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REVIEWS

What recent ribosome structures have revealed about the mechanism of translation

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The high-resolution structures of ribosomal subunits published in 2000 have revolutionized the field of protein translation. They facilitated the determination and interpretation of functional complexes of the ribosome by crystallography and electron microscopy. Knowledge of the precise positions of residues in the ribosome in various states has facilitated increasingly sophisticated biochemical and genetic experiments, as well as the use of new methods such as single-molecule kinetics. In this review, we discuss how the interaction between structural and functional studies over the last decade has led to a deeper understanding of the complex mechanisms underlying translation.

The ribosome is the large ribonucleoprotein particle that synthesizes proteins in all cells, using messenger RNA as the template and aminoacyl-transfer RNAs as substrates. Ribosomes from bacteria consist of a large (50S) and a small (30S) subunit, which together compose the 2.5-megadalton 70S ribosome; their eukaryotic counterparts are the 60S and 40S subunits and the 80S ribosome. The 50S subunit consists of 23S RNA (~2,900 nucleotides), 5S RNA (~120 nucleotides) and about 30 proteins; the 30S subunit consists of 16S RNA (~1,500 nucleotides) and about 20 proteins. In addition, several protein factors act on the ribosome at various stages of translation. In this review, we focus mainly on structural and mechanistic insights into bacterial translation obtained in the last few years. A previous review deals more extensively with earlier work[1].

The essentially complete atomic structures of an archaeal 50S subunit from *Haloarcula marismortui*[2] and a bacterial 30S subunit from *Thermus thermophilus*[3] published in 2000 were the basis for the phasing and/or molecular interpretation of every subsequent structure of the ribosome or its subunits. Such structures include low-resolution structures of the 70S ribosome by crystallography[4] or cryoelectron microscopy (cryoEM)[5], the structure of a bacterial 50S subunit[6], and more recent high-resolution structures of the 70S ribosome[7,8]. Finally, mobile elements of the 50S subunit such as the L1 or L7/L12 stalks that are partly or completely disordered in most high-resolution structures of the ribosome or the 50S subunit have been solved in isolation[9,10].

The basic architecture of the ribosome is shown in Fig. 1. The interface between the two subunits consists mainly of RNA. The mRNA binds in a cleft between the 'head' and 'body' of the 30S subunit, where its codons interact with the anticodons of tRNA. There are three binding sites for tRNA: the A site that binds the incoming aminoacyl-tRNA, the P site that holds the peptidyl-tRNA attached to the nascent polypeptide chain, and the E (exit) site to which the deacylated P-site tRNA moves after peptide-bond formation before its ejection from the ribosome. In the 50S subunit, the 3′ ends of P- and A-site tRNAs are in close proximity in the peptidyl-transferase centre (PTC), whereas the 3′ end of the E-site tRNA is ~50 Å away from the PTC.

Initiation

Bacterial translation can be roughly divided into three main stages, initiation, elongation and termination (Fig. 2; a movie of the process can be seen at http://www.mrc-lmb.cam.ac.uk/ribo/homepage/movies/translation_bacterial.mov). Initiation requires the ribosome to position the initiator fMet-tRNA^{fMet} over the start codon of mRNA in the P site. In bacteria, the ribosome is positioned in the vicinity of the start codon by base pairing between the 3′ end of 16S RNA and an approximately complementary sequence just upstream of the mRNA start codon, called the Shine–Dalgarno sequence. The precise positioning of the start codon in the P site requires the binding of a special initiator fMet-tRNA^{fMet} and three initiation factors, IF1–3. However, exactly how the correct tRNA is selected remains unclear, as are the roles of the various factors.

A probable first step in initiation is the binding of IF3 to the 30S that has been split from the 50S by ribosome recycling factor RRF and elongation factor G (EF-G) after translational termination (see Fig. 2 and the termination section later). This binding stimulates release of the mRNA and deacylated tRNA, leftover from the previous round of translation, from the 30S and prevents the large subunit from re-associating[11,12]. The binding of the 30S–IF3 complex to mRNA, IF1, IF2 and initiator tRNA results in the 30S initiation complex (30S-IC). IF2, a GTPase, promotes subunit joining to form the 70S initiation complex (70S-IC), which is accompanied by IF3 release[13–15]. After GTP hydrolysis and phosphate release from IF2 (refs 16, 17), fMet-tRNA^{fMet} moves into the PTC, readying the ribosome for elongation.

The mechanism of initiation is still unclear, owing to a paucity of structural data. There has been little progress towards high-resolution structures of initiation complexes since the structure of IF1 bound to a 30S subunit[18]. However, recent cryoEM studies have visualized both 30S and 70S initiation complexes. In a 30S-IC (ref. 19), which unfortunately did not contain IF3, IF2 stretches across the subunit interface of the 30S, contacting the acceptor end of fMet-tRNA^{fMet} with its carboxy terminus. The anticodon stem and elbow are shifted towards the E site, resulting in a "30S P/I state". IF1 is visible in the A site, but does not contact IF2. After subunit joining, the G domain of IF2 interacts with the GTPase centre of the large subunit[20]. It maintains its contacts with fMet-tRNA^{fMet}, which has shifted up out of plane from the 30S P/I state to a 70S P/I state, and seems to make a direct contact with IF1 in the 70S-IC. The 30S subunit is rotated relative to the 50S by ~4° anticlockwise, similar to the ratcheting seen during translocation[21].

In the structure of 70S-mRNA–fMet-tRNA^{fMet}–IF2–GDPCP[22], IF2 is still bound to the GTPase centre, but has lost contact with fMet-tRNA^{fMet}, now in the PTC in the canonical P/P state. The

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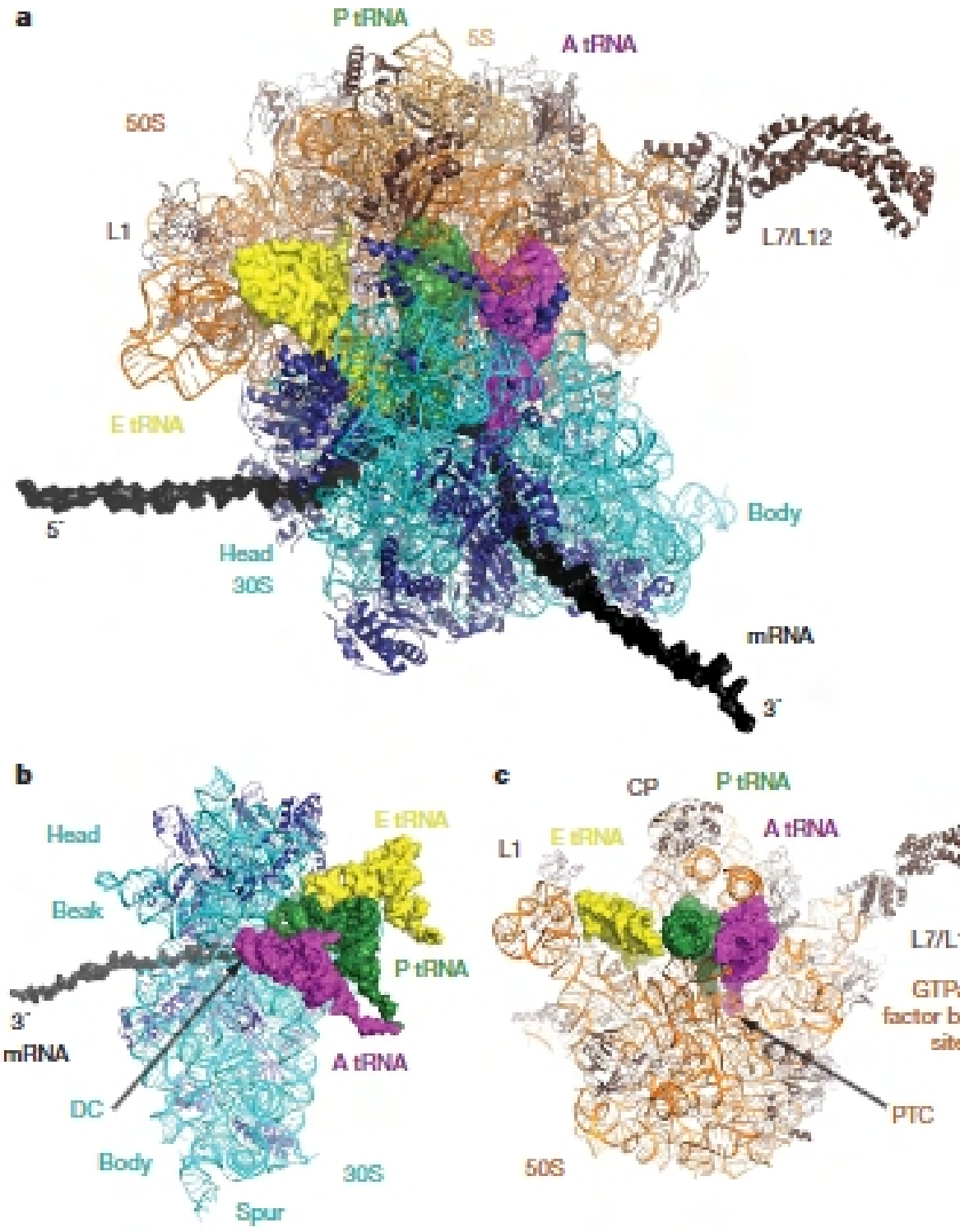

Figure 1 | Structure of the ribosome. a, 'Top' view of the 70S ribosome with mRNA and A- P- and E-site tRNAs. **b, c,** Exploded view of the 30S subunit (**b**) and 50S subunit (**c**). The structure of the L7/L12 arm[10] was fit onto the 70S ribosome[69], with mRNA elongated by modelling. This and all other figures were made with Pymol (Delano Scientific) and Photoshop (Adobe).

authors have suggested that this conformation represents the state after GTP hydrolysis before P_i release. Alternatively, another group has suggested that it is the result of the absence of IF1 and IF3 (ref. 23). The 70S complex with the GDP state of IF2 has the 30S subunit returned to the un-ratcheted state and IF2 largely separated from the GTPase centre, ready to dissociate from a properly initiated 70S ribosome[22]. Single-molecule fluorescence resonance energy transfer (FRET) studies show that this subunit rotation, which readies the ribosome for elongation, requires GTP hydrolysis[24], thus supporting a direct role for the GTPase activity of IF2 in initiation, which has been in dispute[16,25].

The elongation cycle

The elongation cycle consists of the steps involved in sequentially adding amino acids to the polypeptide chain (Fig. 2). At the beginning of the cycle, the ribosome contains a peptidyl-tRNA with a nacent polypeptide chain in the P site and an empty A site. During decoding, the next amino acid is delivered in a ternary complex of elongation factor Tu (EF-Tu), GTP and aminoacyl-tRNA. Decoding is followed by peptide-bond formation, resulting in the elongation of the polypeptide chain by one amino acid. EF-G-catalysed translocation moves the tRNAs and mRNA with respect to the ribosome.

Decoding. Decoding ensures that the correct aminoacyl-tRNA, as dictated by the mRNA codon, is selected in the A site. The binding of the appropriate ternary complex in the A site of the ribosome results in GTP hydrolysis by EF-Tu, the dissociation of the factor from the ribosome and the movement of the aminoacyl end of A-site tRNA into the PTC, termed accommodation (Fig. 3). The many steps of decoding have been dissected by pre-steady state kinetic measurements[26] and single-molecule FRET studies[27].

The high accuracy of tRNA selection cannot be accounted for by just the free energy differences between base pairing and mismatches of the codon and anticodon[28,29], even considering the contribution of proofreading. Instead, interactions made by three universally conserved bases of the ribosome with the minor groove of the first two base pairs of the codon–anticodon helix gives rise to further discrimination (Fig. 3)[30]. Such close monitoring of base-pairing geometry by the ribosome does not occur at the wobble position, consistent with the degeneracy of the genetic code. The binding energy of these extra interactions is not used primarily to increase the relative affinity of cognate versus near-cognate tRNA, but instead to induce a domain closure in the 30S subunit[31], which presumably leads to the acceleration observed in rates of the forward steps in decoding[32].

CryoEM studies of EF-Tu at increased resolution[33,34] show that EF-Tu contacts the shoulder domain of the 30S subunit. Thus, domain closure would move the shoulder domain of the 30S subunit towards the ternary complex[29], potentially stabilizing the transition state for GTP hydrolysis by EF-Tu[31] and leading to an acceleration of GTPase activation and tRNA selection. It seems that mutations or antibiotics that facilitate domain closure decrease the accuracy of the ribosome, whereas mutations that make domain closure more difficult result in increased accuracy[29,31].

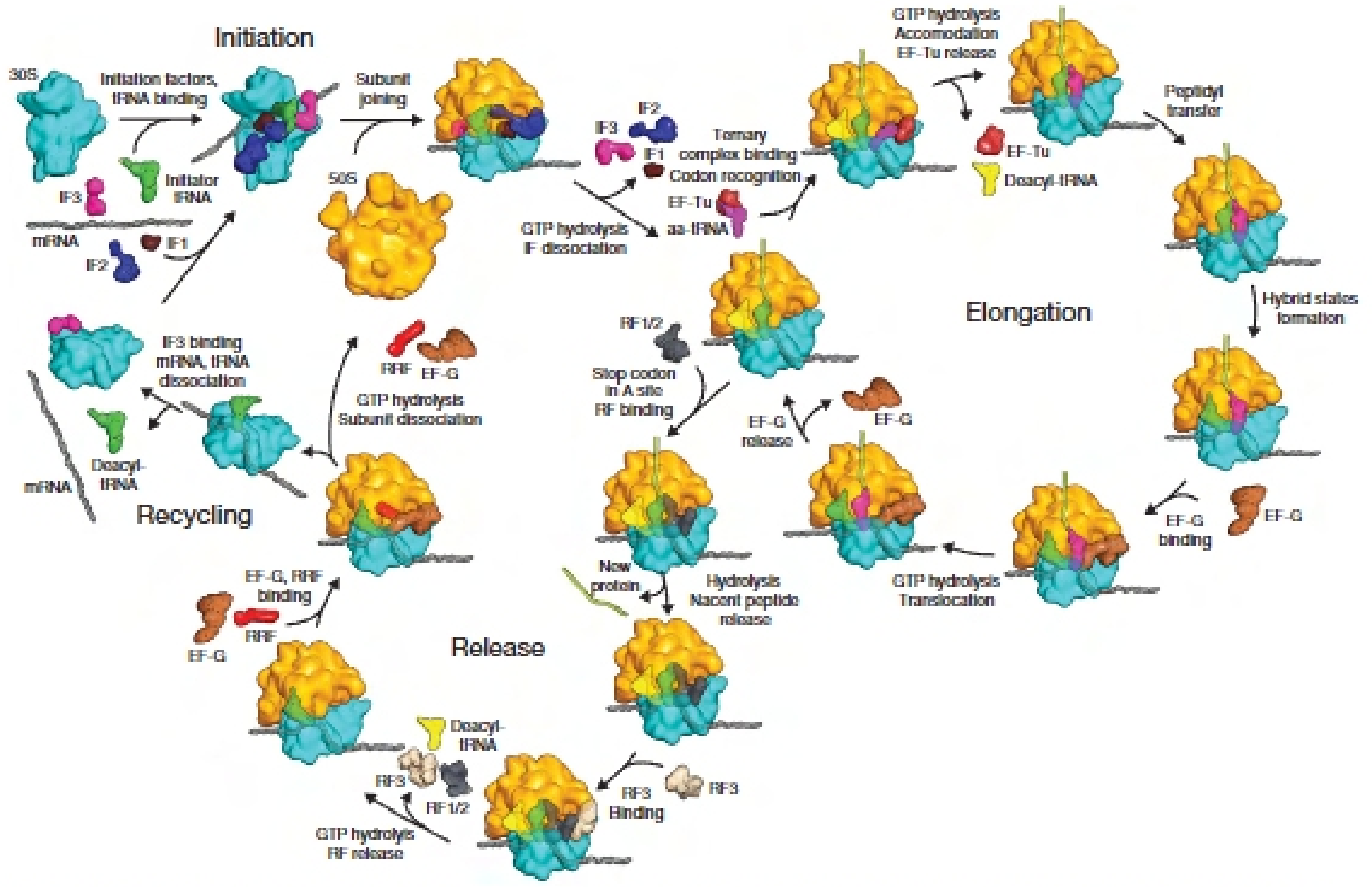

Figure 2 | Overview of bacterial translation. For simplicity, not all intermediate steps are shown. The colour scheme shown here is used consistently throughout this review. aa-tRNA, aminoacyl-tRNA; EF elongation factor; IF, initiation factor; RF, release factor.

These cryoEM structures, the most recent of which are beyond 7 Å resolution[35,36], also show that the tRNA is bent at the anticodon stem (Fig. 3f). The anticodon stem in the decoding centre is very nearly in the orientation acquired after accommodation and movement of the acceptor arm into the PTC. Thus, the binding energy derived from base pairing between the correct codon–anticodon is not only used to induce a conformational change in the ribosome, but also to distort the tRNA. A distorted tRNA may be characteristic of the transition state for GTP hydrolysis by EF-Tu, consistent with experiments showing that a fragmented tRNA is unable to carry out decoding[37]. In addition, recent mutational data on S12, a protein at the shoulder of the 30S subunit with a tail that stretches into the decoding centre, suggest it may be involved in relaying changes induced at the decoding centre to the ternary complex[38].

As this review was going to press, the crystal structure of EF-Tu and tRNA bound to the ribosome was determined[39]. This structure shows details of the tRNA distortion that allows aminoacyl-tRNA to interact with both EF-Tu at the factor-binding site and the decoding centre of the 30S subunit. Furthermore, a series of conformational changes in aminoacyl-tRNA and EF-Tu that occur after productive ribosome binding suggest a communication pathway between the decoding centre and the GTPase centre of EF-Tu, which would trigger GTP hydrolysis after codon recognition.

After release of EF-Tu, the tRNA relaxes into the PTC[31,34]. If the anticodon stem loop is held tightly at the decoding centre (as in the closed form induced by cognate tRNA), accommodation is accelerated[40]. However, recent work on the Hirsh suppressor tRNA (a mutant Trp tRNA that recognizes the UGA stop codon) shows that this tRNA leads to acceleration of GTP hydrolysis and apparently accommodation with a near-cognate codon–anticodon pairing[41]. Thus, the mutant tRNA may be stabilized by additional interactions with the ribosome, rather than simply showing enhanced flexibility.

The discrimination achieved from monitoring the minor groove geometry in the codon–anticodon helix by decoding centre nucleotides though A-minor interactions can potentially yield an accuracy of $\sim 10^{-2}$–10^{-4} in a single step[42]. Should the ribosome use this discrimination, then with proofreading, it would be possible to obtain much higher accuracy than is usually reported. Evidently, the ribosome forgoes accuracy by using the binding energy of codon–anticodon recognition to induce conformational changes in the ribosome and tRNA that result in accelerated GTP hydrolysis and tRNA selection.

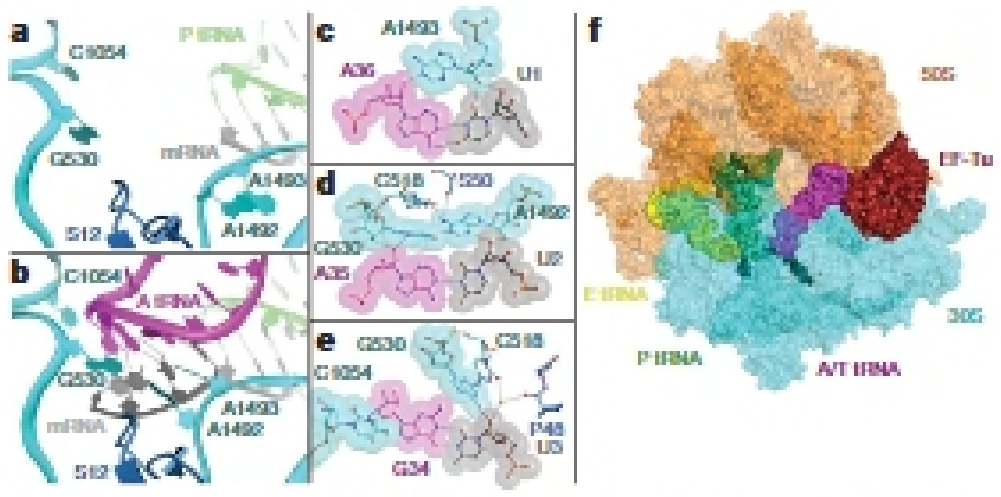

Figure 3 | Decoding by the ribosome. **a**, In the apo ribosome, A1492 and A1493 are stacked in h44. **b**, When a cognate tRNA bind to mRNA in the A site, A1492, A1493 and G530 change conformation to interact with the minor groove of the mRNA–tRNA minihelix[80] **c**–**e**, Interactions of the 30S with the codon–anticodon pair. In the first (**c**) and second (**d**) positions, ribosomal bases monitor the geometry of the minor groove of the base pairs. Protein S12 also interacts with the second and third (**e**) positions. **f**, The ternary complex of EF-Tu and aminoacyl-tRNA with the 70S ribosome shows that the tRNA is bent in the anticodon stem (for example, see refs 35, 36).