REVIEWS

What recent ribosome structures have revealed about the mechanism of translation

T. Martin Schmeing[1] & V. Ramakrishnan[1]

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

[1]MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK.

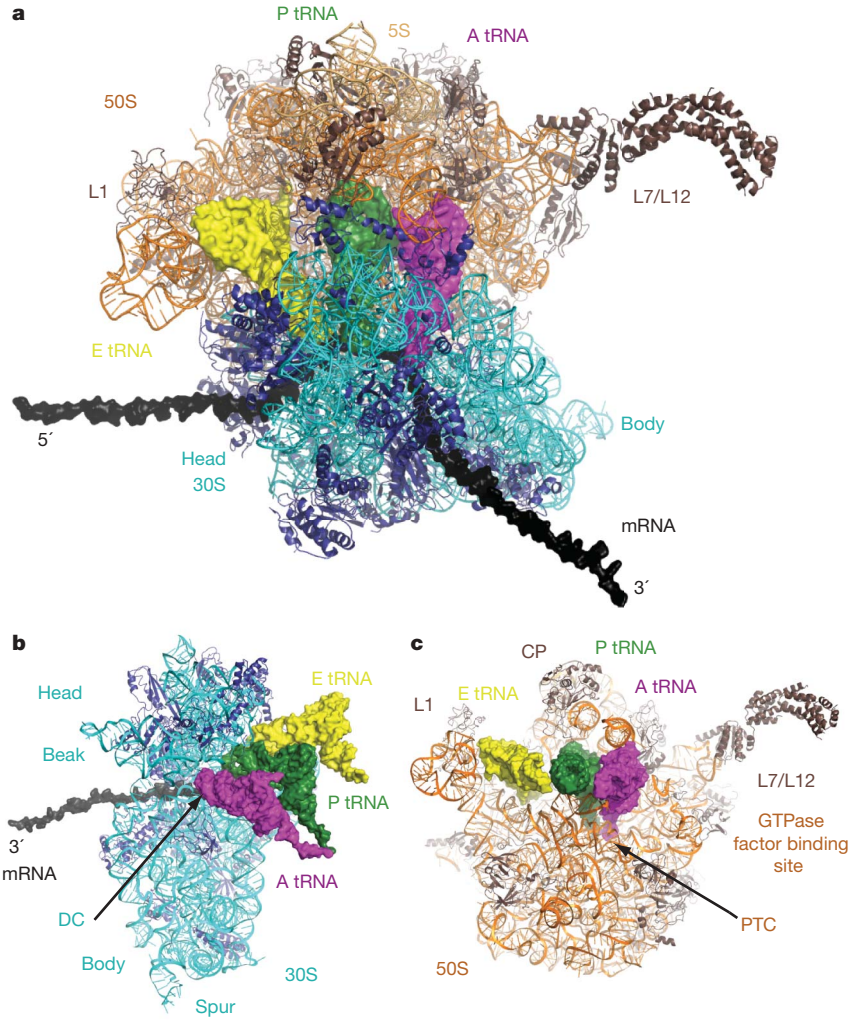

Figure 1 | Structure of the ribosome. **a**, 'Top' view of the 70S ribosome with mRNA and A- P- and E-site tRNAs. **b**, **c**, Exploded view of the 30S subunit (**b**) and 50S subunit (**c**). The structure of the L7/L12 arm[10] was fit onto the 70S ribosome[69], with mRNA elongated by modelling. This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

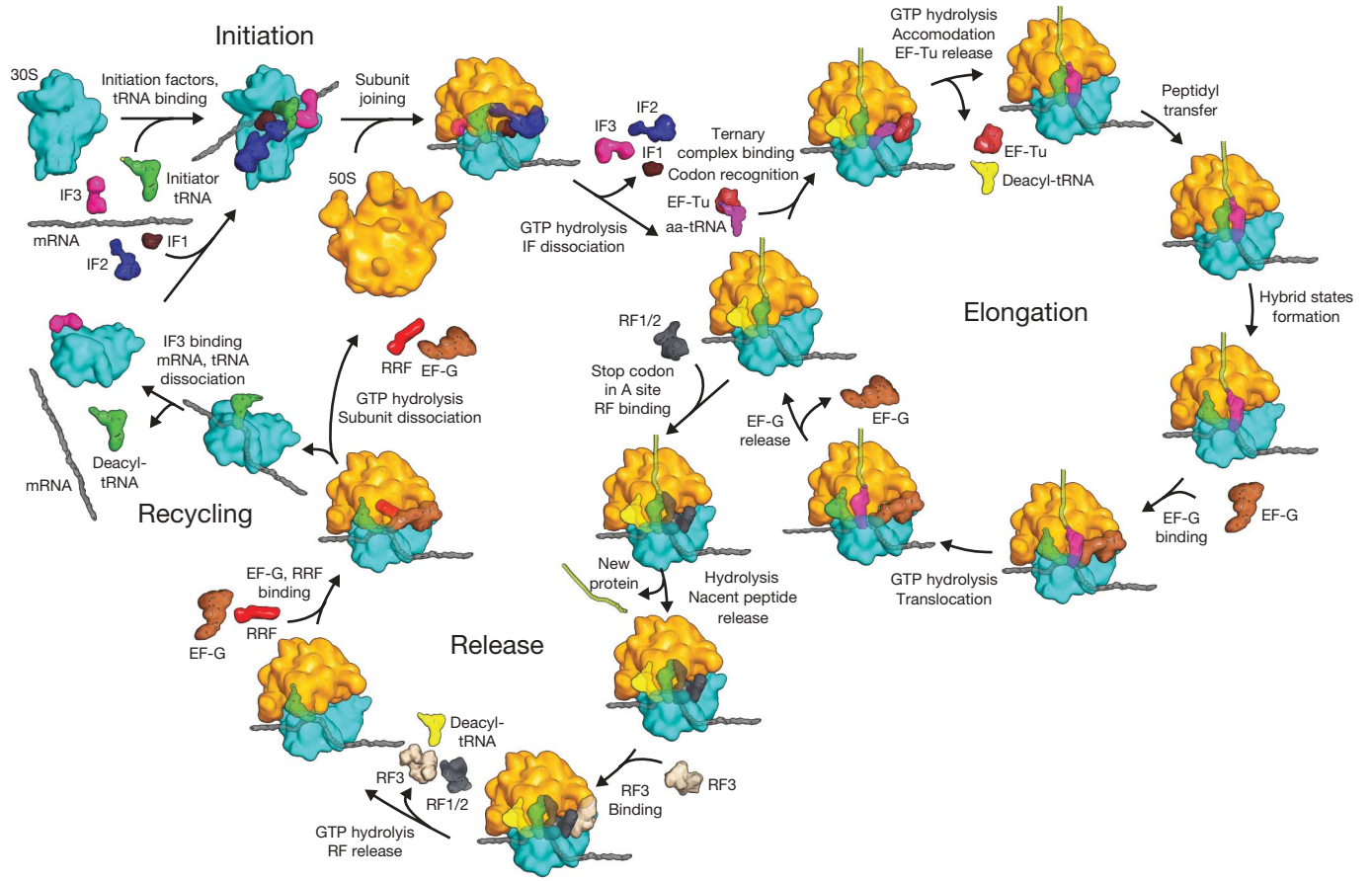

Figure 2 | Overview of bacterial translation. For simplicity, not all intermediate steps are shown. The colour scheme shown here is used consistently throughout this review. aa-tRNA, aminoacyl-tRNA; EF elongation factor; IF, initiation factor; RF, release factor.

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 $\sim 10^3$–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.

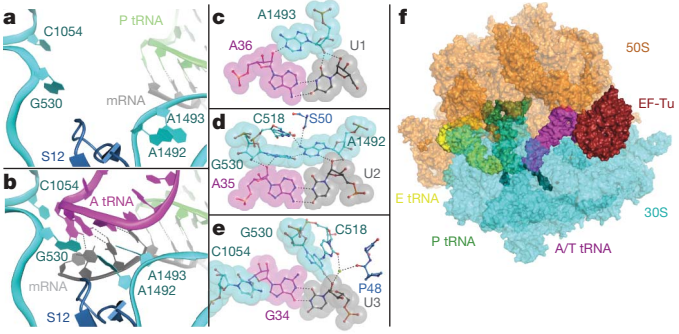

Figure 3 | Decoding by the ribosome. **a**, In the apo ribosome, A1492 and A1493 are stacked in h44. **b**, When a cognate tRNA bind to mRNA in the A site, A1492, A1493 and G530 change conformation to interact with the minor groove of the mRNA–tRNA minihelix[30] **c**–**e**, Interactions of the 30S with 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).

However, a recent result suggests that the ribosome is capable of combining very high accuracy (>10^6) with a speed comparable to that of *in vivo* protein synthesis (~22 amino acids added per second)[43], both of which are much higher than previous measurements *in vitro* (accuracy ~450, speed ~6.6 s^{-1})[32]. In the recent experiments[43], the accommodation of tRNA into the PTC is apparently too fast to allow significant discrimination by proofreading after GTP hydrolysis. If so, the structural basis of how one could have such a high accuracy with little or no proofreading is not clear, nor why measured *in vivo* rates of misincorporation are so much higher (reviewed in ref. 29). Further experiments with other reporters and in varying conditions are required to clarify these differences.

Peptide-bond formation. The central chemical event in protein synthesis is the peptidyl-transferase reaction, in which the α-amino group of the aminoacyl-tRNA nucleophillically attacks the ester carbon of the peptidyl-tRNA to form a new peptide bond (Fig. 4a; see the movie at http://www.sciencedirect.com/science/MiamiMultiMediaURL/B6WSR-4HHX2B2-B/B6WSR-4HHX2B2-B-2/7053/html/d074e3c1ecf8e4064d37dd72bc0b7e93/Movie_S1..mov). The ribosome increases the rate of this reaction by at least ~10^5-fold[44]. The catalytic site is in domain 5 of the 23S RNA, which binds the CCA ends of aminoacyl- and peptidyl-tRNA (Fig. 4b). It was located precisely in crystal structures of the *H. marismortui* 50S subunit[45], at the bottom of a large cleft (Fig. 4b). These structures precipitated many studies aimed at determining the catalytic mechanism of the peptidyl-transferase reaction. An initial proposal for a general acid/base catalytic mechanism involving N3 of A2451—a nucleotide in very close proximity to substrate analogues[45,46]—was disproved by the dispensability of A2451 for the peptidyl-transferase reaction[47–51]. Furthermore, crystal structures with improved resolution and more accurate transition state mimics showed that N3 of A2451 is not within hydrogen-bonding distance of the nucleophile throughout the reaction[52,53]. When the reactive α-amine was substituted with a hydroxyl, making chemistry rate limiting[54], a pH-independent reaction rate was observed. This is strong evidence that there is no general acid/base catalysis involving a group with near-neutral pK_a, on A2451 or any other ribosomal moiety.

If there is no acid/base catalysis, what is the source of catalytic power of the ribosome? As with all enzymes, the precise organization of substrates and the active site plays an important contribution. In the ribosome, this is achieved when the binding of aminoacyl-tRNA induces a conformational change of the PTC and peptidyl-tRNA[55] (http://www.nature.com/nature/journal/v438/n7067/extref/nature04152-s6.mov, http://www.nature.com/nature/journal/v438/n7067/extref/nature04152-s7.mov). The α-amino group of the aminoacyl-tRNA interacts with the N3 of A2451 and the 2′ OH of A76 of the peptidyl-tRNA, as part of an extensive network of hydrogen bonds that position the substrates for reaction (Fig. 4c)[53,56–58]. It had been proposed that binding and orienting of substrates accounts for most of the ribosomal rate enhancement[59]. A comparison of the rate of peptide-bond formation by the ribosome and by a ribosome-free model system suggested that the ribosome accelerated the reaction solely by entropic effects[44], which may include substrate positioning, shielding the reaction from bulk solvent, or organization of the active site[44,57]. A precisely positioned water molecule interacts with the highly polarized transition state, as an oxyanion hole[44,53].

Although structural and biochemical studies have found no ribosomal group that acts in chemical catalysis, a substrate-assisted mechanism is possible. The 2′ OH of the peptidyl-tRNA is well positioned to abstract and donate protons from the nucleophile and leaving group, respectively[52,53,55]. Several studies suggest that this hydroxyl is vital for the reaction[57,60–63], whereas one group proposes it is dispensable[64]. In the most rigorous study, Weinger *et al.*[63] substituted the 2′ OH of A76 of peptidyl-tRNA with H or F (ref. 63), and found a rate reduction of at least 10^6-fold. The importance and proximity of the 2′ OH led to the proposal of a concerted proton shuttling mechanism, whereby it simultaneously accepts a proton from the α-amino group and donates one to the 3′ O leaving group, perhaps as part of a six-membered ring of interactions[53,57,62] (Fig. 4d, e). Such a mechanism may not require perturbation of the pK_a of the 2′ OH pK_a.

Many mechanistic insights and biochemical experiments of the peptidyl-transferase reaction are based on structures of *H. marismortui* 50S complexes. It was questioned whether this reductionist system, which only includes the large subunit and the terminal nucleotides of tRNA, accurately represents the process in the whole ribosome with intact substrates[7,65,66]. However, the 50S can catalyse the peptidyl-transferase reaction at similar rates to the 70S ribosome using a small dinucleotide A-site substrate, provided that a full-length tRNA is present in the P site[67]. This analogue also shows the same robustness against active site mutations, and a pH profile similar to full aminoacyl-tRNAs in 70S ribosomes[68]. Finally, recent structures show that a 70S ribosome with full-length tRNA substrates show that the PTC and substrate conformations are essentially identical to those in structures of the 50S with substrate analogues[69].

Although the ribosome is asymmetric, a pseudo-two-fold axis of symmetry exists at the PTC, relating the A and P sites[65]. It is likely that 23S RNA started as a molecule of around 100 nucleotides, which duplicated to allow the proto-ribosome to bring two (non-coded) substrates into proximity[65,70]. Careful analyses of the tertiary interactions reveal an evolutionary pathway of expansion of this proto-ribosome, giving rise to 23S RNA[70].

Recent studies shed light on the role of two proteins previously implicated in peptidyl transfer. In bacteria, the amino terminus of L27 could be crosslinked to the 3′ end of both A- and P-site tRNAs, showing it was part of the PTC[71,72]. Deletion or N-terminal truncation of L27 results in reduced peptidyl-transferase activity[72] and computer simulations suggest the role of L27 is to aid binding of aminoacyl-tRNA[73]. In addition, deletion of L16 was shown to cause a deficiency in A-site tRNA binding and the rate of peptidyl transfer[74,75]. Recent structures show that the N-terminal tail of L27 is

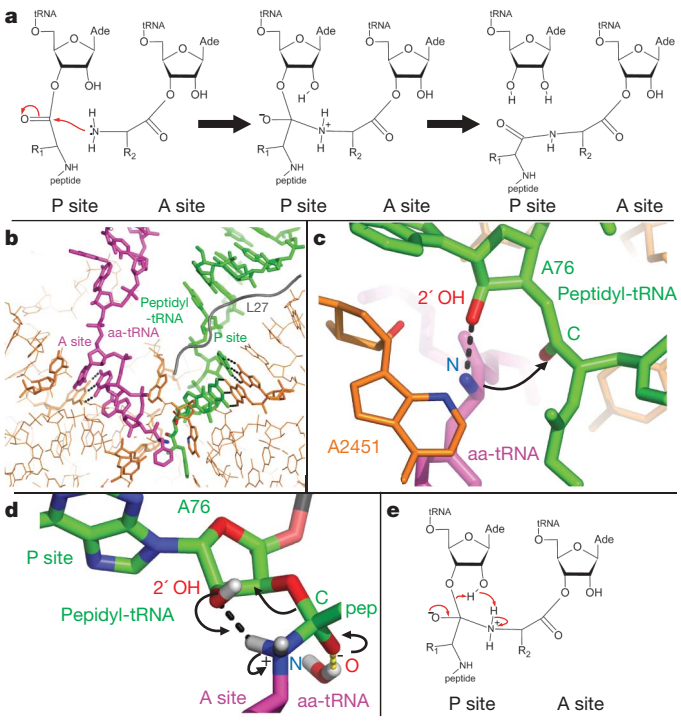

Figure 4 | Peptide-bond formation. **a**, Schematic drawing of the reaction. Ade, adenine. **b**, Binding of tRNAs to the PTC[69]. **c**, The α-amino nucleophile is positioned by interaction with the 2′ OH of A76 of peptidyl-tRNA and N3 of A2451, as part of an extensive network of hydrogen bonds[53]. **d**, **e**, Possible mechanism by which the intermediate of the reaction breaks down into products, by a proton shuttle involving the 2′ OH of A76 of peptidyl-tRNA.

ordered in the PTC where it interacts with the tRNA substrates[8,69] (Fig. 4b), and that L16 becomes ordered owing to its interactions with the acceptor arm of A-site tRNA, rationalizing these findings. Thus, some proteins seem to aid the RNA components that primarily facilitate the peptidyl-transfer activity of the ribosome.

Translocation: the formation of hybrid states. With peptide-bond formation, the nascent peptide chain is transferred to the A-site tRNA leaving a deacylated tRNA in the P site. Before the next round of elongation, the tRNAs and mRNA need to move relative to the ribosome. During translocation the mRNA shifts by precisely one codon, except when either errors or programmed frameshifts occur. The tRNAs must also translocate from the A and P sites to the P and E sites, requiring a movement as large as 50 Å for the 3′ end of the P-site tRNA.

Chemical footprinting showed that movements of the tRNAs occurred first with respect to the 50S subunit. P/E and A/P hybrid tRNA states form spontaneously after peptide-bond formation, and only after the addition of the GTPase elongation factor G (EF-G) did movement occur with respect to the 30S subunit[76] (Fig. 5). The hybrid states were visualized by careful sorting of ribosomal complexes[77,78]. The ribosomal subunits have rotated by ~6° relative to each other[79] (http://www.nature.com/nature/journal/v406/n6793/extref/406318ai1.mov), and this 'ratcheted' ribosome contains both A/P and P/E tRNAs. Single-molecule FRET studies show that although the ribosome is initially in the unratcheted state, it oscillates between the unratcheted and ratcheted states after peptidyl transfer[80,81], until EF-G binding stabilizes the latter. To demonstrate that the ratcheted state of the ribosome is related to translocation, FRET experiments have shown that viomycin, an antibiotic that inhibits translocation, traps the ribosome in a ratcheted state indistinguishable by FRET from that obtained when EF-G is bound[82]. Furthermore, FRET measurements show that concomitantly with the ratcheting of the subunits, the L1 stalk moves to interact with the newly deacylated P-site tRNA, as would be expected if it moves into a P/E hybrid state[83]. The formation of hybrid tRNA states is ordered, with the P/E tRNA state formed first, followed by the A/P state[84,85].

The role of the E site in translocation. The adoption of the P/E hybrid state also explains why the E site may be necessary. The E site is known to have evolved before the divergence of the three kingdoms, as the interactions of the E-site tRNA with the 50S subunit in archaea[86] and bacteria[8,66] are similar. Because the E site binds deacylated but not peptidyl-tRNA, it is able to trap a hybrid P/E tRNA as soon as the P-site tRNA becomes deacylated, facilitating translocation by the formation of hybrid states. This concept is supported by direct kinetic evidence[87] and the observation that tRNA modifications that affect E-site binding also affect translocation[88,89].

The crucial interactions between the ribosome and the terminal adenine of the E-site tRNA require only the 23S rRNA[86]. Thus, the E site may have evolved before the evolution of proteins, such as translational factors that facilitate translocation by hybrid states. Consistent with this theory, ribosomes with modifications in S12 or S13 can perform translation even in the absence of elongation factors[90,91]. These proteins are at the subunit interface, and their modification presumably disrupts contacts between the two subunits, facilitating their rotation relative to each other.

The role of EF-G in translocation relative to the 30S subunit. The second step in translocation is the movement of tRNAs and mRNA with respect to the 30S subunit, which is catalysed by EF-G. It is generally accepted that GTP hydrolysis by EF-G precedes translocation[92].

CryoEM structures (for example, ref. 21) show that EF-G in the GTP-bound form on the ribosome has a significantly altered conformation from that in the GDP or apo form in isolation[93,94], and binds to the ratcheted state of the ribosome. A recent higher-resolution structure shows that the switch I and II regions of the GTPase domain become ordered on binding to the ribosome[95]. Calorimetric studies suggest that EF-G undergoes a conformational change on binding GTP even before binding the ribosome, although full activation occurs only after ribosomal binding[96]. The sarcin–ricin loop is the ribosomal element closest to the switch II region that is functionally important for GTPase activation in both EF-G and EF-Tu[35,36,95].

Ribosomes depleted of the L7/L12 stalk of the 50S subunit can bind EF-Tu or EF-G, but cannot efficiently activate GTP hydrolysis by the factors[97]. L7 is an N-terminally modified form of L12, and by the association of its N-terminal domains exists as a tetramer in *Escherichia coli* or a hexamer in other species[10,98]. This multimer of L12 binds a single copy of L10 to form a stalk that is fully or partially disordered in high-resolution structures of the ribosome. The tip of the stalk containing the C-terminal domain of L12 seems to be too far from the ribosomal GTPase centre to be involved directly in stimulating hydrolysis (Fig. 1). The structure of a hexamer of L12 complexed with L10 has been determined and modelled into the structure of the 50S subunit[10]. An increased rate of initial binding of GTPase factors was observed kinetically, which could be caused by several copies of the C-terminal domain of L12 effectively increasing the local concentration of the binding sites. Because the N- and C-terminal domains of L12 are connected by a flexible linker, the latter could move with the factor close to the sarcin–ricin loop.

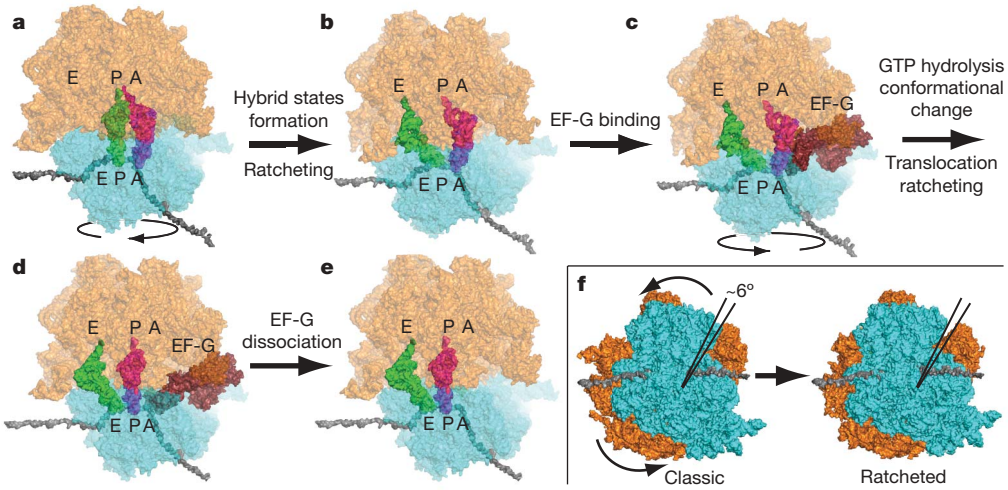

Figure 5 | EF-G catalysed translocation. **a**–**e**, After peptidyl transferase, tRNAs shift spontaneously to the A/P and P/E states in a ratcheted ribosome (**b**), to which EF-G binds. After GTP hydrolysis and tRNA movement, ratcheting reverses (**d**) and EF-G dissociates (**e**). **f**, Ratcheting involves a rotation of the 30S subunit by approximately 6 degrees. See text for details. Note that transitions **a**-to-**b**, and **c**-to-**d** could be divided into sub-steps. No structure exists for **c**, so a domain movement was modelled to prevent EF-G and A/P tRNA from clashing.

The L11 region is in close proximity to the GTPase centre. This region also binds the antibiotics thiostrepton and micrococcin, which inhibit and enhance GTPase activity, respectively. In the structure of the 50S subunit bound with micrococcin, density for the C-terminal domain of L12 is observed adjacent to L11 and would be positioned to interact with EF-G[99]. Thus micrococcin could stabilize the binding of the C-terminal domain of L12 to the GTPase factor.

How GTP hydrolysis leads to movement of mRNA and tRNAs and resets the ratcheted ribosome to its canonical form is still unclear. Presumably, a rearrangement in the ribosome induced by GTP hydrolysis allows movement of the tRNAs and mRNA[85,100]. Direct monitoring of mRNA showed that mRNA and tRNA movements occur at the same rate, and thus are directly coupled[101]. In the GDP form, the conformation of domain IV of EF-G places it in the A site of the 30S subunit[102,103]. Thus, GTP hydrolysis may drive translocation by preventing the reverse movement of tRNA and mRNA. GTP hydrolysis may also allow the ribosome to act as a helicase and unwind the secondary structures formed in mRNA[104].

As this review was going to press, the crystal structure of EF-G·GDP trapped in the post-translocational state on the ribosome by fusidic acid was determined[105]. The structure shows that domain IV makes extensive interactions with the minor groove of the codon–anticodon base pairs at the P site, but not with the A site codon. Fusidic acid seems to trap EF-G in a conformation between that of the GTP and GDP states, and the binding of EF-G in this state stabilizes the L10–L7/L12 stalk as well as (indirectly) the L1 stalk.

Termination of translation

The elongation cycle continues until an mRNA stop codon moves into the A site, signalling the end of the coding sequence. A class I release factor recognizes the stop codon and cleaves the nascent polypeptide chain from the P-site tRNA, resulting in the release of the newly synthesized protein from the ribosome. In bacteria, there are two class I release factors, RF1 and RF2. Whereas both factors recognize the UAA stop codon, UAG and UGA are only recognized by RF1 and RF2, respectively. In eukaryotes, a single eRF1 that is unrelated to RF1 or RF2 (refs 106, 107) recognizes all three stop codons. Tripeptide motifs PXT in RF1 and SPF in RF2 confer specificity for the codons UAG or UGA[108], whereas a universally conserved GGQ motif is implicated in peptide hydrolysis by release factors[106,107].

Unlike the extended structure of eRF1 (ref. 107), the crystal structure of isolated RF2 is compact, with the GGQ and SPF motifs only 23 Å apart[109]. However, low-resolution structures showed that when bound to the ribosome, release factors were in an open form and domain 3, containing the GGQ motif, inserted into the PTC[110–112] (Fig. 6a). The high-resolution crystal form of the ribosome[8] was used to solve structures of class I release factors bound to the bacterial ribosome[113–115], considerably advancing our understanding of the function of RF1 and RF2.

Recognition of the stop codon. Release-factor binding causes the conserved decoding centre bases G530, A1492 and A1493 to change conformation and form crucial interactions (Fig. 6b)[113–115]. The changes are distinct from those during decoding of tRNA in which the bases monitor base-pairing geometry (Fig. 3c–e)[30], in a conformation incompatible with the binding of release factors[116]. Instead, A1493 stacks on A1913 of 23S RNA, forming a new contact between the two subunits, and G530 stacks onto the third stop codon base.

Although the structures can rationalize the specificity of RF1 and RF2 for their respective stop codons, the tripeptide motifs implicated in conferring specificity of RF1 or RF2 (ref. 108) make only limited interactions with the stop codon (Fig. 6b)[113–115], so that their role is still unclear. Bases after the stop codon affect release-factor efficiency (for example, ref. 117), and a single mutation in RF2 distant from the stop codon allows it to recognize all three stop codons[118]. Furthermore, it has been shown that a mismatch in the P site (after a near-cognate tRNA has been accepted and translocated) leads to release factor recognition of sense codons with increased efficiency[119]. Thus, release factor function may involve a subtle balance between the energetics of binding and conformational changes, similar to that during decoding by tRNA. After stop-codon recognition, peptide release is triggered, but the mechanism of signal transduction in unclear.

Catalysis of peptide release. The conserved GGQ motif is positioned in the PTC in a conformation only allowed because of its glycines (Fig. 6c)[113–115], explaining the drastic reduction in the activity of release factors when they are mutated[120,121]. Furthermore, after release-factor binding U2585 shifts to expose the ester bond between the nascent peptide and P-site tRNA as is observed upon binding of A-site tRNA or its analogues[55,69]. This shift has been proposed to be catalytically important for both peptidyl transfer and release[55], as in both cases it exposes the ester bond to attack by a nucleophile. Further supporting this theory, a variety of nucleophiles are effective to varying degrees during catalysis of peptide release by release factors[121].

The glutamine in the GGQ motif makes a hydrogen bond through its main-chain amide with the 3′ OH of A76 of deacylated P-site tRNA[113–115], which represents the product state after catalysis and release of the nascent peptide chain. One group has proposed product stabilization as part of the catalytic mechanism of release factors,

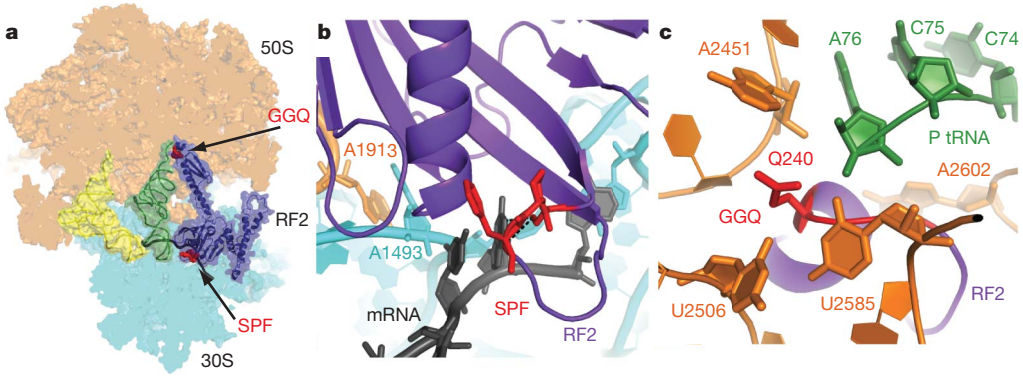

Figure 6 | Termination of translation by class I release factors. a, Overview of class I release-factor binding to the 70S ribosome[113–115]. The view shows RF2; the GGQ motif implicated in catalysis of peptide release at the PTC and the SPF motif implicated in stop-codon recognition at the decoding centre are highlighted in red. **b**, RF2 in the decoding centre, with the SPF motif highlighted. **c**, The PTC of the ribosome showing the GGQ motif of RF2 and deacylated P-site tRNA.

similar to certain proteases[113,115]. Although the mutation of the glutamine to alanine results in only a modest 5–10-fold reduction in the catalytic rate[120,121], several other observations argue that it has a specific role in catalysis. The glutamine is universally conserved, which for glutamine is rarely for purely structural reasons. It is required for viability in bacteria and in eukaryotes[107,122]. Whereas the mutation of the glutamine does not affect the rate of catalysis by other nucleophiles, it does specifically affect the rate for peptide release by water[121]. Finally, the side-chain amino group of the glutamine is methylated, and the loss of methylation was shown to reduce the efficiency of peptide release[123]. Consistent with these data, one of the structural studies proposed a model in which the glutamine side chain directly coordinates a water molecule for nucleophilic attack[114]. Structures of the substrate and transition state complexes will address this question.

The role of RF3. The class II release factor RF3 accelerates the dissociation of class I factors from the ribosome after peptide release. The binding of RF3 to the ribosome–RF1/2 complex in the GDP form is thought to induce RF3 to exchange GDP for GTP[124]. The crystal structure of RF3–GDP resembles EF-Tu in the GTP form[125]. The same study showed that the binding of RF3 in the GTP form to the ribosome induces conformational changes likely to destabilize the binding of class I release factors, thus leading to their dissociation from the ribosome.

Recycling of ribosomes before reinitiation. After hydrolysis of GTP by RF3, the factor dissociates from the ribosome, leaving mRNA and a deacylated tRNA in the P site. The ribosome must be recycled into subunits for a new round of protein synthesis to begin. In bacteria, an essential protein called ribosome recycling factor (RRF) works together with EF-G to carry out this process[126].

Chemical probing, cryoEM and crystallography all suggest similar interactions of RRF with the ribosome[127–132]. However, the location of RRF in these studies would be incompatible with a P-site tRNA in the 50S subunit, as it would clash with the tip of domain I of RRF. Therefore, a probable model is that RRF binds to a ribosome containing a deacylated hybrid P/E tRNA. EF-G would then bind, similar to the way in which it binds the ribosome in a pre-translocation state. However, this view is complicated by studies suggesting that RRF can even act on ribosomes with a peptidyl-tRNA[133,134]. CryoEM studies on the 50S subunit with RRF and both RRF and EF-G suggest the type of changes that might occur before and after RRF activity[129], but it is unclear if they represent any specific state of recycling. So far, there is no structure of the entire ribosome with both EF-G and RRF.

GTP hydrolysis seems to be required to promote the separation of subunits[12], yielding a 50S subunit and a complex of 30S, mRNA and deacylated tRNA, which requires IF3 to dissociate[11]. The action of IF3 to remove mRNA and tRNA from the 30S subunit is attractive because it couples the last step in protein synthesis to the first, by preparing the 30S subunit for a new round of initiation (Fig. 2).

Conclusions

In this review, we have focused on the main aspects of bacterial translation that are common to the synthesis of all proteins. Although even this basic pathway is very complicated, translation involves many other features that have also been the subject of structural and functional studies in recent years. These include the rescue of stalled ribosomes, programmed frameshifting, the interaction of the nascent peptide with the exit tunnel, the modification of the peptide as it emerges from the ribosome, its folding and its transport across or insertion into membranes, and the regulation of translation. Nevertheless, one can only look back in wonder at the rate of progress in the last decade in our understanding of many key aspects of the translation pathway.

This progress is likely to continue unabated, with cryoEM now yielding increasingly high resolution and an increasing number of functional states becoming amenable to crystallographic studies. Two areas that would particularly benefit from high-resolution structures are initiation and translocation. These, as well as other stages of translation involve GTPase factors. The very recent crystal structures of EF-Tu[39] and EF-G[105] represent the first high-resolution structures of GTPase factors bound to the ribosome. By showing that such complexes are accessible to crystallography, they allow us to be optimistic that similar structures in other states will lead to an understanding of how the ribosome specifically activates GTP hydrolysis by these factors at precise stages that differ for each GTPase. In addition to structural studies, increasingly sophisticated biochemical methods such as single-molecule studies will help to dissect the various steps of complicated processes. Although the structures of key states of the ribosome will be welcome, an understanding of how the ribosome proceeds from one state to the next will be aided by molecular dynamics, which is now able to tackle larger and more complex problems as a result of advances in computing and methodology. Finally, the extremely complicated field of eukaryotic translation, especially initiation, is sure to be increasingly targeted by biophysical and biochemical techniques.

Published online 18 October 2009.

1. Ramakrishnan, V. Ribosome structure and the mechanism of translation. *Cell* **108,** 557–572 (2002).
2. 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).
3. Wimberly, B. T. *et al.* Structure of the 30S ribosomal subunit. *Nature* **407,** 327–339 (2000).
4. Yusupov, M. M. *et al.* Crystal structure of the ribosome at 5.5 Å resolution. *Science* **292,** 883–896 (2001).
5. Gao, H. *et al.* Study of the structural dynamics of the *E. coli* 70S ribosome using real-space refinement. *Cell* **113,** 789–801 (2003).
6. Harms, J. *et al.* High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell* **107,** 679–688 (2001).
7. Schuwirth, B. S. *et al.* Structures of the bacterial ribosome at 3.5 Å resolution. *Science* **310,** 827–834 (2005).
8. Selmer, M. *et al.* Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* **313,** 1935–1942 (2006).
   This high-resolution structure of a functional complex of the ribosome has paved the way for many other studies.
9. Nikulin, A. *et al.* Structure of the L1 protuberance in the ribosome. *Nature Struct. Biol.* **10,** 104–108 (2003).
10. Diaconu, M. *et al.* Structural basis for the function of the ribosomal L7/12 stalk in factor binding and GTPase activation. *Cell* **121,** 991–1004 (2005).
11. Karimi, R., Pavlov, M. Y., Buckingham, R. H. & Ehrenberg, M. Novel roles for classical factors at the interface between translation termination and initiation. *Mol. Cell* **3,** 601–609 (1999).
12. Peske, F., Rodnina, M. V. & Wintermeyer, W. Sequence of steps in ribosome recycling as defined by kinetic analysis. *Mol. Cell* **18,** 403–412 (2005).
13. Antoun, A., Pavlov, M. Y., Lovmar, M. & Ehrenberg, M. How initiation factors maximize the accuracy of tRNA selection in initiation of bacterial protein synthesis. *Mol. Cell* **23,** 183–193 (2006).
14. Grigoriadou, C., Marzi, S., Pan, D., Gualerzi, C. O. & Cooperman, B. S. The translational fidelity function of IF3 during transition from the 30 S initiation complex to the 70 S initiation complex. *J. Mol. Biol.* **373,** 551–561 (2007).
15. Milon, P., Konevega, A. L., Gualerzi, C. O. & Rodnina, M. V. Kinetic checkpoint at a late step in translation initiation. *Mol. Cell* **30,** 712–720 (2008).
16. Tomsic, J. *et al.* Late events of translation initiation in bacteria: a kinetic analysis. *EMBO J.* **19,** 2127–2136 (2000).
17. Grigoriadou, C., Marzi, S., Kirillov, S., Gualerzi, C. O. & Cooperman, B. S. A quantitative kinetic scheme for 70 S translation initiation complex formation. *J. Mol. Biol.* **373,** 562–572 (2007).
18. Carter, A. P. *et al.* Crystal structure of an initiation factor bound to the 30S ribosomal subunit. *Science* **291,** 498–501 (2001).
19. Simonetti, A. *et al.* Structure of the 30S translation initiation complex. *Nature* **455,** 416–420 (2008).
    Refs 19 and 20 have shed light on the location of initiation factors in the ribosome.
20. Allen, G. S., Zavialov, A., Gursky, R., Ehrenberg, M. & Frank, J. The cryo-EM structure of a translation initiation complex from *Escherichia coli*. *Cell* **121,** 703–712 (2005).
21. Valle, M. *et al.* Locking and unlocking of ribosomal motions. *Cell* **114,** 123–134 (2003).
22. Myasnikov, A. G. *et al.* Conformational transition of initiation factor 2 from the GTP- to GDP-bound state visualized on the ribosome. *Nature Struct. Mol. Biol.* **12,** 1145–1149 (2005).
23. Allen, G. S. & Frank, J. Structural insights on the translation initiation complex: ghosts of a universal initiation complex. *Mol. Microbiol.* **63,** 941–950 (2007).
24. Marshall, R. A., Aitken, C. E. & Puglisi, J. D. GTP hydrolysis by IF2 guides progression of the ribosome into elongation. *Mol. Cell* **35,** 37–47 (2009).

25. Antoun, A., Pavlov, M. Y., Andersson, K., Tenson, T. & Ehrenberg, M. The roles of initiation factor 2 and guanosine triphosphate in initiation of protein synthesis. *EMBO J.* **22,** 5593–5601 (2003).
26. Rodnina, M. V. & Wintermeyer, W. Fidelity of aminoacyl-tRNA selection on the ribosome: kinetic and structural mechanisms. *Annu. Rev. Biochem.* **70,** 415–435 (2001).
27. Blanchard, S. C., Gonzalez, R. L., Kim, H. D., Chu, S. & Puglisi, J. D. tRNA selection and kinetic proofreading in translation. *Nature Struct. Mol. Biol.* **11,** 1008–1014 (2004).
28. Xia, T. *et al.* Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs. *Biochemistry* **37,** 14719–14735 (1998).
29. Ogle, J. M. & Ramakrishnan, V. Structural insights into translational fidelity. *Annu. Rev. Biochem.* **74,** 129–177 (2005).
30. Ogle, J. M. *et al.* Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* **292,** 897–902 (2001).
31. Ogle, J. M., Murphy, F. V., 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).
32. Gromadski, K. B. & Rodnina, M. V. Kinetic determinants of high-fidelity tRNA discrimination on the ribosome. *Mol. Cell* **13,** 191–200 (2004).
33. 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).
34. Valle, M. *et al.* Incorporation of aminoacyl-tRNA into the ribosome as seen by cryo-electron microscopy. *Nature Struct. Biol.* **10,** 899–906 (2003).
35. Villa, E. *et al.* Ribosome-induced changes in elongation factor Tu conformation control GTP hydrolysis. *Proc. Natl Acad. Sci. USA* **106,** 1063–1068 (2009).
This paper and ref. 36 report cryoEM structures of an important state of the ribosome at and beyond 7 Å resolution.
36. Schuette, J. C. *et al.* GTPase activation of elongation factor EF-Tu by the ribosome during decoding. *EMBO J.* **28,** 755–765 (2009).
37. Piepenburg, O. *et al.* Intact aminoacyl-tRNA is required to trigger GTP hydrolysis by elongation factor Tu on the ribosome. *Biochemistry* **39,** 1734–1738 (2000).
38. Gregory, S. T., Carr, J. F. & Dahlberg, A. E. A signal relay between ribosomal protein S12 and elongation factor EF-Tu during decoding of mRNA. *RNA* **15,** 208–214 (2009).
39. Schmeing, T. M. *et al.* The structure of the ribosome bound to EF-Tu and tRNA. *Science* doi:10.1126/science.1179700 (in the press).
40. Pape, T., Wintermeyer, W. & Rodnina, M. Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *EMBO J.* **18,** 3800–3807 (1999).
41. Cochella, L. & Green, R. An active role for tRNA beyond codon:anticodon base pairing. *Science* **308,** 1178–1180 (2005).
42. Battle, D. J. & Doudna, J. A. Specificity of RNA-RNA helix recognition. *Proc. Natl Acad. Sci. USA* **99,** 11676–11681 (2002).
43. Johansson, M., Bouakaz, E., Lovmar, M. & Ehrenberg, M. The kinetics of ribosomal peptidyl transfer revisited. *Mol. Cell* **30,** 589–598 (2008).
44. Sievers, A., Beringer, M., Rodnina, M. V. & Wolfenden, R. The ribosome as an entropy trap. *Proc. Natl Acad. Sci. USA* **101,** 7897–7901 (2004).
45. Nissen, P., Hansen, J., Ban, N., Moore, P. B. & Steitz, T. A. The structural basis of ribosome activity in peptide bond synthesis. *Science* **289,** 920–930 (2000).
46. Muth, G. W., Ortoleva-Donnelly, L. & Strobel, S. A. A single adenosine with a neutral pK_a in the ribosomal peptidyl transferase center. *Science* **289,** 947–950 (2000).
47. Beringer, M., Adio, S., Wintermeyer, W. & Rodnina, M. The G2447A mutation does not affect ionization of a ribosomal group taking part in peptide bond formation. *RNA* **9,** 919–922 (2003).
48. Polacek, N., Gaynor, M., Yassin, A. & Mankin, A. S. Ribosomal peptidyl transferase can withstand mutations at the putative catalytic nucleotide. *Nature* **411,** 498–501 (2001).
49. Thompson, J. *et al.* Analysis of mutations at residues A2451 and G2447 of 23S rRNA in the peptidyltransferase active site of the 50S ribosomal subunit. *Proc. Natl Acad. Sci. USA* **98,** 9002–9007 (2001).
50. Katunin, V. I., Muth, G. W., Strobel, S. A., Wintermeyer, W. & Rodnina, M. V. Important contribution to catalysis of peptide bond formation by a single ionizing group within the ribosome. *Mol. Cell* **10,** 339–346 (2002).
51. 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).
52. 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).
53. 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).
54. Bieling, P., Beringer, M., Adio, S. & Rodnina, M. V. Peptide bond formation does not involve acid-base catalysis by ribosomal residues. *Nature Struct. Mol. Biol.* **13,** 423–428 (2006).
55. 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).
This paper shows that peptidyl-transferase activity involves an induced conformational change that opens the ester bond between the peptide and tRNA to nucleophilic attack.
56. Lang, K., Erlacher, M., Wilson, D. N., Micura, R. & Polacek, N. The role of 23S ribosomal RNA residue A2451 in peptide bond synthesis revealed by atomic mutagenesis. *Chem. Biol.* **15,** 485–492 (2008).
57. Trobro, S. & Aqvist, J. Mechanism of peptide bond synthesis on the ribosome. *Proc. Natl Acad. Sci. USA* **102,** 12395–12400 (2005).
58. Sharma, P. K., Xiang, Y., Kato, M. & Warshel, A. What are the roles of substrate-assisted catalysis and proximity effects in peptide bond formation by the ribosome? *Biochemistry* **44,** 11307–11314 (2005).
59. Nierhaus, K. H., Schulze, H. & Cooperman, B. S. Molecular mechanisms of the ribosomal peptidyltransferase center. *Biochem. Int.* **1,** 185–192 (1980).
60. Hecht, S. M., Kozarich, J. W. & Schmidt, F. J. Isomeric phenylalanyl-tRNAs. Position of the aminoacyl moiety during protein biosynthesis. *Proc. Natl Acad. Sci. USA* **71,** 4317–4321 (1974).
61. Quiggle, K., Kumar, G., Ott, T. W., Ryu, E. K. & Chladek, S. Donor site of ribosomal peptidyltransferase: investigation of substrate specificity using 2′(3′)-*O*-(*N*-acylaminoacyl)dinucleoside phosphates as models of the 3′ terminus of *N*-acylaminoacyl transfer ribonucleic acid. *Biochemistry* **20,** 3480–3485 (1981).
62. Dorner, S., Panuschka, C., Schmid, W. & Barta, A. Mononucleotide derivatives as ribosomal P-site substrates reveal an important contribution of the 2′-OH to activity. *Nucleic Acids Res.* **31,** 6536–6542 (2003).
63. 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. Mol. Biol.* **11,** 1101–1106 (2004).
64. Koch, M., Huang, Y. & Sprinzl, M. Peptide-bond synthesis on the ribosome: no free vicinal hydroxy group required on the terminal ribose residue of peptidyl-tRNA. *Angew. Chem. Int. Edn Engl.* **47,** 7242–7245 (2008).
65. Bashan, A. *et al.* Structural basis of the ribosomal machinery for peptide bond formation, translocation, and nascent chain progression. *Mol. Cell* **11,** 91–102 (2003).
66. Korostelev, A., Trakhanov, S., Laurberg, M. & Noller, H. F. Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. *Cell* **126,** 1065–1077 (2006).
67. Wohlgemuth, I., Beringer, M. & Rodnina, M. V. Rapid peptide bond formation on isolated 50S ribosomal subunits. *EMBO Rep.* **7,** 699–703 (2006).
68. Brunelle, J. L., Youngman, E. M., Sharma, D. & Green, R. The interaction between C75 of tRNA and the A loop of the ribosome stimulates peptidyl transferase activity. *RNA* **12,** 33–39 (2006).
69. Voorhees, R. M., Weixlbaumer, A., Loakes, D., Kelley, A. C. & Ramakrishnan, V. Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. *Nature Struct. Mol. Biol.* **16,** 528–533 (2009).
70. Bokov, K. & Steinberg, S. V. A hierarchical model for evolution of 23S ribosomal RNA. *Nature* **457,** 977–980 (2009).
71. Wower, J., Hixson, S. S. & Zimmermann, R. A. Labeling the peptidyltransferase center of the *Escherichia coli* ribosome with photoreactive tRNA(Phe) derivatives containing azidoadenosine at the 3′ end of the acceptor arm: a model of the tRNA-ribosome complex. *Proc. Natl Acad. Sci. USA* **86,** 5232–5236 (1989).
72. Maguire, B. A., Beniaminov, 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).
73. Trobro, S. & Aqvist, J. Role of ribosomal protein L27 in peptidyl transfer. *Biochemistry* **47,** 4898–4906 (2008).
74. Moore, V. G., Atchison, R. E., Thomas, G., Moran, M. & Noller, H. F. Identification of a ribosomal protein essential for peptidyl transferase activity. *Proc. Natl Acad. Sci. USA* **72,** 844–848 (1975).
75. Kazemie, M. Binding of aminoacyl-tRNA to reconstituted subparticles of *Escherichia coli* large ribosomal subunits. *Eur. J. Biochem.* **67,** 373–378 (1976).
76. Moazed, D. & Noller, H. F. Intermediate states in the movement of transfer RNA in the ribosome. *Nature* **342,** 142–148 (1989).
77. Agirrezabala, X. *et al.* Visualization of the hybrid state of tRNA binding promoted by spontaneous ratcheting of the ribosome. *Mol. Cell* **32,** 190–197 (2008).
This paper and ref. 78 show direct structural evidence for hybrid states following peptide-bond formation.
78. Julián, P. *et al.* Structure of ratcheted ribosomes with tRNAs in hybrid states. *Proc. Natl Acad. Sci. USA* **105,** 16924–16927 (2008).
79. Frank, J. & Agrawal, R. K. A ratchet-like inter-subunit reorganization of the ribosome during translocation. *Nature* **406,** 318–322 (2000).
80. Blanchard, S. C., Kim, H. D., Gonzalez, R. L. Jr, Puglisi, J. D. & Chu, S. tRNA dynamics on the ribosome during translation. *Proc. Natl Acad. Sci. USA* **101,** 12893–12898 (2004).
A ground-breaking paper on the use of single molecule techniques to study dynamics in the ribosome.
81. Cornish, P. V., Ermolenko, D. N., Noller, H. F. & Ha, T. Spontaneous intersubunit rotation in single ribosomes. *Mol. Cell* **30,** 578–588 (2008).
82. Ermolenko, D. N. *et al.* The antibiotic viomycin traps the ribosome in an intermediate state of translocation. *Nature Struct. Mol. Biol.* **14,** 493–497 (2007).
83. Fei, J., Kosuri, P., MacDougall, D. D. & Gonzalez, R. L. Jr. Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation. *Mol. Cell* **30,** 348–359 (2008).
84. Munro, J. B., Altman, R. B., O'Connor, N. & Blanchard, S. C. Identification of two distinct hybrid state intermediates on the ribosome. *Mol. Cell* **25,** 505–517 (2007).
85. Pan, D., Kirillov, S. V. & Cooperman, B. S. Kinetically competent intermediates in the translocation step of protein synthesis. *Mol. Cell* **25,** 519–529 (2007).

86. Schmeing, T. M., Moore, P. B. & Steitz, T. A. Structures of deacylated tRNA mimics bound to the E site of the large ribosomal subunit. *RNA* **9,** 1345–1352 (2003).
87. Savelsbergh, A., Mohr, D., Wilden, B., Wintermeyer, W. & Rodnina, M. V. Stimulation of the GTPase activity of translation elongation factor G by ribosomal protein L7/12. *J. Biol. Chem.* **275,** 890–894 (2000).
88. Lill, R., Robertson, J. M. & Wintermeyer, W. Binding of the 3′ terminus of tRNA to 23S rRNA in the ribosomal exit site actively promotes translocation. *EMBO J.* **8,** 3933–3938 (1989).
89. Feinberg, J. S. & Joseph, S. Identification of molecular interactions between P-site tRNA and the ribosome essential for translocation. *Proc. Natl Acad. Sci. USA* **98,** 11120–11125 (2001).
90. Gavrilova, L. P., Koteliansky, V. E. & Spirin, A. S. Ribosomal protein S12 and 'non-enzymatic' translocation. *FEBS Lett.* **45,** 324–328 (1974).
91. Cukras, A. R., Southworth, D. R., Brunelle, J. L., Culver, G. M. & Green, R. Ribosomal proteins S12 and S13 function as control elements for translocation of the mRNA:tRNA complex. *Mol. Cell* **12,** 321–328 (2003).
92. Rodnina, M. V., Savelsbergh, A., Katunin, V. I. & Wintermeyer, W. Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. *Nature* **385,** 37–41 (1997).
93. Ævarsson, A. *et al.* Three-dimensional structure of the ribosomal translocase: elongation factor G from *Thermus thermophilus*. *EMBO J.* **13,** 3669–3677 (1994).
94. Czworkowski, J., Wang, J., Steitz, T. A. & Moore, P. B. The crystal structure of elongation factor G complexed with GDP, at 2.7 Å resolution. *EMBO J.* **13,** 3661–3668 (1994).
95. 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).
96. Hauryliuk, V. *et al.* The pretranslocation ribosome is targeted by GTP-bound EF-G in partially activated form. *Proc. Natl Acad. Sci. USA* **105,** 15678–15683 (2008).
97. Mohr, D., Wintermeyer, W. & Rodnina, M. V. GTPase activation of elongation factors Tu and G on the ribosome. *Biochemistry* **41,** 12520–12528 (2002).
98. Ilag, L. L. *et al.* Heptameric $(L12)_6$/L10 rather than canonical pentameric complexes are found by tandem MS of intact ribosomes from thermophilic bacteria. *Proc. Natl Acad. Sci. USA* **102,** 8192–8197 (2005).
99. Harms, J. M. *et al.* Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin. *Mol. Cell* **30,** 26–38 (2008).
100. Savelsbergh, A. *et al.* An elongation factor G-induced ribosome rearrangement precedes tRNA-mRNA translocation. *Mol. Cell* **11,** 1517–1523 (2003).
101. Studer, S. M., Feinberg, J. S. & Joseph, S. Rapid kinetic analysis of EF-G-dependent mRNA translocation in the ribosome. *J. Mol. Biol.* **327,** 369–381 (2003).
102. Agrawal, R. K., Penczek, P., Grassucci, R. A. & Frank, J. Visualization of elongation factor G on the *Escherichia coli* 70S ribosome: the mechanism of translocation. *Proc. Natl Acad. Sci. USA* **95,** 6134–6138 (1998).
103. Stark, H., Rodnina, M. V., Wieden, H. J., van Heel, M. & Wintermeyer, W. Large-scale movement of elongation factor G and extensive conformational change of the ribosome during translocation. *Cell* **100,** 301–309 (2000).
104. Takyar, S., Hickerson, R. P. & Noller, H. F. mRNA helicase activity of the ribosome. *Cell* **120,** 49–58 (2005).
105. Gao, Y.-G. *et al.* The structure of the ribosome with elongation factor G trapped in the post-translocational state. *Science* doi:10.1126/science.1179709 (in the press).
106. Frolova, L. Y. *et al.* Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. *RNA* **5,** 1014–1020 (1999).
107. Song, H. *et al.* The crystal structure of human eukaryotic release factor eRF1–mechanism of stop codon recognition and peptidyl-tRNA hydrolysis. *Cell* **100,** 311–321 (2000).
108. Ito, K., Uno, M. & Nakamura, Y. A tripeptide 'anticodon' deciphers stop codons in messenger RNA. *Nature* **403,** 680–684 (2000).
109. Vestergaard, B. *et al.* Bacterial polypeptide release factor RF2 is structurally distinct from eukaryotic eRF1. *Mol. Cell,* (2001).
110. Klaholz, B. P. *et al.* Structure of the *Escherichia coli* ribosomal termination complex with release factor 2. *Nature* **421,** 90–94 (2003).
111. Rawat, U. B. *et al.* A cryo-electron microscopic study of ribosome-bound termination factor RF2. *Nature* **421,** 87–90 (2003).
112. 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,** 1255–1266 (2005).
113. Laurberg, M. *et al.* Structural basis for translation termination on the 70S ribosome. *Nature* **454,** 852–857 (2008).
This paper and ref. 114 provide insights into the recognition of stop codons by release factors.
114. Weixlbaumer, A. *et al.* Insights into translational termination from the structure of RF2 bound to the ribosome. *Science* **322,** 953–956 (2008).
115. Korostelev, A. *et al.* Crystal structure of a translation termination complex formed with release factor RF2. *Proc. Natl Acad. Sci. USA* **105,** 19684–19689 (2008).
116. Youngman, E. M., He, S. L., Nikstad, L. J. & Green, R. Stop codon recognition by release factors induces structural rearrangement of the ribosomal decoding center that is productive for peptide release. *Mol. Cell* **28,** 533–543 (2007).
117. Poole, E. S., Major, L. L., Mannering, S. A. & Tate, W. P. Translational termination in *Escherichia coli*: three bases following the stop codon crosslink to release factor 2 and affect the decoding efficiency of UGA-containing signals. *Nucleic Acids Res.* **26,** 954–960 (1998).
118. Ito, K., Uno, M. & Nakamura, Y. Single amino acid substitution in prokaryote polypeptide release factor 2 permits it to terminate translation at all three stop codons. *Proc. Natl Acad. Sci. USA* **95,** 8165–8169 (1998).
119. Zaher, H. S. & Green, R. Quality control by the ribosome following peptide bond formation. *Nature* **457,** 161–166 (2009).
120. Zavialov, A. V., Mora, L., Buckingham, R. H. & Ehrenberg, M. Release of peptide promoted by the GGQ motif of class 1 release factors regulates the GTPase activity of RF3. *Mol. Cell* **10,** 789–798 (2002).
121. Shaw, J. J. & Green, R. Two distinct components of release factor function uncovered by nucleophile partitioning analysis. *Mol. Cell* **28,** 458–467 (2007).
122. Mora, L. *et al.* The essential role of the invariant GGQ motif in the function and stability *in vivo* of bacterial release factors RF1 and RF2. *Mol. Microbiol.* **47,** 267–275 (2003).
123. Dinçbas-Renqvist, V. *et al.* A post-translational modification in the GGQ motif of RF2 from *Escherichia coli* stimulates termination of translation. *EMBO J.* **19,** 6900–6907 (2000).
124. Zavialov, A. V., Buckingham, R. H. & Ehrenberg, M. A posttermination ribosomal complex is the guanine nucleotide exchange factor for peptide release factor rf3. *Cell* **107,** 115–124 (2001).
125. Gao, H. *et al.* RF3 induces ribosomal conformational changes responsible for dissociation of class I release factors. *Cell* **129,** 929–941 (2007).
126. Hirashima, A. & Kaji, A. Role of elongation factor G and a protein factor on the release of ribosomes from messenger ribonucleic acid. *J. Biol. Chem.* **248,** 7580–7587 (1973).
127. Lancaster, L., Kiel, M. C., Kaji, A. & Noller, H. F. Orientation of ribosome recycling factor in the ribosome from directed hydroxyl radical probing. *Cell* **111,** 129–140 (2002).
128. Agrawal, R. K. *et al.* Visualization of ribosome-recycling factor on the *Escherichia coli* 70S ribosome: functional implications. *Proc. Natl Acad. Sci. USA* **101,** 8900–8905 (2004).
129. Gao, N. *et al.* Mechanism for the disassembly of the posttermination complex inferred from cryo-EM studies. *Mol. Cell* **18,** 663–674 (2005).
130. Wilson, D. N. *et al.* X-ray crystallography study on ribosome recycling: the mechanism of binding and action of RRF on the 50S ribosomal subunit. *EMBO J.* **24,** 251–260 (2005).
131. Borovinskaya, M. A. *et al.* Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nature Struct. Mol. Biol.* **14,** 727–732 (2007).
132. Weixlbaumer, A. *et al.* Crystal structure of the ribosome recycling factor bound to the ribosome. *Nature Struct. Mol. Biol.* **14,** 733–737 (2007).
133. Heurgué-Hamard, V. *et al.* Ribosome release factor RF4 and termination factor RF3 are involved in dissociation of peptidyl-tRNA from the ribosome. *EMBO J.* **17,** 808–816 (1998).
134. Rao, A. R. & Varshney, U. Specific interaction between the ribosome recycling factor and the elongation factor G from Mycobacterium tuberculosis mediates peptidyl-tRNA release and ribosome recycling in *Escherichia coli*. *EMBO J.* **20,** 2977–2986 (2001).

Acknowledgements We thank R. Voorhees for a critical reading of this manuscript, and J. Frank and X. Aggirrezabala for providing coordinates of a hybrid state complex. Work in V.R.'s laboratory is supported by the Medical Research Council (UK), the Wellcome Trust, the Louis-Jeantet Foundation and the Agouron Institute. T.M.S. was supported by fellowships from the Human Frontiers Science Program and Emmanuel College, Cambridge. Part of this review was written when V.R. was a G. N. Ramachandran Visiting Professor at the Indian Institute of Science, Bangalore, where he thanks U. Varshney for his hospitality and useful discussions.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to V.R. (ramak@mrc-lmb.cam.ac.uk) or T.M.S. (schmeing@mrc-lmb.cam.ac.uk).