to the P site with sparsomycin but no A-site substrate, the ester group of CCApcb is both positioned and sequestered by three bases of the 50S subunit of Hma: U2620 (2585), A2486 (2451) and C2104 (2063) (the nucleotide numbers in parentheses correspond to those of the *E. coli* rRNA sequence). Furthermore, the carbonyl oxygen of the ester group points towards the position that is eventually occupied by the α-amino group of the A-site substrate, and the carbonyl-carbon is sequestered from water. However, when the analogue CC-hydroxy puromycin binds to the A site, it induces a series of conformational changes in the rRNA that result in the movement of base U2620 (2585) away from the ester group. This allows the exposure and reorientation of its carbonyl group in a manner that is favourable for nucleophilic attack by the nearby α-amino group. On binding of both the A- and P-site substrates, the rRNA — particularly the A loop and P loop, which interact with the 3′ CCAs of the A-site and P-site tRNAs — properly position the attacking α-amino group and ester-linked carbonyl-carbon for reaction. The proper orientation of the two substrates — as in all enzymes — is likely to make the major contribution to the catalytic power of the ribosome in the peptidyl-transferase reaction[27,28].

Catalysing the peptidyl-transfer reaction. The structure of a complex that manifests the pre-attack conformation of the substrates bound to the PTC (FIG. 4c) shows that only two residues are hydrogen-bonded to the attacking α-amino group: the N3 of A2486 (2451) and the 2′ hydroxyl group of the A76 of peptidyl-tRNA. Thus, these are the only possible candidates that might serve an additional chemical role in catalysis[9]. The hypothesis that the N3 of A2486 (2451) might serve as a general base to activate the attacking α-amino group[8] was conclusively eliminated by genetic and biochemical experiments by Green and co-workers. They showed that mutation of A2486 to any other base has no effect on the rate of peptide bond formation when full aminoacyl-tRNA substrates were used in the 70S ribosome[29].

By contrast, the 2′ hydroxyl group of residue A76 of the peptidyl-tRNA has been shown to have a prominent role in the mechanism of ribosome catalysis of peptide bond formation. Evidence from a series of structural, biochemical and genetic experiments led to the conclusion that the 2′ hydroxyl group functions as a vital proton shuttle in substrate-assisted catalysis (FIG. 4d). It receives the proton either directly from the attacking α-amino group or from an intervening water molecule, while providing a proton to the leaving 3′ hydroxyl group of the tRNA on completion of peptide bond formation[9,10,22,30,31]. The 3′ hydroxyl group may also have a substrate-assisted catalysis role to deprotonate the 2′ hydroxyl group. In a modelled complex of the 50S subunit with a peptidyl-tRNA that lacks the 2′ hydroxyl group (2′ deoxy-A76), the bulk solvent water still has no access to the 3′ hydroxyl group of the peptide-linked tRNA. This will presumably hamper the peptidyl transfer to the attacking α-amino group because there is no source from which the 3′ hydroxyl group can receive a proton upon deacylation[10]. Strobel and colleagues[31] found that the rate of peptide bond formation by peptidyl-tRNAs containing 2′ deoxy-A76 was at least 10^6-fold slower than the ribo-A76, which highlights the pivotal importance of the 2′ hydroxyl group in peptide bond formation. These data support the proposal that the 2′ hydroxyl group functions as a proton shuttle that receives a proton ultimately from the α-amino group and donates it to the 3′ hydroxyl group. However, it might be inappropriate to consider that the 2′ hydroxyl group provides a 10^6-fold enhancement when compared with the uncatalysed reaction. A 2′ deoxy-A76 would have a 100-fold reduced rate owing to the vicinal effect of the 2′ hydroxyl group even in the absence of the ribosome. Furthermore, in solution, the 3′ hydroxyl group of A76 would have access to bulk solvent, thereby allowing it to be protonated on deacylation, as the reaction requires. By contrast, water has no access to this 3′ hydroxyl group on the ribosome.

Stabilization of the transition state of the peptidyl-transferase reaction by the ribosome might contribute to catalysis. This possibility has been examined in structures of several complexes of the Hma large subunit using analogues in which either a phosphodiester or triester mimics the tetrahedral transition state that occurs during peptide bond formation[10]. The structures of numerous analogues, some of which have a protein side-chain mimic and none of which contain a non-bridging phosphorothioate, were determined at resolutions between 2.4 Å and 2.8 Å. They showed that the oxyanion mimic of the intermediate points away from A2486 (2451) and interacts with a water molecule, which in turn is positioned by interactions with two nucleotides. It is possible that the electropositive hydrogen from the polar water molecule partially stabilizes the negative charge that is formed in the oxyanion transition state and thereby contributes to catalysis.

A thorough examination of the binding of both divalent and monovalent cations using manganese and potassium ions, among others, clearly demonstrates that no metal ion is directly involved in catalysis at the PTC[10]. Therefore, the ribosome achieves catalysis of peptide bond formation by three mechanisms: first, by properly positioning the two substrates that are to react; second, by using the 2′ hydroxyl group of the P-site A76 as a general acid–base proton shuttle; and third, by electrostatically stabilizing the oxyanion transition state.

Therefore, at present, the most convincing biochemical and structural data lead to the conclusion that there are no significant structural differences between the PTC of the 50S subunit and the 70S ribosome when they are engaged in catalysing the peptidyl-transferase reaction. In addition, the mechanistic conclusions that have been drawn from structural studies of the 50S subunit also apply to the whole ribosome (BOX 1).
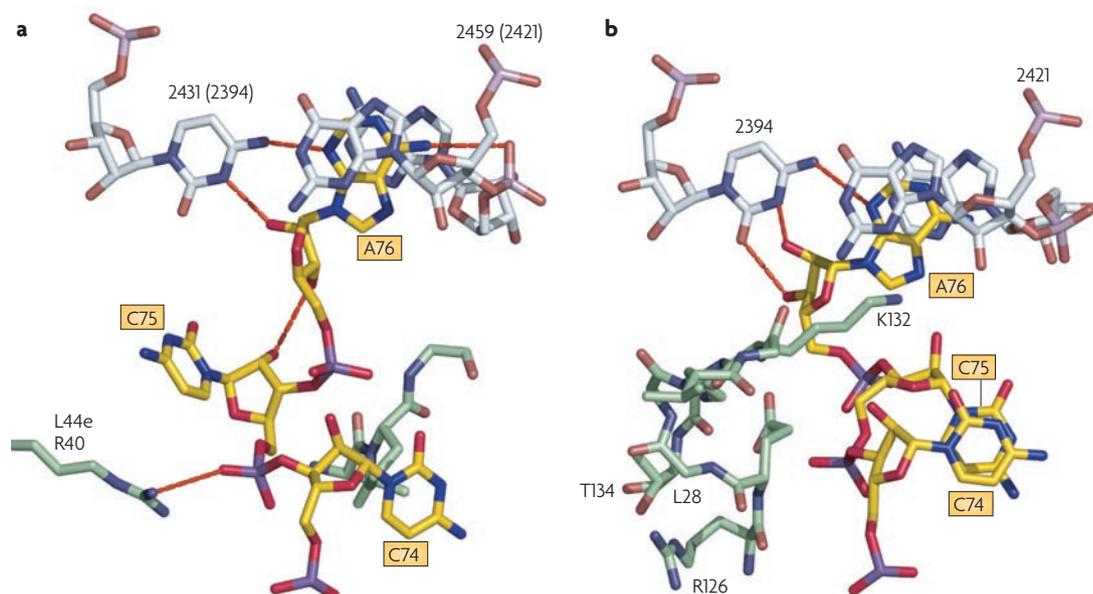
Figure 5 | **Different interactions of the tRNA with the E site across kingdoms. a** | The CCA motif (yellow) of a tRNA bound to the ribosome E site of the archaeon *Haloarcula marismortui*. Bases C74 and C75 are splayed out and interact extensively with a polypeptide loop of the ribosomal protein L44e (green), which is only found in archaea and eukaryotes. A76 is inserted between two conserved ribosomal (r)RNA bases (white). The pocket that binds the sugar of A76 is of limited volume, which ensures that the E site can only accept deacylated tRNA because even a tRNA charged with the smallest amino acid, Gly, would sterically clash with rRNA. The nucleotide numbers in parentheses are the numbers in the *E. coli* rRNA sequence. **b** | The CCA portion of the tRNA adopts a different conformation when bound to the eubacterial ribosome from *Thermus thermophilus*, which lacks L44e but instead has L28. Whereas the binding site of A76 is conserved, C74 and C75 show a continuously stacked conformation, presumably because there is no L44e homologue or analogue for these bases to interact with, and L28 overlaps with the splayed conformation of C75 seen in *Haloarcula marismortui*. The addition of ribosomal proteins L28 and L44e to ribosomes must have occurred after the split of the kingdoms. The nucleotide numbers refer to the *E. coli* rRNA sequence.

Translocation of the tRNAs and mRNA

After the peptidyl-transfer reaction is completed, the 3′ end of the deacylated tRNA in the P site and the peptidyl-tRNA in the A site move to the E site and P site, respectively. It has been concluded that their CCA ends, which interact with the large 50S subunit, are the first parts to move and that they do so spontaneously. This results in the formation of a hybrid state[32], which was shown initially by biochemical experiments[32] and seen more recently in cryo-EM studies[33]. In response to the binding of EF-G, the anticodon stem-loop on the small subunit, as well as the mRNA with which it interacts, are also translocated[34].

Although several atomic structures of EF-G exist either with or without bound GDP[35,36], the current understanding of the structural basis of translocation driven by EF-G is derived entirely from cryo-EM studies, which have been interpreted in the context of biochemical and kinetic studies. The translocation step requires GTP hydrolysis by ribosome-bound EF-G, which is observed to precede translocation[37]. This has led Rodnina *et al.*[37] to suggest that the chemical energy of GTP hydrolysis is transformed into mechanical energy during translocation in a manner similar to the actin–myosin power stroke in muscle that is activated by ATP hydrolysis. The largest conformational change in the ribosome is a ratchet-like rotation of the small subunit

relative to the large one, which occurs on binding of the GTP complex with EF-G[34,38]. This rotation advances the mRNA in the direction that is required for translocation on the 30S subunit. In the structure of its complex with the 70S ribosome, the domains of EF-G are observed to be rearranged when compared with the crystal structure. An elongated domain IV is seen to extend to the A site on the small subunit, while the GTPase domain is seen interacting with the large subunit. A recent 7.3-Å resolution map of the complex shows details of the interactions between the RNA of the large subunit and the ordered loops 1 and 2 of EF-G[39].

Binding of tRNAs to the E site

The structures of the Hma large subunit in complex with the CCA end of tRNA or with an RNA stem-loop mimic of the acceptor stem[40] show that the unstacked bases of the CCA end interact with conserved E-site nucleotides. In archaea and eukaryotes, these bases also interact with ribosomal protein L44e, which is only found in these kingdoms. The terminal base, A76 of the tRNA, is inserted between two conserved rRNA bases and is hydrogen bonded to a conserved base of the rRNA. The bases of C74 and C75 are, however, unstacked, splayed out and interact extensively with a polypeptide loop of the L44e protein (FIG. 5a). The E site can only bind deacylated tRNA because the 3′ hydroxyl group of A76
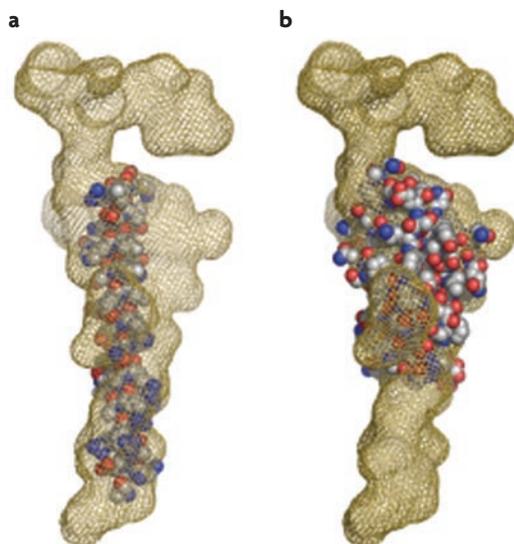
Figure 6 | **Space-filling models of the polypeptide exit tunnel. a** | An α helix, the surface of which is delineated by the mesh, can be accommodated by the polypeptide tunnel. **b** | An immunoglobulin domain modelled in the polypeptide shows many steric clashes, which give rise to the spheres on the outside of the mesh, and therefore cannot be accommodated.

sits in a pocket of limited volume, and even esterification by a Gly residue would sterically clash with rRNA. Presumably, deacylated tRNAs spontaneously move their 3′ ends into the E site due to the ability of the D stem to adapt an energetically more stable unkinked conformation and, perhaps, an intrinsically tighter tRNA binding to the E site that can exclude acylated tRNAs.

The binding site for the CCA portion of tRNA is not, however, universally conserved. In eubacterial *T. thermophilus* ribosomes, the CCA portion of tRNA that is bound in the E site adapts a different, continuously stacked conformation[5,6] (FIG. 5b). This is presumably the case because there is no protein analogue or homologue of L44e in eubacteria with which C74 and C75 can interact. Furthermore, protein L28, which is present in eubacteria but not archaea or eukaryotes, overlaps with the splayed-out conformation of C75 that is seen in the Hma 50S subunit complex, thereby preventing this adaptation in eubacterial ribosomes. Likewise, the stacked conformation of the CCA that is observed in the eubacterial E site is blocked by L44e, which overlaps with the stacked conformation of C75. Because proteins L44e and L28 must have been added to the protein portfolio of the ribosome after the split of the kingdoms, their introduction is presumably responsible for the different CCA conformations. One might posit that the last common ancestor was able to bind CCA to the E site as seen in eubacteria, interacting with universally conserved rRNA bases. And only after the addition of L44e to the archaeal and eukaryotic lineage did the unstacked protein interaction arise. This protein difference also accounts for the observation that antibiotics (such as 13-deoxytedanolide, which targets the E site) inhibit eukaryotic and archaeal ribosomes, but not

eubacterial ribosomes: this is because some antibiotics are seen to interact in part, but significantly, with L44e[41]. Also importantly, protein L28 significantly overlaps with the 13-deoxytedanolide-binding site.

What's up in the tunnel?

Unwin and co-workers first observed in EM images of the ribosome what has become known as the polypeptide exit tunnel[42]. Since its identification in the early 1980s, the polypeptide exit tunnel has been the subject of extensive study and much speculation concerning its nature and function. Emanating from the PTC, the tunnel is 12–20 Å in diameter and traverses through the centre of the 50S subunit, exiting on the other side ~80 Å away[43]. Cryo-EM reconstructions have been interpreted to show a main tunnel and a side tunnel that leads to a second exit[44]. This led to the proposal that this side tunnel might be used for certain categories of polypeptides to exit from the ribosome, such as secreted proteins, whereas the main tunnel would be used for the soluble majority of proteins. The crystal structure of the 50S subunit does not support the existence of a second tunnel exit[45].

No role for the tunnel in protein folding. Numerous EM studies of the ribosome over the past two decades have been interpreted to suggest that the folding of polypeptide chains could occur within the tunnel, which might function analogously to a chaperone protein[46]. However, polypeptides being synthesized by the ribosome are protected from digestion by proteases; depending on the protein being studied, somewhere between 30 and 50 amino acids are protected from protease digestion, presumably because they have not yet exited the tunnel[47]. This suggests that at least half of the polypeptide is in an extended conformation and less than half could form a simple α-helix.

A detailed analysis of the tunnel using the coordinates derived from the refined 2.4-Å resolution crystal structure of the Hma 50S subunit[48] shows that, even though the tunnel is extremely porous for molecules the size of water, there are no side tunnels that are large enough to allow the passage of a polypeptide, even one as narrow as poly-Ala[45]. Furthermore, analysis of the structure in the polypeptide exit tunnel strongly suggests that protein folding beyond the level of formation of an α-helix is extremely unlikely. Most certainly, it rules out the possibility that an immunoglobulin domain could fold within the tunnel (FIG. 6b) and be released, as has been concluded from a cryo-EM study[46].

It has been suggested that the tunnel may be flexible and might be able to expand in diameter to accommodate a folding polypeptide[46]. However, expansion appears to be unlikely because structures of the RNAs surrounding the tunnel are identical in both of the 50S ribosomal subunit structures that have been determined as well as three 70S structures[4–6], and the required expansion of the tunnel would be 10–20 Å. The extremely porous nature of the ribosomal RNA structure leads one to compare it to that of a sponge; however, the possible robustness of the structure may be more appropriately compared
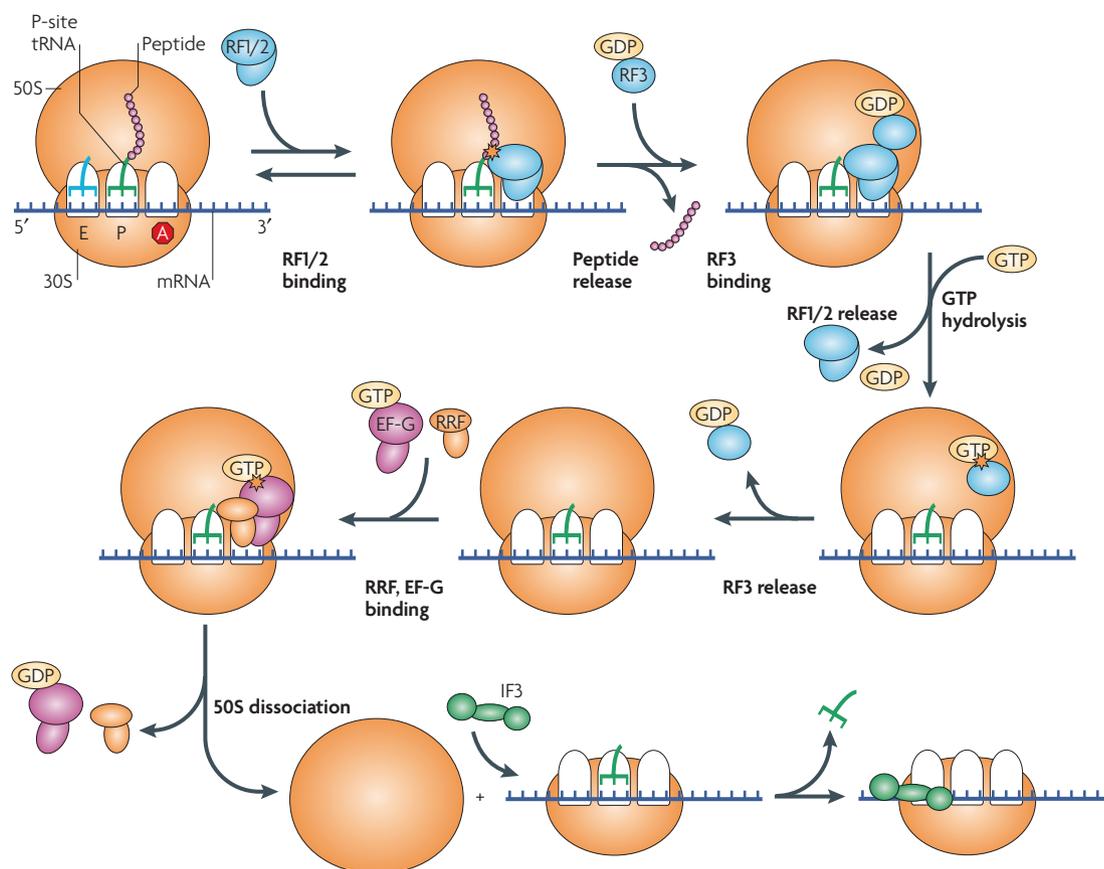
Figure 7 | **An overview of termination of translation.** A stop codon in the mRNA A site (red hexagon) recruits either release factor-1 (RF1) or RF2 to mediate the hydrolysis and release of the peptide from the tRNA in the P site. This functions as a signal to recruit RF3–GDP, which induces the release of RF1/2. Exchange of GDP for GTP on RF3 and subsequent hydrolysis is thought to release RF3. The ribosome is left with mRNA and a deacylated tRNA in the P site. This complex is disassembled by the binding of ribosomal release factor (RRF) and the EF-G elongation factor[62]. GTP hydrolysis causes the dissociation of the 50S ribosomal subunit, and initiation factor-3 (IF3) is required to dissociate the deacylated tRNA from the P site. Figure adapted from REF. 61.

to that of the Eiffel Tower. The many crisscrossing RNA helical rods make specific interactions with each other and with proteins to stabilize and reinforce a particular structure, thereby making it extremely unlikely that the tunnel can expand and contract by 10–20 Å.

Protein folding at the end of the tunnel. Some co-translational protein folding does appear to occur, which is assisted by a protein bound at the end of the polypeptide exit tunnel. Models of the 50S subunit bound to trigger factor (which were derived from co-crystal structures of the 50S ribosomal subunit with fragments of the trigger factor protein) lead to the conclusion that the trigger factor forms a significant interior volume at the bottom of the tunnel that is sufficient in size to allow the protected folding of modest-sized protein domains[49,50]. The structure of the N-terminal binding domain of the *Deinococcus radiodurans* trigger factor bound to the *D. radiodurans* large ribosomal subunit shows that it interacts with proteins L23 and L29 and 23S rRNA at the end of the tunnel[50]. Superposition of the full-length trigger factor on the N-terminal domain shows that there is a hydrophobic

crevice at the end of the tunnel that is large enough to accommodate the nascent polypeptide chain. Taken together with cryo-EM models of the signal recognition particle (SRP) bound to the ribosome, it appears that simultaneous binding of both trigger factor and the SRP to the tunnel exit is possible[50].

Protein synthesis termination
When a stop codon in the mRNA reaches the A site of the ribosome at the end of the elongation phase of protein synthesis, translocational release factors (RF1, RF2 and RF3 in bacteria) catalyse the hydrolysis and release of the ester-linked polypeptide on the P-site tRNA (FIG. 7). One primary mechanistic question that remains is how the release factor proteins are able to recognize the stop codon in the mRNA on the small subunit and thereby enable the other end of the factor to catalyse release on the large subunit, some 70 Å away. Furthermore, is the hydrolysis achieved by inducing the same PTC conformational change that is produced by the A site substrate[9] that positions an attacking water molecule for nucleophilic attack, direct chemical catalysis or some combination thereof?

Trigger factor
A prokaryotic protein that binds to the ribosome tunnel exit and assists in nascent polypeptide folding.

Tantalizing insights into the structural basis of release factor function derive from low-resolution crystal structures of the 70S ribosome in complex with tRNA molecules bound in a codon-specific manner to the P sites and either RF1 or RF2 bound in the A site[51]. They found that the RFs exhibit the overall shape of a tRNA. A loop in domain 2 of RF1 containing a PVT sequence (in RF1) is observed in the vicinity of the UGA stop codon[21], consistent with genetic data[52]. Domain 4 interacts with the N-terminal domain of L11, and the GGQ-containing loop of domain 1 is seen in the PTC near the site of the peptide link to the P-site tRNA. The low resolution, however, prevents a detailed understanding of the structural and chemical mechanism by which the GGQ loop promotes hydrolysis of the ester-linked peptide.

Recently, two structures of the bacterial 70S ribosome in complex with the ribosomal recycling factor (RRF) have been determined that have led to hypotheses about its mechanism of action[53,54].

Conclusions and future directions

Excellent cryo-EM maps of the ribosome have been obtained, capturing it at various stages in the process of protein synthesis. These include complexes of the bacterial 70S ribosome with bound EF-G–GTP, EF-Tu with aminoacyl-tRNA or RF, as well as a 5-Å resolution X-ray map of an RF complex. However, none of these complexes is known at atomic resolution. The structural basis for how proteins are co-translationally secreted has begun to be addressed by the crystal structure of the translocon alone[55] as well as by a cryo-EM map of a ribosome secreting a polypeptide through a translocon dimer that is bound at the end of the exit tunnel[56]. One model for the mechanism by which proteins are co-translationally secreted is based on the crystal structure of a monomer as well as biochemical studies, and posits the existence of a channel in the centre of the monomer through which the polypeptide passes. However, a second model based on the cryo-EM data proposes that secretion occurs between a bound translocon dimer. Clearly, a detailed atomic-resolution view of the polypeptide passing through the translocon is required.

Although the core aspects of decoding and protein synthesis are highly conserved across the three kingdoms, other aspects, such as the initiation of protein synthesis, differ significantly. So far, only low-resolution cryo-EM studies of eukaryotic ribosome structures have been successful. High-resolution structural studies of eukaryotic ribosomes and, most importantly, complexes of the 40S ribosomal subunit captured in the act of initiation in complex with initiation factors, fRNA[fmet] and mRNA have not yet been accomplished. Structural insights into this aspect of protein synthesis will be of particular interest because of its crucial role in the regulation of translation[57,58].

1. Palade, G. E. A small particulate component of the cytoplasm. *J. Biophys. Biochem. Cytol.* **1**, 59–68 (1955).
2. Watson, J. D. Involvement of RNA in the synthesis of proteins. *Science* **140**, 17–26 (1963).
3. Lake, J. A. Ribosomal structure determined by electron microscopy of *E. coli* small subunits, large subunits and monomeric ribosomes. *J. Mol. Biol.* **105**, 131–159 (1976).
4. Schuwirth, B. S. *et al*. Structures of the bacterial ribosome at 3.5 Å resolution. *Science* **310**, 827–834 (2005).
   Presents the first complete atomic structure of the 70S ribosome from *E. coli* derived from a high-resolution map, but without bound substrates.
5. Selmer, M. *et al*. Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* **313**, 1935–1942 (2006).
   The most complete and accurate structure of the 70S ribosome published to date also has bound mRNA, as well as tRNAs at the A site (partial), P site and E site.
6. Korostelev, A., Trakhanov, S., Laurberg, M. & Noller, H. F. Crystal structure of a 70S ribosome–tRNA complex reveals functional interactions and rearrangements. *Cell* **126**, 1066–1077 (2006).
7. Ogle, J. M. *et al*. Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* **292**, 897–902 (2001).
   The structure of the 30S subunit with mRNA and an anticodon stem-loop RNA mimic of tRNA shows how decoding occurs in the A site.
8. Nissen, P., Ban, N., Hansen, J., Moore, P. B. & Steitz, T. A. The structural basis of ribosome activity in peptide bond synthesis. *Science* **289**, 920–930 (2000).
9. Schmeing, T. M., Huang, K. S., Strobel, S. A. & Steitz, T. A. An induced-fit mechanism to promote peptide bond formation and exclude hydrolysis of peptidyl-tRNA. *Nature* **438**, 520–524 (2005).
   Shows that the binding of an appropriate A-site substrate to the 50S subunit complex with a P-site substrate induces an active site conformational change that is essential for catalysis.
10. Schmeing, T. M., Huang, K. S., Kitchen, D. E., Strobel, S. A & Steitz, T. A. Structural insights into the roles of water and the 2′ hydroxyl of the P site tRNA in the peptidyl transferase reaction. *Mol. Cell* **20**, 437–448 (2005).
11. Schmeing, T. M. *et al*. A pre-translocational intermediate in protein synthesis observed in crystals of enzymatically active 50S subunits. *Nature Struct. Biol.* **9**, 225–230 (2002).
12. Green, R. & Noller, H. F. Ribosomes and translation. *Annu. Rev. Biochem.* **66**, 679–716 (1997).
13. Nissen, P., Ippolito, J. A., Ban, N., Moore, P. B. & Steitz, T. A. RNA tertiary interactions in the large ribosomal subunit: the A-minor motif. *Proc. Natl Acad. Sci. USA* **98**, 4899–4903 (2001).
14. Ogle, J. M., Murphy, F. V. I., Tarry, M. J. & Ramakrishnan, V. Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell* **111**, 721–732 (2002).
15. Rodnina, M. V. & Wintermeyer, W. Fidelity of aminoacyl-tRNA selection on the ribosome's kinetic and structural mechanisms. *Annu. Rev. Biochem.* **70**, 415–435 (2001).
16. Valle, M. *et al*. Cryo-EM reveals an active role for aminoacyl tRNA in the accommodation process. *EMBO J.* **21**, 3557–3567 (2002).
17. Stark, H. *et al*. Ribosome interactions of aminoacyl-tRNA and elongation factor Tu in the codon-recognition complex. *Nature Struct. Biol.* **9**, 849–854 (2002).
18. Valle, M. *et al*. Incorporation of aminoacyl-tRNA into the ribosome as seen by cryo-electron microscopy. *Nature Struct. Biol.* **10**, 889–906 (2003).
19. Berk, V., Zhang, W., Pai, R. D. & Cate, J. H. D. Structural basis for mRNA and tRNA positioning on the ribosome. *Proc. Natl Acad. Sci. USA* **103**, 15830–15834 (2006).
20. Maguire, B. A., Benaminov, A. D., Ramu, H., Mankin, A. S. & Zimmermann, R. A. A protein component at the heart of an RNA machine: the importance of protein L27 for the function of the bacterial ribosome. *Mol. Cell.* **20**, 427–435 (2005).
21. Moore, P. B. & Steitz, T. A. The structural basis of large ribosomal subunit function. *Annu. Rev. Biochem.* **72**, 813–850 (2003).
22. Hansen, J. L., Schmeing, T. M., Moore, P. B. & Steitz, T. A. Structural insights into peptide bond formation. *Proc. Natl Acad. Sci. USA* **99**, 11670–11675 (2002).
23. Koshland, D. E. Mechanism of transfer enzymes. In *The Enzymes* (Boyer, P. D., Lardy, H. & Myrback, K., eds) 305–346 (Academic Press, New York, 1959).
24. Bennett, W. S. & Steitz, T. A. Glucose-induced conformational change in yeast hexokinase. *Proc. Natl Acad. Sci. USA* **75**, 4848–4852 (1976).
25. Beringer, M. & Rodnina, M. V. The ribosomal peptidyl transferase. *Mol. Cell* **26**, 311–321 (2007).
26. Caskey, C. T., Beaudet, A. L., Scolnick, E. M. & Rosman, M. Hydrolysis of fMet-tRNA by peptidyl transferase. *Proc. Natl Acad. Sci. USA* **68**, 3163–3167 (1971).
27. Pape, T., Wintermeyer, W. & Rodnina, M. V. Conformational switch in the decoding region of 16S rRNA during aminoacyl-tRNA selection on the ribosome. *Nature Struct. Biol.* **7**, 104–107 (2000).
28. Paige, M. I. & Jencks, W. P. Entropic contributions to rate acceleration in enzymatic and intramolecular reactions and the chelate effect. *Proc. Natl Acad. Sci. USA* **68**, 1678–1683 (1971).
29. Youngman, E. M., Brunelle, J. L., Kochaniak, A. B. & Green, R. The active site of the ribosome is composed of two layers of conserved nucleotides with distinct roles in peptide bond formation and peptide release. *Cell* **117**, 589–599 (2004).
30. Dorner, S., Panuschka, F., Schmid, W. & Barta, A. Mononucleotide derivatives as ribosomal P-site substrates reveal an important contribution of the 2′-OH activity. *Nucl. Acids Res.* **31**, 6536–6542 (2003).
31. Weinger, J. S., Parnell, K. M., Dorner, S., Green, R. & Strobel, S. A. Substrate-assisted catalysis of peptide bond formation by the ribosome. *Nature Struct. Biol.* **11**, 1101–1106 (2004).
   Biochemical demonstration of the large contribution of the 2′ hydroxyl group of A76 of the P-site substrate to peptide bond formation.
32. Moazed, D. & Noller, H. F. Intermediate states in the movement of transfer RNA in the ribosome. *Nature* **342**, 142–148 (1989).

33. Gao, N. *et al*. Mechanism for the disassembly of the post termination complex inferred from cryo-EM studies. *Mol. Cell* **18**, 663–674 (2005).

34. Valle, M., Zavialov, A., Sengupta, J., Rawat, U., Ehrenberg, M. & Frank, J. Locking and unlocking of ribosomal motions. *Cell* **114**, 123–134 (2003).

35. Ævarsson, A. *et al*. Three-dimensional structure of the ribosomal translocase: elongation factor G from *Thermus thermophilus*. *EMBO J*. **13**, 3669–3677 (1994).

36. Czworkowski, J., Wang, J., Steitz, T. A. & Moore, P. B. The crystal structure of elongation factor G complexed with DGP, at 2.7 Å resolution. *EMBO J*. **13**, 3661–3668 (1994).

37. Rodnina, M., Savelsbergh, A., Katunin, V. I. & Wintermeyer, W. Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. *Nature* **385**, 37–41 (1979).

38. Frank, J. & Agrawal, R. K. A ratchet-like inter-subunit reorganization of the ribosome during translocation. *Nature* **406**, 318–322 (2000).
    The important rotation of the small subunit relative to the large subunit on EFG–GTP binding is shown in this cryo-EM study.

39. Connell, S. R. *et al*. Structural basis for interaction of the ribosome with the switch regions of GTP-bound elongation factors. *Mol. Cell* **25**, 751–764 (2007).
    The highest-resolution cryo-EM structure of the 70S ribosome with bound EFG gives insights into the mechanism of its function in translocation.

40. Schmeing, T. M., Moore, P. B. & Steitz, T. A. Structure of deacylated tRNA mimics bound to the E site of the large ribosomal subunit. *RNA* **9**, 1345–1352 (2003).

41. Schroeder, S., Blaha, G., Tirado-Rives, J., Steitz, T. A. & Moore, P. B. The structures of antibiotics bound to the E-site region of the 50S ribosomal subunit of *Haloarcula marismortui*; 13-deoxytedanolide and girodazole. *J. Mol. Biol*. **367**, 1471–1479 (2007).

42. Milligan, R. A. & Unwin, P. N. *In vitro* crystallization of ribosomes from chick embryos. *J. Cell Biol*. **95**, 648–653 (1982).

43. Ban, N., Nissen, P., Hansen, J., Moore, P. B. & Steitz, T. A. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* **289**, 905–920 (2000).

44. Gabashvili, I. S. *et al.* The polypeptide tunnel system in the ribosome and its gating in erythromycin mutants of L4 and L22. *Mol. Cell* **8**, 181–188 (2001).

45. Voss, N. R., Gerstein, M., Steitz, T. A. & Moore, P. B. The geometry of the ribosomal exit tunnel. *J. Mol. Biol*. **360**, 893–906 (2006).

46. Gilbert, R. J. *et al*. Three dimensional structures of translating ribosomes by cryo-EM. *Mol. Cell* **14**, 57–66 (2004).

47. Malkin, L. I. & Rich, A. Partial resistance of nascent polypeptide chains to proteolytic digestion due to ribosomal shielding. *J. Mol. Biol*. **26**, 329–346 (1967).

48. Klein, D. J., Moore, P. B. & Steitz, T. A. The roles of ribosomal proteins in the structure, assembly and evolution of the large ribosomal subunit. *J. Mol. Biol*. **340**, 141–177 (2004).

49. Ferhtz, L. *et al*. Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. *Nature* **431**, 590–596 (2004).

50. Schlünzen, F. *et al*. The binding mode of the trigger factor in the ribosome: implications for protein folding and SRP interaction. *Structure* **13**, 1685–1694 (2005).

51. Petry, S. *et al*. Crystal structures of the ribosome in complex with release factors RF1 and RF2 bound to a cognate stop codon. *Cell* **123**, 1256–1266 (2005).

52. Ito, K., Uno, M. & Nakamura, Y. A tripeptide "anticodon" deciphers stop codons in messenger RNA. *Nature* **403**, 680–684 (2000).

53. Borovinskaya, M. A. *et al*. Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nature Struct. Mol. Biol*. **14**, 727–732 (2007).

54. Weixlbaumer, A. *et al*. Crystal structure of the ribosome recycling factor bound to the ribosome. *Nature Struct. Mol. Biol*. **14**, 733–737 (2007).

55. van den Berg, B. *et al*. X-ray structure of a protein-conducting channel. *Nature* **427**, 36–44 (2004).

56. Mitra, K. *et al*. Structure of the *E. coli* protein-conducting channel bound to a translating ribosome. *Nature* **438**, 318–324 (2005).

57. Dever, T. E. Gene-specific regulation by general translation factors. *Cell* **108**, 545–556 (2002).

58. Sonenberg, N. & Dever, T. E. Eukaryotic translation initiation factors and regulators. *Curr. Opin. Struct. Biol*. **13**, 56–63 (2003).

59. Simonovic, M. & Steitz, T. A. Cross-crystal averaging reveals that the structure of the peptidyl-transferase center is the same in the 70S ribosome and 50S subunit. *Proc. Natl Acad. Sci. USA* (in the press).

60. Brunnelle, J. L. *et al*. The interaction between C75 of tRNA and the A loop of the ribosome stimulates peptidyl transferase activity. *RNA* **12**, 33–39 (2006).

61. Ramakrishnan, V. Ribosome structure and the mechanism of translation. *Cell* **108**, 557–572 (2002).

62. Janosi, L., Shimizu, I., Kaji, A. Ribosome recycling factor (ribosome releasing factor) is essential for bacterial growth. *Proc. Natl Acad. Sci. USA* **91**, 4249–4253 (1994).