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Ribosome Structure and the Mechanism of Translation

Review

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The publication of crystal structures of the 50S and 30S ribosomal subunits and the intact 70S ribosome is revolutionizing our understanding of protein synthesis. This review is an attempt to correlate the structures with biochemical and genetic data to identify the gaps and limits in our current knowledge of the mechanisms involved in translation.

Introduction

In translation, the sequence of codons on mRNA directs the synthesis of a polypeptide chain. This process takes place on the ribosome, and the movement of tRNA and mRNA through the ribosome is a complicated process that combines high speed with high accuracy (reviewed in Green and Noller, 1997). The ribosome, a large ribonucleoprotein particle, consists of two subunits in all species. In bacteria, the subunits are designated 30S and 50S, and together make up the 70S ribosome. Each subunit has three binding sites for tRNA, designated the A (aminoacyl), which accepts the incoming aminoacylated tRNA; P (peptidyl), which hold the tRNA with the nascent peptide chain; and E (exit), which holds the deacylated tRNA before it leaves the ribosome. The 30S subunit binds mRNA and the anticodon stem-loops of tRNA, and contributes to the fidelity of translation by monitoring base pairing between codon and anticodon in the decoding process. The 50S subunit binds the acceptor arms of tRNA and catalyzes peptide bond formation between the incoming amino acid on A-site tRNA and the nascent peptide chain attached to the P-site tRNA. Both subunits are involved in translocation, in which the tRNAs and mRNA move precisely through the ribosome, one codon at a time. Translation involves not only the ribosome, but additional protein factors, many of which are GTPases activated by the ribosome. The study of ribosomes is also important for medical reasons, since it is the target of many important antibiotics.

The overall scheme of translation was determined about four decades ago, but a detailed mechanistic understanding of translation has proved elusive for several reasons. Because ribosomes were considered too large for high-resolution structural analysis until recently, and biochemical and genetic tools were not as sophisticated as they are now, qualitative progress toward an understanding of mechanisms proved difficult. As a result, translation took a back seat to other problems in molecular biology after its golden age in the 1960s and 70s.

In the last decade, however, rapid progress has been made due to a convergence of various approaches. Increasingly sophisticated biochemical and genetic tools have been applied to translation. Pre-steady-state kinetics has allowed the dissection of steps in the translation pathway (Pape et al., 1998; Rodnina et al., 1997). The advent of single particle reconstruction techniques, combined with cryoelectron microscopy (cryoEM) began to provide images of ribosomes in increasing detail. Because no crystals are required and the amount of material required is minuscule compared to crystallography, this technique has been used to determine the structure of ribosomes in complex with tRNA and mRNA ligands and various factors (reviewed in Agrawal and Frank, 1999; van Heel, 2000).

Finally, nearly two decades after the first report of crystals of 50S subunits (Yonath et al., 1980), rapid progress has been made in the crystallography of ribosomes. This required not only improved crystals of both subunits and the whole ribosome, but also that a number of crystallographic difficulties be overcome (reviewed in Ramakrishnan and Moore, 2001). As a result, atomic structures of both subunits and a molecular model at 5.5 Å of the entire 70S ribosome have been published recently. Together, these crystal structures provide a tremendous amount of information on the global architecture and details of protein-RNA interactions in each subunit, as well as details of the interaction of the ribosome with ligands such as initiation factors, mRNA, and tRNA.

This review is an attempt to revisit both classical and recent biochemical data in light of the structures and in the context of the translation pathway. As judged by the remarkably conserved nature of critical regions of the ribosome and the universal structure of tRNAs, many of the central processes such as tRNA recognition, peptide bond formation, and possibly translocation, are likely to be the same across all kingdoms of life. However, eukaryotic translation is more complex and far more subject to regulation. In this review, we focus entirely on translation in bacteria, where most of the biochemical information comes from work using *E. coli*, and we use *E. coli* numbering for residues unless otherwise stated.

Crystal Structures of the Ribosome

It would be useful at the outset to specify the various ribosomal crystal structures that have been determined, along with their limitations. The first of the high-resolution ribosome structures to be published was the 2.4 Å structure of the 50S subunit from the archaean *Haloarcula marismortuii* (Ban et al., 2000). The structure comprises most of the 50S subunit, including all of the important peptidyl transferase center, but at high resolution, prominent features such as the L1 stalk, the L11-RNA region, and the L7/L12 stalk, all of which are functionally important in factor binding and translocation, appear to be disordered. Some stem-loops of RNA that are involved in contacts with the 30S subunit are also disordered. The *Haloarcula* 50S subunit is also the only structure to date with sufficient resolution to see water molecules, metal ions, and base modifications with any degree of certainty. They are likely to be crucial to our

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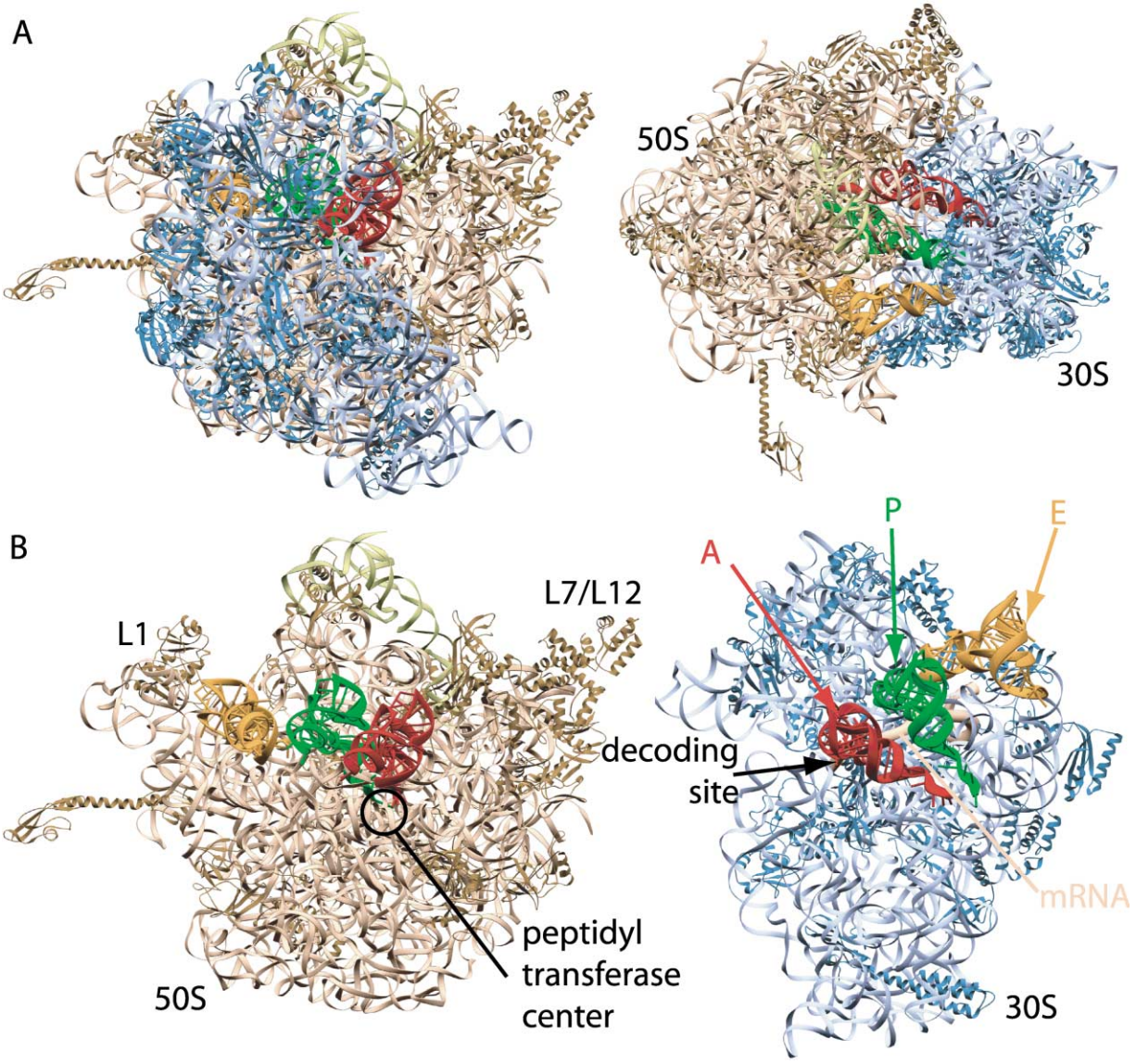

Figure 1. Crystal Structures of the Ribosome

The structure of the 70S ribosome shown was facilitated by atomic structures of the 30S and 50S subunits (see text).

(A) Two views of the 70S ribosome complexed with mRNA and tRNA (Yusupov et al., 2001), with the "top" view on the left and the view from the 30S side on the right.

(B) Exploded view of the 50S (left) and 30S (right) subunits in the 70S ribosome, showing the locations of A-, P-, and E-site tRNAs. This and the other molecular figures in this paper were made using RIBBONS (Carson, 1991) or MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merritt and Bacon, 1997).

understanding of the folding and stability of RNA and may also play important functional roles. Recently, the structure of a 50S subunit at 3.1 Å resolution from a mesophilic bacterium *Deinococcus radioduarans* has been reported (Harms et al., 2001). The RNA in this 50S has a very similar conformation to that reported originally for the *Haloarcula* 50S, but the structure includes some of the regions that were disordered in the *Haloarcula* 50S structure, such as the L1 stalk, the L11/ RNA region, and some of the RNA stem loops that make bridges to the 30S subunit.

Two independent structures of the 30S subunit from the bacterium *Thermus thermophilus*, one at 3.3 Å from a group at the Max Planck/Weizmann Institutes (Schluenzen et al., 2000) and another at 3.05 Å from a group at the MRC (Wimberly et al., 2000), were reported last year. Differences between the two structures have been discussed elsewhere (Ramakrishnan and Moore, 2001). Briefly, the MRC structure represents an essentially complete atomic model of the 30S subunit, and there are a number of significant differences in interpretation of both the RNA and protein components between the two structures. However, more recent structures from the Max Planck/Weizmann group (Pioletti et al., 2001) are in good agreement with the MRC structure originally published.

The subunit structures have made it possible to reveal details of antibiotics bound to the ribosome from crystallographic data on subunit-antibiotic complexes (Brodersen et al., 2000; Carter et al., 2000; Pioletti et al., 2001; Schlünzen et al., 2001). They have also made it possible to study at high resolution the interactions of functional ligands and factors with the 30S and 50S subunits (Carter et al., 2001; Nissen et al., 2000; Ogle et al., 2001; Pioletti et al., 2001; Schmeing et al., 2002)

At anything worse than about 3.5 Å resolution, it would normally not be possible to construct an accurate model of a macromolecule de novo. However, the availability of atomic structures of both subunits facilitated the construction of a model for the RNA and protein backbone in the *Thermus thermophilus* 70S ribosome at 5.5 Å resolution (Yusupov et al., 2001) (Figure 1). Many parts of the 50S subunit that were disordered in the *Haloarcula* 50S structure are ordered in the 70S ribosome. Moreover, the 70S structure is a complex with mRNA and tRNA, so interactions with these ligands, as well as intersubunit interactions, are interpreted in molecular terms. The published 70S structure is a composite of two structures. The first is a 5.5 Å structure of the 70S with mRNA and tRNA in the P and E sites. The second is a structure at 6.5 Å resolution, obtained by adding A-site tRNA to preformed crystals of the 70S ribosome with P- and E-site tRNAs. This results in some non-isomorphism and consequent loss of diffraction when compared with the original structure, but has the advantage that the relative orientations of the three tRNAs can be determined in the context of the ribosome. Structures of the 70S ribosome in the presence of tRNA, but with and without mRNA, has also been determined (Yusupova et al., 2001). This work allows the visualization of even poorly ordered parts of mRNA from difference Fourier maps, so that the extended path of the mRNA in the ribosome can be seen.

Initiation

Initiation in bacteria involves the interaction of the 30S subunit with the Shine-Dalgarno sequence on mRNA that is complementary to the 3′ end of 16S RNA. The process also involves three initiation factors, IF1, IF2, and IF3 (reviewed in Gualerzi and Pon, 1990). IF3 is known to bind strongly to the 30S subunit and prevent its association with the 50S subunit. It also helps in the selection of initiator tRNA (fMet-tRNA^{fMet}) by destabilizing the binding of other tRNAs in the P site of the ribosome (Hartz et al., 1990). In a possibly related function, IF3 has been found to dissociate deacylated tRNA from the 30S subunit in the last step of termination before it is recycled in a new round of protein synthesis (Karimi et al., 1999). IF2 is a GTPase that binds preferentially to fmet-tRNA^{fmet}, and its affinity for the ribosome is increased by IF1 (Zucker and Hershey, 1986). Surprisingly, recent kinetic data indicate that the GTPase activity of IF2 is required neither for the proper placement of initiator tRNA in the P site nor for IF2 release (Tomsic et al., 2000). Structures of bacterial IF1 (Sette et al., 1997), IF3 (Biou et al., 1995; Garcia et al., 1995a, 1995b), and an archaebacterial IF2 homolog (Roll-Mecak et al., 2000) have been solved (Figure 2a)

The crystal structure of the 30S-IF1 complex shows that IF1 binds to the A site of the 30S ribosomal subunit (Carter et al., 2001), consistent with previous biochemical data. In doing so, it prevents tRNA binding in the A site, but also induces a conformational change that may represent the transition state in the equilibrium between subunit association and dissociation. The location of IF3 is controversial. Difference density attributed to IF3 in a cryoEM study was located in the interface side of the platform and neck of the 30S (McCutcheon et al., 1999). All of the difference density and the putative location of the C-terminal (but not the N-terminal) domain are consistent with conclusions derived recently from hydroxyl radical cleavage data (Dallas and Noller, 2001). This location provides a direct explanation for the role of IF3 in preventing subunit association, since the interface side of the platform is involved in extensive contacts with the 50S subunit. However, difference Fourier maps from X-ray crystallography on crystals of the 30S subunit soaked with the C-terminal domain of IF3 suggested that the domain was on the opposite side of the platform, away from the interface (Pioletti et al., 2001). This implies that its effect on subunit association is indirect, consistent with some recent biochemical data (Petrelli et al., 2001). The crystallographic result is not definitive because the binding site of IF3 indicated by cryoEM and footprinting is occluded by lattice packing contacts in this crystal form, so that the location determined crystallographically could be a nonspecific binding site. The cocrystallization of a complex of IF3 with the 30S subunit needs to be done to settle this question unambiguously. No direct location of IF2 has been determined, but since it is known to bind the aminoacyl end of initiator tRNA in the P site, as well as interact with IF1, a model could be proposed in which it binds over IF1 in the A site (Roll-Mecak et al., 2000). In addition, presumably its GTPase domain binds in the vicinity of the factor binding site of the 50S subunit where the corresponding domains of elongation factors G and Tu (EF-G and EF-Tu) also bind, since it is known to footprint some of the same residues in 23S RNA (La Teana et al., 2001).

When one combines the current structural and biochemical data, a view emerges in which IF1 binds in the A site, IF2 binds over the A site, the P site is occupied by initiator tRNA, and IF3 occupies the E site (Figure 2b). Thus, all of the tRNA sites are occupied in the initiation complex, presumably "setting" the correct conformation of the 30S for the initiation of protein synthesis. However, this raises a number of questions. Why do all of the tRNA binding sites need to be occupied? How does IF3 preferentially destabilize elongator tRNAs? If the GTPase activity of IF2 is not required for P-site tRNA binding or for IF2 release (Tomsic et al., 2000), what is its role? When does the 50S subunit become associated with the initiation complex? Finally, despite many years of work, the order in which the factors bind and are released in vivo, and what they have to do with the conformation of the ribosome, have not been definitively elucidated.

Overview of the Elongation Cycle

The end of the initiation process leaves an aminoacylated initiator tRNA in the P site of the ribosome and an empty A site, which serves to start the elongation cycle.

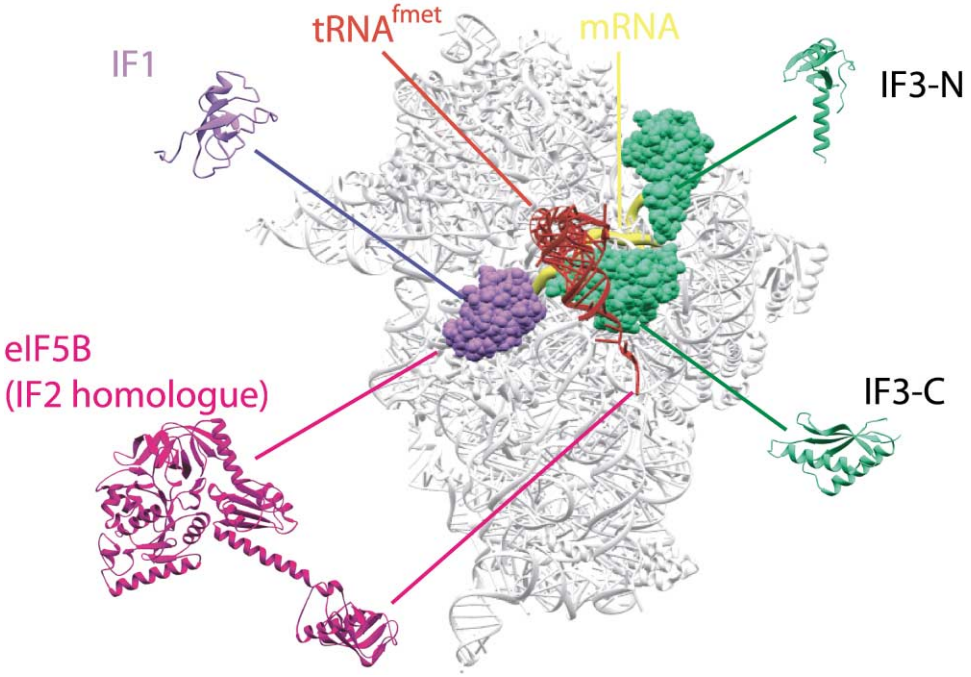

Figure 2. Structure and Interaction of Initiation Factors with the 30S Subunit

The structures of IF1 (Sette et al., 1997), IF2 (Roll-Mecak et al., 2000), and IF3 (Biou et al., 1995) are shown along with their locations in the 30S subunit. The crystal structure of the 30S-IF1 complex (Carter et al., 2001) is shown with the approximate orientation of IF3 derived from hydroxyl-radical cleavage data (Dallas and Noller, 2001), while the interactions of IF2 are indicated. The locations of P-site (initiator) tRNA (red) and mRNA (yellow) are those derived from the 70S structure in Figure 1.

An outline of the elongation cycle is shown in Figure 3. Briefly, aminoacylated tRNA is brought into the A site as a ternary complex with EF-Tu and GTP. Correct codon-anticodon interactions result in conformational changes in the ribosome which stabilize tRNA binding and trigger GTP hydrolysis by EF-Tu. This leads to the release of the aminoacyl end of A-site tRNA by EF-Tu; the tRNA then swings into the peptidyl transferase site of the 50S subunit in a process called accommodation. Peptide bond formation, which involves the deacylation of P-site tRNA and the transfer of the peptide chain to A-site tRNA, is then essentially spontaneous. Following peptidyl transfer, the ribosome has a deacylated tRNA in the P site and peptidyl tRNA in the A site. Translocation of the tRNAs and mRNA is facilitated by EF-G, which is also a GTPase. The result is a ribosome ready for the next round of elongation, with deacylated tRNA in the E site, peptidyl tRNA in the P site, and an empty A site that is ready to receive the next cognate ternary complex.

Decoding

Base pairing between the codon on mRNA and the anticodon on tRNA is the ultimate basis for selection of the correct tRNA for participation in the addition of a new amino acid to the growing polypeptide chain. However, the energy difference in base pairing of cognate tRNA, which has a perfect match to the codon, and that of near-cognate tRNA, which generally only has a single mismatch, is too small to account for the accuracy of selection, which has an error rate of 10^{-3} to 10^{-4}. For example, the free energy of formation of a noncanonical GU base pair at the first position should be very similar to that of an AU pair, yet the ribosome is able to discriminate accurately between these two cases. Moreover, from base pairing alone, the interaction between the phenylalanine UUU codon and the GAA anticodon of $tRNA^{Phe}$ should actually be less stable than the incorrect pairing between the serine UGC codon and the GCG anticodon for $tRNA^{Arg}$, because the stronger GC pairs in the latter should more than compensate for the noncanonical GU pair at the first position. Yet the ribosome strongly prefers a correct $tRNA^{Phe}$ to an incorrect $tRNA^{Arg}$.

What is the basis for the ribosome's selectivity? It has long been suggested that the ribosome contains a "decoding site" that recognizes the geometry of codon-anticodon base pairing and sterically discriminates against mismatches, in much the same way that an enzyme discriminates against a substrate that is nearly correct (Davies et al., 1964; Potapov, 1982). In a second view, often termed "kinetic proofreading," the accuracy comes from splitting the selection step into an initial selection and a proofreading step (Hopfield, 1974; Ninio, 1975), which are separated by an irreversible step, such as the hydrolysis of GTP by EF-Tu. In this scheme, the tRNA has two chances to dissociate (red arrows in Figure 3), once during initial selection, and once after GTP hydrolysis. But because the cellular concentration of free aminoacyl tRNA is small, tRNA can only enter the ribosome at the beginning of the sequence, as part of the ternary complex, and must remain bound through the entire sequence of events to participate in peptidyl trans-

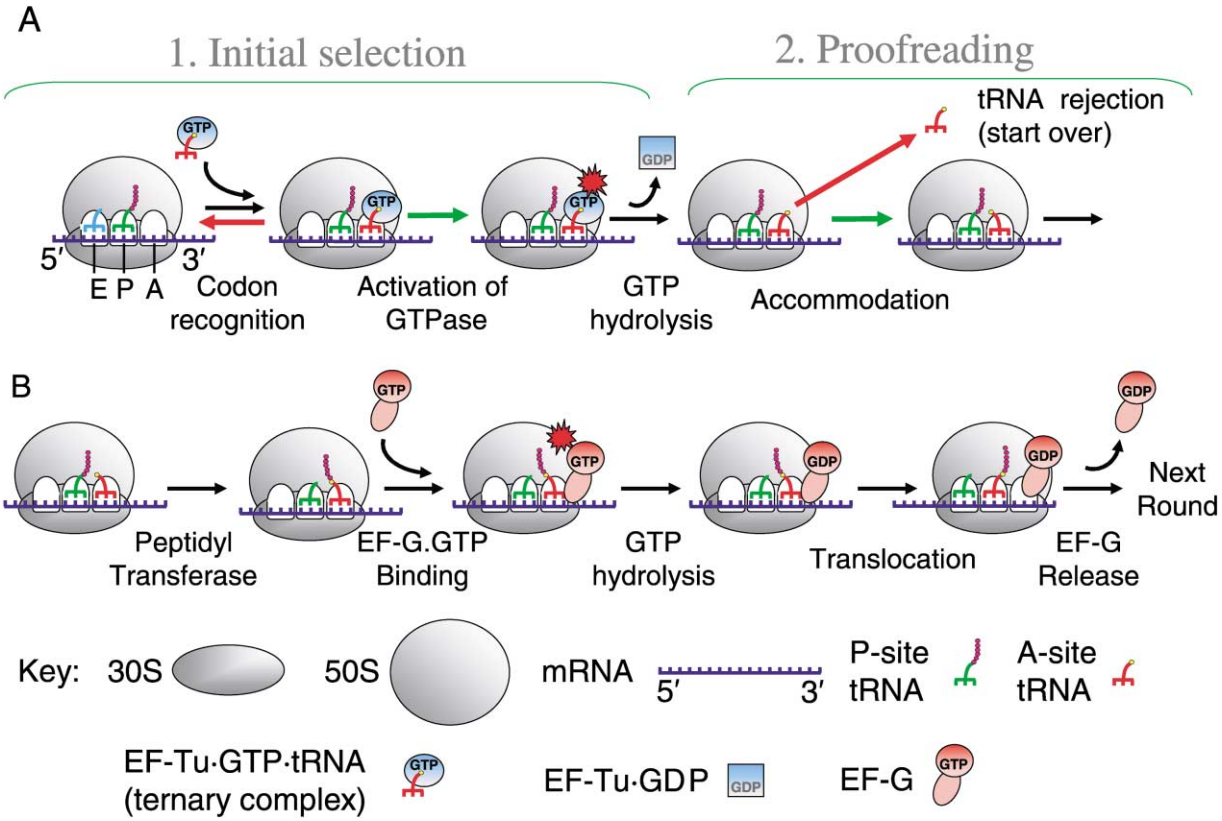

Figure 3. Overview of Elongation Pathway

For simplicity, not all of the steps discerned in kinetic experiments are shown.

ferase. Theoretically, this process can result in a selectivity that is the product of the selectivity at each step. But in practice, the relative rates of the forward reaction and dissociation rate of tRNA at each stage will determine how much of the selectivity can be used. Although steric recognition and kinetic proofreading are often thought of as two distinct possibilities, recent work (see below) shows that they almost certainly work in concert. It is unlikely that the energy discrimination inherent in base pairing alone could account for the accuracy of protein synthesis, even with kinetic proofreading.

Pre-steady-state kinetic experiments have dissected the selection process (Pape et al., 1999). One surprising result of these studies is that in addition to having lower dissociation rates, cognate tRNA also has much faster forward rates of GTPase activation and accommodation than near-cognate tRNA (green arrows in Figure 3). Based on this result, it was proposed that cognate tRNA induces a conformational change in the ribosome. An induced conformational change is also a feature of the allosteric three site model (Nierhaus, 1990). In this model, the affinities of A- and E-site tRNAs are reciprocally coupled. In the presence of E-site tRNA, only cognate ternary complex has enough affinity for the A site to induce a conformational change in the ribosome on binding, leading to the release of E-site tRNA.

Three universally conserved bases of 16S RNA—G530, A1492, and A1493—are footprinted by A-site tRNA (Moazed and Noller, 1986) and are required for viability in *E. coli* (Powers and Noller, 1994 Yoshizawa et al., 1999). A1492 and A1493 lie in an internal loop of helix 44 of 16S RNA that is the binding site for aminoglycosides such as paromomycin that cause increased levels of incorporation of amino acids from near-cognate tRNA. Biochemical experiments also suggested a direct interaction of the N1 of these adenines with the 2′ OH of mRNA (Yoshizawa et al., 1999), but as shown below, this has not been borne out by recent structural data. An NMR structure of an RNA fragment of helix 44 with paromomycin suggested that the antibiotic induces a conformation of the ribosome that mimics the tRNA-bound form (Fourmy et al., 1996), while kinetic experiments showed that paromomycin increases both the affinity and GTPase activation rate of near-cognate tRNA (Pape et al., 2000). However, until recently, the structural basis for recognition of cognate tRNA by the ribosome remained unclear.

In a crystal structure of the 30S complexed with antibiotics (Carter et al., 2000), paromomycin was found to bind in the internal loop of helix 44 approximately in agreement with the earlier NMR structure (Fourmy et al., 1996). However, instead of being modestly displaced into the minor groove as in the NMR structure, A1492 and A1493 were found to be completely flipped out of the helix so that they were in a position to interact directly with the minor groove of the codon-anticodon helix in the A site. It was proposed that the binding of cognate tRNA would induce a similar flip in these bases,

which would hydrogen bond with both strands of the minor groove of the codon-anticodon helix in a way that would be sensitive to Watson-Crick base pairing. Such tertiary interactions of conserved adenines in the minor grooves of adjacent helices were seen elsewhere in the 16S RNA structure. These ideas were tested in a recent structure of the 30S subunit complexed with an oligonucleotide for mRNA and the anticodon stem-loop of cognate tRNA (Ogle et al., 2001). The structure shows that the binding of cognate tRNA indeed induces a conformational change in A1492 and A1493, but unexpectedly, it also causes a flip of G530 from a *syn* to an *anti* conformation (Figure 4). The result is that A1493 interacts with the minor groove of the first codon-anticodon base pair to form a type I A minor motif found elsewhere in tertiary interactions in large RNA structures (Doherty et al., 2001; Nissen et al., 2001). A1492 and G530 also tightly pack into the second base pair of the codon-anticodon helix. Unlike the first two base pairs, the third base pair between codon and anticodon is not closely monitored by the ribosome. The degeneracy of the genetic code at the third position originally led to the "wobble hypothesis" that the same tRNA could recognize codons that differ at the third position, with the third base pair often consisting of certain non-canonical base pairs such as a GU wobble (Crick, 1966). Consistent with this, the structure shows that the ribosome stringently monitors base pairing at the first two positions, but is able to tolerate non-canonical base pairs such as a GU wobble base pair at the third position. There is now little doubt that the ribosome plays a major role in the selectivity of tRNA by direct recognition of the geometry of codon-anticodon base pairing.

The Role of EF-Tu

Although crucial, the structural recognition of codon-anticodon base pairing addresses only one aspect of the decoding process. In translation, selection of tRNA begins with the binding of the EF-Tu ternary complex, which presents the tRNA to the decoding site at a very different angle (Stark et al., 1997), as seen in Figure 7B. Footprinting data show that the same three bases are footprinted by A-site tRNA, regardless of whether the ternary complex or tRNA alone is bound (Powers and Noller, 1994). This suggests that initial selection involves the same mode of recognition by these bases, but they must be capable of rotating with the anticodon of tRNA as it rotates into the peptidyl transferase site after release by EF-Tu. An examination of the structure suggests that such a change in the orientation of the bases is possible, but a high-resolution structure of a ternary complex of EF-Tu bound to the 70S ribosome is required to settle this issue. A second and more difficult question is how codon-anticodon recognition triggers the hydrolysis of GTP by EF-Tu. This step requires the transmission of a signal from the decoding site on the 30S subunit where codon-anticodon interactions are recognized, to the 50S subunit where the GTPase domain of EF-Tu binds. Such a signal could be transmitted not only through the ribosome, but also through tRNA itself, since intact tRNA is required for the process (Piepenburg et al., 2000). The nature of the transmission of the signal, and exactly how this leads to activation of GTP hydrolysis, both remain pressing questions. In the presence of kirromycin, EF-Tu is able to hydrolyze GTP but does not release tRNA or dissociate from the ribosome. In the presence of aurodox (related to kirromycin) and GDP, the structure of EF-Tu is very similar to its complex with GTP, rather than GDP, showing that kirromycin probably prevents the conformational change in EF-Tu on GTP hydrolysis (Vogeley et al., 2001). This suggests that the structure of the kirromycin-stalled complex of EF-Tu with the ribosome (Stark et al., 1997) is the one just after GTP hydrolysis but before accommodation.

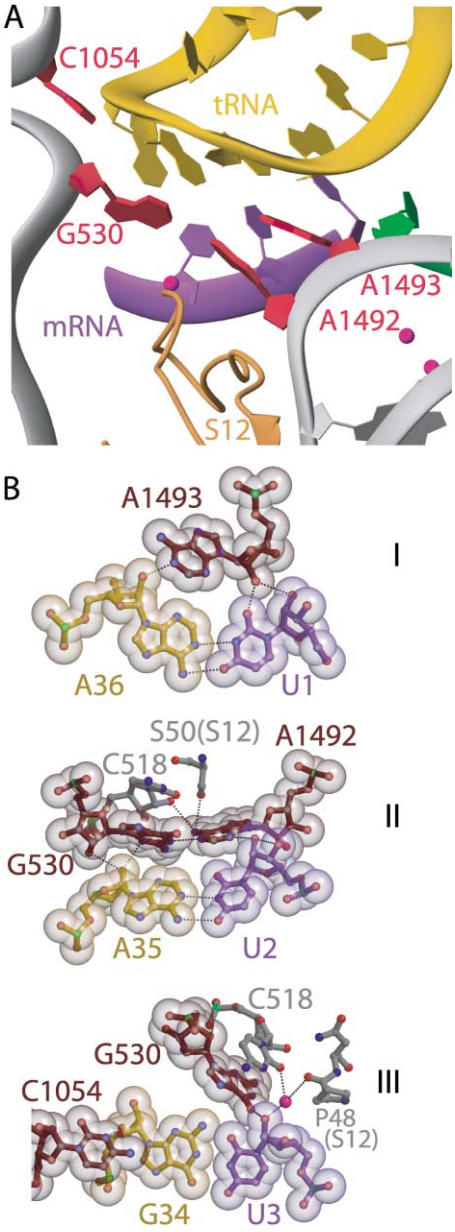

Figure 4. Recognition of Codon-Anticodon Interactions by the Ribosome

(A) Cartoon of the decoding site of the 30S subunit, showing the A-site codon (blue) and the tRNA anticodon stem-loop (gold). Critical bases of 16S RNA that bind to the tRNA-mRNA complex are shown in red. The magenta spheres are probably magnesium ions.

(B) Details of minor groove recognition at the first (I), second (II), and third (III) base pairs between codon and anticodon.

The figures are reproduced with permission from Ogle et al. (2001)

During the accommodation or proofreading step, the tRNA has a second chance to dissociate. Genetic analysis suggests that helix 27 of 16S RNA can switch the ribosome from a hyperaccurate state that has reduced affinity for tRNA to a "*ram*" or permissive state that has higher affinity for tRNA (Lodmell and Dahlberg, 1997). Mutants that preferentially stabilize one or other form appear to have distinct conformations (Gabashvili et al., 1999). Although this region is highly conserved, similar mutations in helix 27 in a eukaryotic system do not confer a *ram* phenotype but do increase accuracy (Velichutina et al., 2000).

An extended interface in the 30S subunit is formed between the shoulder and platform domains. Mutations that destabilize one end of the interface, such as the *ram* mutations in S4 and S5, result in an error-prone phenotype, while mutations that destabilize the other end, such as in S12 or in helix 27 (Lodmell and Dahlberg, 1997; Velichutina et al., 2000), result in a hyperaccurate phenotype. By contrast, streptomycin appears to stabilize the S12-H27 region (Carter et al., 2000), and has the opposite phenotype of increased error rate. All of this suggests that a relative movement of the two domains is involved in the accommodation or proofreading step, and that the lifetime of the various states, as well as the activation barrier during the process, contribute to accuracy. However, not only do we not know the nature of the structural changes involved, but we do not even know where the putative *ram* and restrictive states lie on a kinetic pathway. In addition to high-resolution structural data of the various states, it will be important to measure kinetics for various mutants in the presence and absence of streptomycin and correlate it with structural data.

Peptidyl Transferase

When the aminoacyl end of A-site tRNA enters the peptidyl transferase center, peptide bond formation occurs rapidly and spontaneously (Pape et al., 1998). For a long time, nucleic acids were thought not to be capable of catalysis, so the notion that ribosomal RNA could catalyze peptidyl transfer was not taken seriously, despite hints to the contrary. This changed following the discovery of catalytic RNA (Kruger et al., 1982; Zaug et al., 1983), and biochemical evidence for the role of 23S RNA in peptidyl transferase began to accumulate (reviewed in Green and Noller, 1997). In the most notable of these experiments, 50S subunits from *Thermus aquaticus* were extracted with phenol after treatment with proteinase K in the presence of SDS, and retained most of their peptidyl transferase activity even after losing more than 80% of the protein composition, strongly suggesting a catalytic role for RNA (Noller et al., 1992). However, even after such harsh treatment, the 50S subunits retained some proteins and peptide fragments (Khaitovich et al., 1999); among these were L2 and L3, proteins that had previously been shown to be among those required for reconstitution of peptidyl transferase activity (Hampl et al., 1981). Complete removal of proteins could only be accomplished under conditions that unfolded the RNA, and resulted in loss of peptidyl transferase activity. Thus, in these various biochemical studies, a definitive catalytic role for RNA could not be established.

Thus, the high-resolution crystal structures of the 50S subunit and its complex with a potent inhibitor of peptidyl transferase called the "Yarus inhibitor" represented a major breakthrough (Ban et al., 2000; Nissen et al., 2000). In many respects, the Yarus inhibitor, C-C-dA-phosphoramide-puromycin, mimics a transition state analog (Welch et al., 1995), so its location defines the peptidyl transferase site. An immediate and unambiguous conclusion from the structure is that there is no protein moiety observable within about 18 Å of this site. As had been suspected for some time, the ribosome is a ribozyme.

Based on the complex with the Yarus inhibitor and an A-site substrate analog, a number of possible contributions to catalysis that have counterparts in protein enzymes were proposed (Nissen et al., 2000). Peptidyl transfer is energetically a downhill reaction, and as has long been suggested for enzymes, it was suggested that a sufficient catalytic enhancement for peptidyl transferase could come just from the precise orientation of substrates (Nierhaus et al., 1980). In agreement with earlier biochemical work (reviewed in Green and Noller, 1997), the structures show that the ribosome precisely positions the CCA ends of the A- and P-site tRNAs through interactions with the highly conserved A- and P-loops, respectively, of 23S RNA (Figure 5a). A second source of catalysis suggested by the Yarus inhibitor complex is transition state stabilization. A third source of catalytic enhancement was proposed to involve the direct participation of two residues, A2451 and G2447, in acid-base catalysis. In the structure, the N3 of A2451 was found to be 3 Å away from, and presumably hydrogen bonded to, one of the non-bridging phosphoramide oxygens of the Yarus inhibitor (Figure 5b). This would require that the N3 of A2451 be protonated, leading to a proposal that it could remove a proton from the amino group of aminoacyl tRNA and donate it to the 2′ OH group of peptidyl tRNA. Protonation of the N3 of A2451 would require an unusually high pK_a, proposed to be facilitated by a charge-relay network that involved G2447. The pH dependence of dimethyl sulfate (DMS) modification experiments (Muth et al., 2000) supported such an elevated pK_a for the N3 of A2451.

More recently, a number of experiments have questioned a direct role for these bases in peptidyl transferase. The pH dependence of DMS modification of A2451 mentioned above occurs only in *E. coli* 50S subunits in an inactive conformation (Bayfield et al., 2001). Moreover, the modification probably occurs at the N1 rather than N3 position, and therefore probes the conformation of the adenine rather than the protonation state of its N3 atom (Muth et al., 2001). Two reports show that ribosomes in which A2451 and G2447 have been mutated to other residues are active in peptidyl transferase (Polacek et al., 2001; Thompson et al., 2001). However, the N3 of guanine or the O2 of pyrimidines could serve a role analogous to the N3 or A2451, so the mutational data cannot be regarded as conclusive in themselves. Structural data will be very useful to see whether features proposed to be essential for catalysis are actually disrupted in the mutants.

The peptidyl transferase center in the recent *D. radiodurans* 50S structure is very similar to the *Haloarcula* 50S, but there are differences in the orientation of key

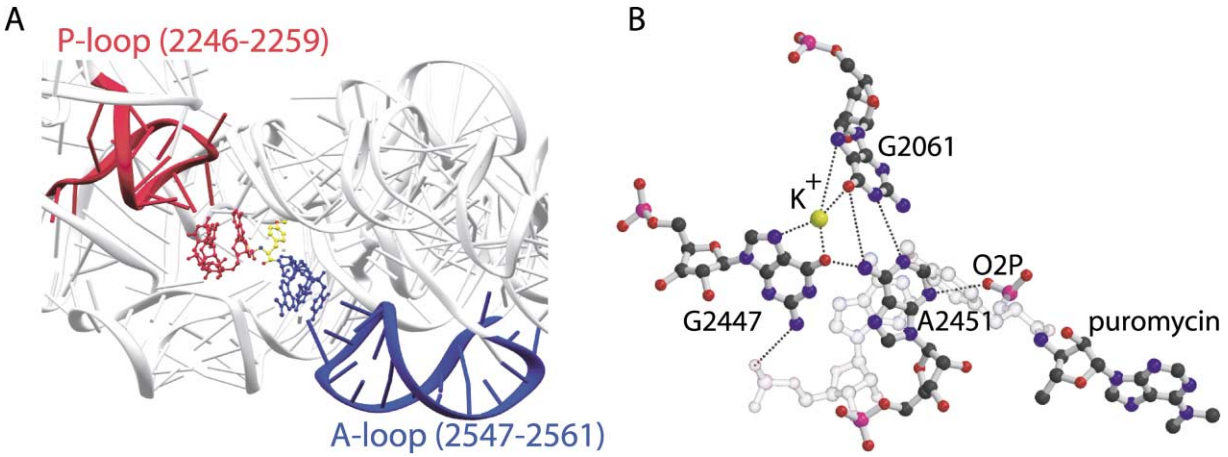

Figure 5. Role of the Ribosome in Peptidyl Transfer

(A) The peptidyl transferase center in the structure of the 50S subunit from *Haloarcula marismortuii* (Nissen et al., 2000). The P loop and A loop of 23S RNA are shown in red and blue. The coordinates of the Yarus inhibitor (Welch et al., 1995) have been combined with those of an A-site substrate to show the CCA ends of P- and A-site tRNAs in red and blue, respectively.

(B) Details of the interaction of key bases of 23S RNA with the Yarus inhibitor (see text).

bases, including A2451, that may be functionally significant (Harms et al., 2001). On the other hand, in all the substrate/product structures of the *Haloarcula* 50S subunit obtained so far (Nissen et al., 2000, Schmeing et al., 2002), the critical A2451 is in the same position, and that position is consistent with its hydrogen bonding to substrates through its N3. In addition, peptidyl transferase activity can be demonstrated in the crystal itself (Schmeing et al., 2002). Finally, mutations of A2451 result in a dominant-lethal phenotype (Thompson et al., 2001), so even though its exact role remains a matter of debate, it is crucial for translation.

While details of the catalytic mechanism continue to be debated, it is astonishing how quickly and dramatically the structure of the 50S subunit has changed the study of peptidyl transferase. Rather than arguing about broad questions such as whether RNA or protein is the catalyst, and which parts of the ribosome are involved, the new studies involve the sort of detailed mechanistic analysis that is applied to small enzymes such as serine proteases. However, it is sobering to see that a covalent intermediate in the action of lysozyme has been shown just this year (Vocadlo et al., 2001), about 35 years after the structure of the enzyme was first determined.

Translocation

Following peptidyl transferase, the P-site tRNA is deacylated and the A-site tRNA has a peptide chain that has one additional residue. To prepare the ribosome for a new round of peptide chain elongation, the tRNAs have to move: the deacylated tRNA needs to be moved from the P site to the E site and eventually ejected from the ribosome, while the peptidyl tRNA has to move from the A site to the P site. Moreover, this movement has to be precise, and the reading frame on mRNA has to be preserved.

Models for Translocation

It was proposed that translocation would involve a relative movement of the two subunits, and could occur in separate steps relative to the 50S and 30S subunits (Bretscher, 1968). This would result in "hybrid states" of the tRNA molecules which would be bound in the A site of the 30S and the P site of the 50S. This model has the advantage that only one end of the tRNA-mRNA complex has to move in each step, while the other fixed end acts as an anchor. It also rationalizes the universal existence of two subunits in all species. Since this paper was entirely theoretical, there was no evidence for whether movement occurred first relative to the 30S or 50S subunit. Moreover, at the time the model was proposed, the E site had not been discovered. Recently, a relative movement between the subunits was inferred by comparing EF-G-bound and -free forms of the ribosome (Frank and Agrawal, 2000).

Experimental evidence for the nature of tRNA movement during translocation had to await the development of base footprinting techniques for the ribosome, pioneered by Noller and colleagues. In a landmark paper, the characteristic footprints of tRNA in each site were used to follow the movement of tRNA through the elongation cycle (Figure 6) (Moazed and Noller, 1989). When puromycin (a mimic of aminoacyl tRNA in the A site) is added to ribosomes with an aminoacylated tRNA in the P site, the tRNA footprint on the 50S disappears and is replaced by an E site footprint, while the footprint in the 30S subunit remains unchanged, suggesting that the tRNA is in a "P/E" hybrid state. As far as the A-site tRNA is concerned, there is initially an "A/T" hybrid which represents the binding of the ternary complex to the A site of the ribosome, in which the aminoacyl end of the tRNA is attached to EF-Tu. On release by EF-Tu, the acceptor arm of the tRNA swings into the peptidyl transferase site of the 50S, resulting in the characteristic footprint of tRNA in the nonhybrid A/A state. However, after peptidyl transferase, the tRNAs footprint their respective A and P sites on the 30S subunit, but their acceptor arms have moved over to the P and E sites on the 50S subunit, consistent with A/P and P/E hybrid

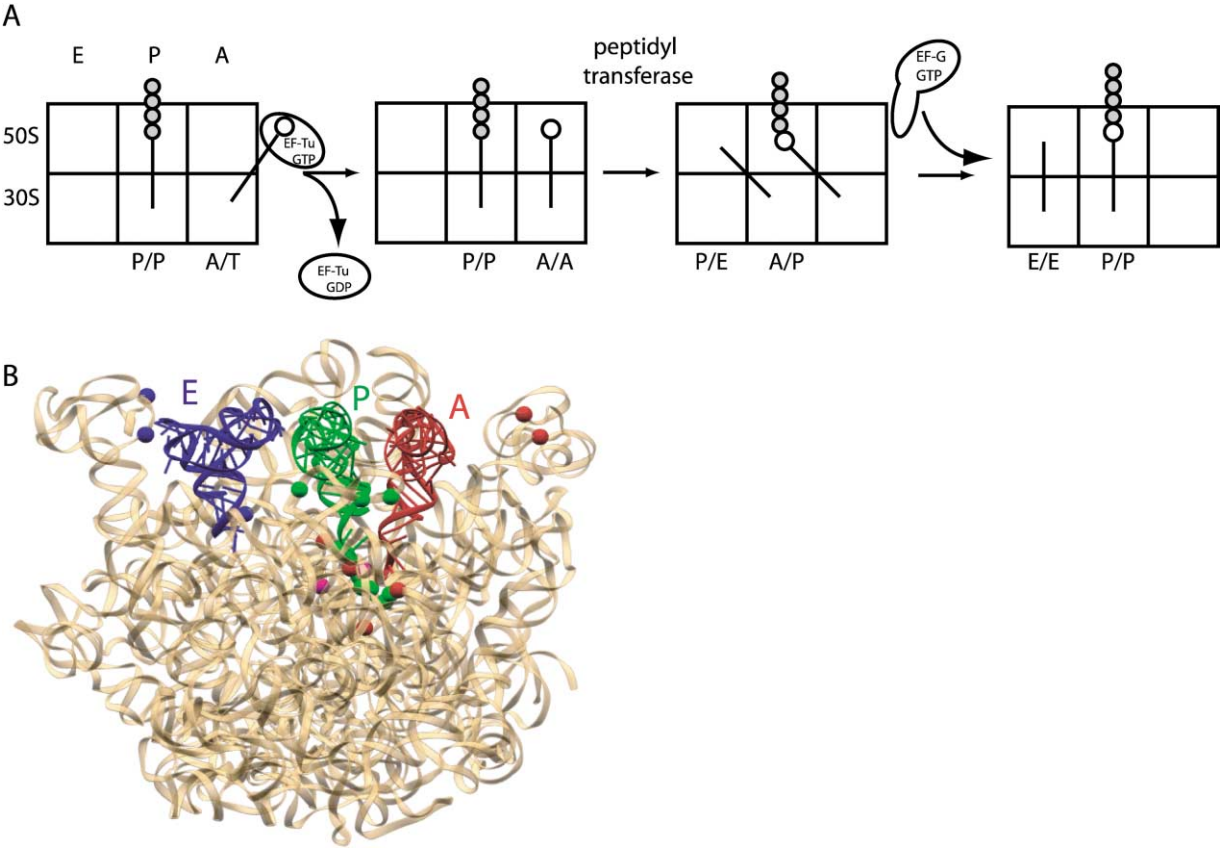

Figure 6. The Hybrid States Model for Translocation

(A) Cartoon of the hybrid states model of translocation as proposed by Moazed and Noller (1989), but now including an E site in the 30S subunit.

(B) The E-, P- and A-site tRNAs in the 50S subunit of the 70S structure (Yusupov et al., 2001), shown in blue, green, and red respectively. The base footprints from each tRNA (Moazed and Noller, 1989) are mapped onto the 70S structure and shown as spheres of the corresponding color. The two magenta spheres are bases affected by both A- and P-site tRNAs.

states for the tRNA. Interestingly, this state does not react with puromycin, even though the 50S A site should have been vacated with a peptidyl tRNA in the 50S P site. After translocation by EF-G, the result is an empty A site, and tRNAs in the E and P sites. The relative movements of the tRNA with respect to each subunit in this model could also involve the movement of one subunit relative to the other, as has been seen in cryoEM.

An alternative "α-ϵ" model for translocation has been proposed by Nierhaus and colleagues (Spahn and Nierhaus, 1998). By measuring the protection by the ribosome of phosphothiorated tRNAs against iodine-mediated cleavage, they concluded that the pattern of protection characteristic of A- and P-site tRNAs does not change during translocation (Dabrowski et al., 1998), suggesting that the ribosomal contacts of A- and P-site tRNAs do not change during translocation. This implies that there are movable domains within the ribosome that transport the tRNAs through the ribosome.

It is informative to revisit these ideas in light of the crystal structure of the 70S ribosome, which accurately defines the ribosomal contacts of tRNAs in the A, P, and E sites (Yusupov et al., 2001). One interesting observation is that the E site is occupied by endogenous tRNA that copurified with the ribosomes used for crystallization, implying that it remains stably bound to the ribosome throughout the extensive purification procedures. The few base footprints observed for E-site tRNA in the 50S subunit come from direct contacts with its elbow or CCA end. Two features of the E site in the crystal structure also address previous uncertainties. First, there is no longer any doubt that the 30S subunit has an E site. Its existence was questioned previously because it does not have a base footprint on 16S RNA, but it is now apparent that this is because all of the contacts with the 30S occur with the backbone of 16S RNA or with proteins S7 and S11 (Yusupov et al., 2001). Second, the E-site tRNA appears to be locked in the ribosome by the L1 stalk in the 50S and protein S7 in the 30S subunit. Different orientations of the L1 stalk have been seen in cryoEM studies (Agrawal et al., 1999a). The L1 stalk has a different orientation in the 70S ribosome as compared to isolated 50S subunits (Harms et al., 2001), where it would not block E-site tRNA from leaving, suggesting that a pivotal movement of this stalk may be required to release E-site tRNA. Earlier work in *E. coli* on the stability of E-site tRNA has been controversial, but the *Thermus* 70S structure supports a view that E-site tRNA is stably bound and requires an active conformational change in the ribo-

some for its release, as suggested in the allosteric three-site model (Nierhaus, 1990). The question of whether codon-anticodon interaction takes place in the E site as required by the model is still unanswered. In the crystal structure, there is only a single possible base pair between codon and anticodon, but the E-site tRNA in the crystal is probably a mixture of endogenous tRNAs rather than a single cognate tRNA.

The A- and P-site tRNA footprints on the 30S subunit are in good agreement with base footprinting and cross-linking data (Carter et al., 2000; Ogle et al., 2001; Yusupov et al., 2001). In the 50S subunit, the characteristic A- and P-site footprints have a spatial distribution that is overlapping rather than distinct, so that those footprints that come from direct contact such as with the A loop and P loop need to be distinguished from those that are possibly the result of indirect conformational changes. While the base footprints come mainly from the CCA ends of the tRNAs, the 50S also makes extensive contacts throughout the acceptor arm and elbow of A- and P-site tRNAs (especially the latter). Interestingly, they involve stem-loops of 23S RNA that form bridges with the 30S subunit, many of which were disordered in the *Haloarcula* 50S structure, suggesting that they could move during translocation. These contacts, because they involve the backbone of 23S RNA and tRNA, could not be probed by measuring base protection. Extensive contacts of the ribosome with tRNA have also been inferred from protection of phosphothiorated tRNA against iodine cleavage as discussed earlier (Dabrowski et al., 1998), but a close comparison reveals discrepancies with the contacts in the crystal structure.

How do these data address the various models for translocation? First, the 50S E-site base protections are completely consistent with the structure. Because these protections are seen along with 30S P-site protections after the puromycin reaction, this is strong evidence for a P/E hybrid state. Interestingly, elements of the 3′ end of P-site tRNA were shown to be required for EF-G-dependent translocation (Lill et al., 1989). These elements have been recently defined as the 2′ OH groups at positions 71 and 76 (Feinberg and Joseph, 2001). These groups do not make contact with the ribosome in the P site, but if the tRNA were present as a P/E hybrid, they would interact with 1892 and 2433–2434 in the E site of 23S RNA. So one way to rationalize the result is by postulating that a P/E hybrid state is required for EF-G function. This may be related to the requirement of peptide release and by implication a deacylated tRNA in a P/E state for RF3 function (see below). Structurally, a P/E hybrid state for deacylated tRNA in the P site has been seen under some buffer conditions but not others (Agrawal et al., 1999b), but the fact that it can be seen at all is evidence that it could occur during translation.

The situation with the A/P hybrid is less straightforward. The base protection data that postulate an A/P hybrid mainly come from the CCA end of tRNA (Figure 6B). So, they do not address the crucial question of how or when the extensive backbone contacts of the tRNA with the ribosome are broken and reformed as it goes from the A site to the P site. The timing of the movement in the 50S subunit is also not clear. A recent cryoEM structure shows that there is only a slight movement of the CCA end toward the P site from the A site immediately after peptidyl transferase (Agrawal et al., 2000). Crosslinking studies with the 3′ end of P-site tRNA (Wower et al., 2000) conclude that the movement into the E site does not immediately follow peptidyl transferase. Finally, a recent crystal structure of the 50S shows that the product after peptidyl transferase remains bound to the A loop (Schmeing et al., 2002). Thus, translocation of the tRNAs in the 50S subunit may require some additional step after peptidyl transferase. In this connection, it is interesting that the ribosome is not puromycin reactive right after peptidyl transferase, but becomes so on the action of EF-G (Borowski et al., 1996).

If there are uncertainties with aspects of the hybrid states model, this is also true for the other possibilities. If there indeed is a movable "boxcar" that transports tRNAs through the ribosome as proposed in the α-epsilon model, what does it consist of? One candidate is helix 44 in the 30S subunit, which is known to undergo significant movement around its tRNA binding region (VanLoock et al., 2000). Another is helix 69 in the 50S subunit, which makes extensive contact with P-site tRNA but is in a different conformation in the 70S and the bacterial 50S structures (Harms et al., 2001; Yusupov et al., 2001). However, it is not clear from the structure how these and other elements can move with a tRNA from one site to the next without breaking contact.

The putative requirement for the binding of deacylated tRNA in the E site of the 50S subunit for efficient EF-G function or RF3 function suggests that there are conformational changes in the 50S associated with E-site occupancy that affect the structure in the vicinity of the factor binding site, where EF-Tu also binds. This would also be consistent with the idea that there is some coupling between the A and E sites of the ribosome. We also do not know what resets the "ratchet" to return the ribosome to its pretranslocation state. In the α-ϵ model, this is thought to be triggered by the binding of the ternary complex in the A site, which induces a conformational change and concomitantly releases E-site tRNA through negative allosteric coupling between the A and E sites (Spahn and Nierhaus, 1998).

The hybrid states and α-ϵ models have sometimes been considered mutually exclusive, but they may in fact be addressing different aspects of the translocation problem. As we have seen, recent structural data provide support for some aspects of both models. But so far, the data do not clearly reveal how tRNA breaks and remakes ribosomal contacts as it moves $\sim$50 Å from one site to the next.

The mechanism of mRNA translocation is even less clear. The observation that translocation can occur even in the absence of messenger RNA (Belitsina et al., 1981) suggests that tRNAs play the primary role in translocation, possibly "dragging" the mRNA with them. There is a kink between the A- and P-site codons (Ogle et al., 2001; Yusupov et al., 2001), and it is possible that this plays some role in the maintenance of the reading frame.

The Role of EF-G

Translocation is catalyzed by EF-G, a ribosome-activated GTPase. However, translation in the absence of added factors has been observed (Gavrilova et al., 1976). This implies that translocation is a property inherent to ribosome, and that EF-G may act by lowering a kinetic barrier on binding to the ribosome. Consistent with this,

it has been known that single rounds of translocation can occur even with nonhydrolyzable analogs of GTP, suggesting that GTP hydrolysis is not required for translocation, but may be required for EF-G release. However, kinetic experiments have shown that GTP hydrolysis precedes translocation, suggesting that the energy of hydrolysis is probably used to drive translocation (Rodnina et al., 1997). Translocation is much slower with nonhydrolyzable analogs than with GTP. These results are compatible with an alternative view that EF-G is a motor protein that drives translocation, but to prove this, one would have to establish that it exerted a force using the energy of GTP hydrolysis, and define the nature of the "stroke" of the motor.

Two antibiotics have been useful to dissect the action of EF-G on the ribosome. Fusidic acid allows GTP hydrolysis but prevents turnover of EF-G. It was thought originally that thiostrepton, which binds to the L11-23S RNA complex even in isolation, was an inhibitor of GTPase. However, kinetic experiments show that thiostrepton does not prevent GTP hydrolysis by EF-G (Rodnina et al., 1999). Rather, it affects subsequent steps, including release of inorganic phosphate and subsequent release of EF-G. Presumably, thiostrepton prevents a conformational change in the ribosome that is required for these subsequent steps, and acts at an earlier step than fusidic acid. In addition to conformational changes in the ribosome, changes in the conformation of EF-G also appear to be required. An EF-G molecule with engineered intramolecular disulfides was active in GTP hydrolysis but could not facilitate translocation and could not turn over (Peske et al., 2000).

The structures of EF-G with and without GDP were solved by crystallography (Aevarsson et al., 1994; Czworkowski et al., 1994). They revealed an odd shape for EF-G, with a long domain IV protruding out of the body of a more globular GTPase domain (Figure 7A). When the structure of the ternary complex of EF-Tu with tRNA and GTP was subsequently determined (Nissen et al., 1995), it became clear that the shapes of EF-G and the ternary complex were so similar that they suggested a case of "molecular mimicry." Domain IV of EF-G appeared to mimic the anticodon stem-loop of tRNA in the ternary complex both in shape and overall charge distribution. The mapping of EF-G on the ribosome by the technique of hydroxyl radical probing from tethered Fe on engineered cysteines (Wilson and Noller, 1998), as well as its direct visualization by cryoEM (Agrawal et al., 1998), supported the view that EF-G bound to the ribosome in approximately a similar way as the ternary complex observed by cryoEM (Stark et al., 1997) (Figure 7B).

Over the last two years, several EF-G complexes trapped in the pre- and posttranslocational states have been studied by cryoEM. Frank and coworkers determined the structures of the 70S with two tRNAs bound, with EF-G in the presence of a nonhydrolyzable GTP analog (representing the pretranslocational state), and that with fusidic acid (representing the posttranslocational state) (Agrawal et al., 1999a). A comparison of the two structures showed that EF-G binds in approximately the same place, but there are significant conformational changes in both EF-G and the ribosome, most notably in the structure of the L7/L12 stalk.

Wintermeyer, van Heel, and coworkers have also studied EF-G·ribosome·tRNA complexes but in the presence of thiostrepton (before and after translocation) and fusidic acid (Stark et al., 2000). The fusidic acid complex has approximately the same orientation for EF-G as in the corresponding structure from the Frank group. However, the conformation and orientation of EF-G in the presence of thiostrepton before and after translocation is markedly different, and each is different from that in the fusidic acid complex. The conformations involve a nearly 270 degree change in the orientation of domain IV of EF-G, as well as a different binding site on the ribosome before and after translocation. Such a dramatic large-scale conformational change in EF-G, as well as its migration from one position to another on the ribosome, is extraordinary. There does not appear to be enough space between the 30S and 50S subunits to accommodate such a dramatic rotation in the domain of EF-G, so the intersubunit space would have to open up. Secondly, the structures defined by the Frank group, EF-G with a nonhydrolyzable analog and one with fusidic acid, probably represent the beginning and end point of the involvement of EF-G with the ribosome. Since they show EF-G bound to the same site of the 70S in an approximately similar conformation, the finding by the Wintermeyer/van Heel group that the intermediate states represented by the thiostrepton complexes have EF-G binding to different sites on the ribosome in very different conformations is surprising. The resolution in the cryoEM reconstructions is such that the assignment of protein domains is subjective to some extent. A high priority therefore should be to reach a resolution where the secondary structure elements of individual protein domains can be distinguished, so that both the assignment and orientation of domains will be unambiguous. In passing, we also note that the definition of resolution by the Frank and van Heel groups is not the same, with the Frank group applying a more conservative criterion that results in a worse nominal value to describe the same actual resolution.

Despite these uncertainties, the cryoEM structures provide a broad framework for understanding the role of EF-G. It is clear that domain IV is inserted into the A site of the 30S, where at least in some states, it could directly displace tRNA. A simplistic view is that translocation occurs slowly even without factors, and EF-G, by binding to the A site, prevents the back reaction in addition to lowering any kinetic barriers. But we are a long way from understanding the precise role of EF-G in translocation.

Termination

The process of termination begins when a stop codon on mRNA is encountered in the A site (Figure 8). In bacteria, recognition of the stop codon involves two "class I" release factors, RF1 and RF2 (reviewed in Kisselev and Buckingham, 2000). Both factors recognize UAA; however, UAG is recognized by RF1 while UGA is recognized by RF2. In eukaryotes, a single factor, eRF1, recognizes all three stop codons. A "class II" release factor, RF3, binds to the complex of RF1/2 with the ribosome and is a GTPase.

The binding of RF1/2 to a ribosome with the appro-

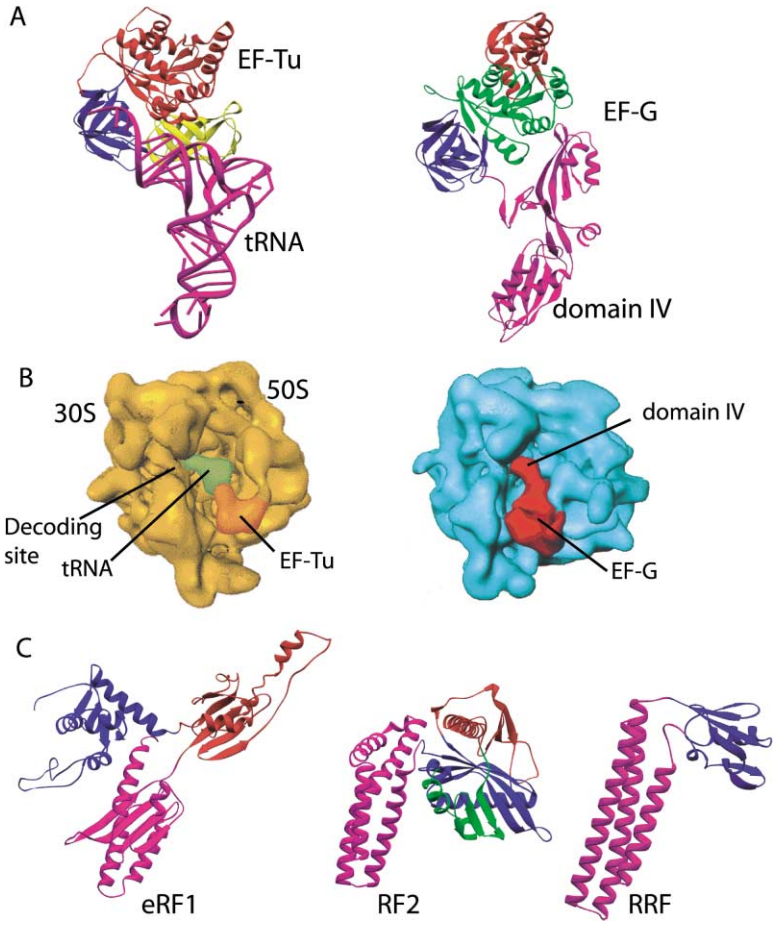

Figure 7. Mimicry among Translation Factors

(A) Crystal structures of (left) the ternary complex of EF-Tu, tRNA, and GDPNP (Nissen et al., 1995) and (right) EF-G (Aevarsson et al., 1994; Czworkowski et al., 1994).

(B) CryoEM reconstructions (left) of the ribosome with the ternary complex (Stark et al., 1997) and EF-G (Agrawal et al., 1998). Reproduced with permission.

(C) Structures of class I release factors eRF1 and RF2 and ribosome recycling factor RRF. The domains that are thought to mimic tRNA are shown in magenta, as is domain IV of EF-G in (A).

priate stop codon in the A site triggers the hydrolysis and release of the peptide chain from tRNA in the P site. It is not clear whether RF1/2 participates directly in catalysis or whether it induces catalysis by the ribosome. RF3 promotes rapid dissociation of RF1 and RF2. Originally, it was thought that the binding of RF3 to the ribosome triggered its GTPase activity with concomitant release of RF1/2. However, more recent work shows that the hydrolysis of peptidyl tRNA by RF1/2 is required for binding GTP to RF3 on the ribosome. This in turn leads to a conformation of RF3 with high affinity for ribosomes and the dissociation of RF1/2 (Zavialov et al., 2001). The hydrolysis of GTP is required for subsequent dissociation of RF3. Surprisingly, RF3 is not essential in bacteria, and does not bind RF1/2 outside the ribosome (Kisselev and Buckingham, 2000).

Genetic experiments show that swapping a conserved tripeptide between RF1 and RF2 can also swap their stop codon specificities (Ito et al., 2000), suggesting that the tripeptide mimics an anticodon in tRNA; this tripeptide was thought to lie at the tip of an anticodon domain of RF1/2.

The emergence of crystal structures of release factors (Figure 7C) suggests that the picture is not quite as straightforward. A structure of human eRF1 did indeed suggest that the protein could mimic tRNA (Song et al., 2000). It could be modeled so that a highly conserved GGQ motif would be close to the CCA end of P-site tRNA, consistent with the requirement of the motif for peptide release activity in class I release factors (Frolova et al., 1999; Song et al., 2000). But it is not clear from the structure how elements of the protein would decode the stop codon. The structure of RF2 from *E. coli* has recently been determined (Vestergaard et al., 2001). It has a completely different structure from eRF1, consistent with the lack of sequence homology between the two, but bears an even more striking resemblance to tRNA. However, the tripeptide that switches specificity (Ito et al., 2000) is quite far from the tip that appears to mimic the anticodon stem-loop, suggesting that it cannot play the role of an anticodon directly. It is also not clear that the structure can be modeled into a 70S ribosome in a manner compatible with contacts inferred from hydroxyl-radical cleavage data (Wilson et al., 2000). Thus, the crucial questions of the catalytic mechanism of peptide release as well as stop codon recognition remain unanswered.

Ribosome Recycling Factor

After release of the peptide chain, the ribosome is left with mRNA and a deacylated tRNA in the P site (Figure 8). This complex needs to be disassembled to prepare the ribosome for a new round of protein synthesis. Another factor called ribosome recycling factor (RRF) along with EF-G is required for this process (reviewed in Janosi et al., 1996). A recent study shows that RRF and EF-G lead to the dissociation of ribosomes into subunits on

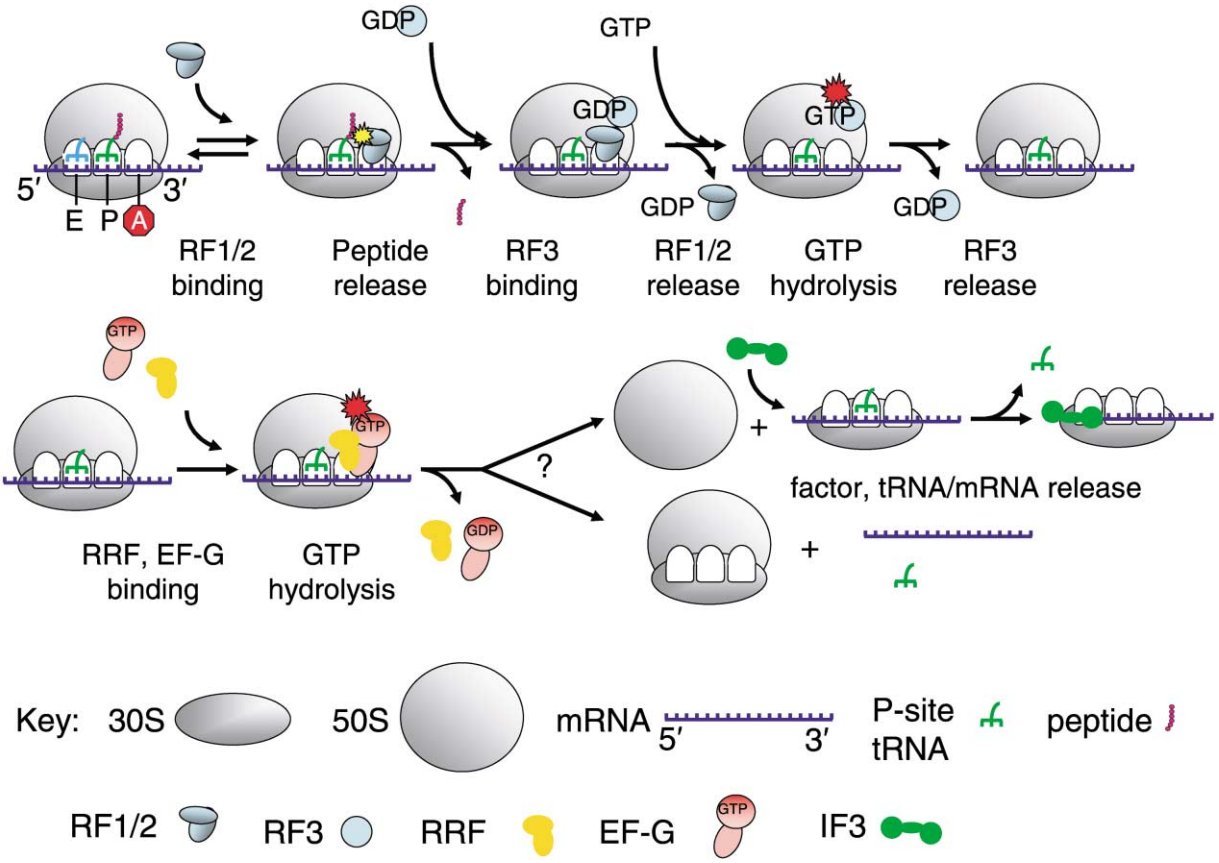

Figure 8. Overview of Termination in Translation

GTP hydrolysis (Karimi et al., 1999). Subsequently, initiation factor IF3 is required for removal of the deacylated tRNA from the 30S subunit. However, recently Kaji and coworkers have shown that hydrolysis of GTP in the ribosome-RRF-EF-G complex leads to a dissociation of the intact 70S ribosome from mRNA and tRNA (Kaji et al., 2001). Given the path of the message through the 70S ribosome (Yusupova et al., 2001), this would require a large-scale conformational change that breaks a significant number of intersubunit contacts to be topologically feasible. As has been suggested by Kaji et al., it is possible that whether subunit dissociation or mRNA release occurs first may depend on the affinity of the particular mRNA sequence for the ribosome.

The structure of RRF (Selmer et al., 1999) is the closest in shape and charge distribution to tRNA of any of the factors determined so far, supporting suggestions that it mimics tRNA in the A site. Presumably, it is required for EF-G function in much the same way that at least the anticodon stem loop of tRNA is required in the A site for translocation by EF-G (Joseph and Noller, 1998).

Conclusions

The crystal structures of the ribosome have already revolutionized the field of protein synthesis by providing a three-dimensional reference for interpreting existing biochemical and genetic data and designing new experiments, but nagging questions remain at each step. When the problem is sufficiently localized, such as peptidyl transferase or the recognition of codon-anticodon base pairing, there has been a dramatic change in our understanding. But other steps such as the triggering of GTP hydrolysis by factors, or translocation, remain mysterious. The hope is that progress in these areas will now be accelerated. Nevertheless, we are only beginning to understand the fundamental mechanisms of the bacterial translation pathway. Moreover, in this review, we have not even touched on some of the more exotic phenomena in translation, such as programmed frameshifting, hopping, or incorporation of selenocysteine. For proteins which have a signal sequence, there is the added complication of interactions with the signal recognition particle and the translocon, which are involved in moving proteins into or through membranes. Understanding eukaryotic translation will pose major additional challenges.

Future work will necessarily involve multiple approaches. The structures of 70S complexes representing clearly defined points on the translation pathway will go a long way to clarifying the nature of the structural changes involved as the ribosome goes from one step to the next. Initially, these will almost certainly come from cryoEM, but as the questions become more detailed and mechanistic, there is unfortunately no substitute for high-resolution crystallography, a technique that is hostage to obtaining suitable crystals. It has often been asserted that we need the “movie” for translation

rather than the few "frames" we have. However, there is no camera fast enough to create this movie. Even detailed structures of the ribosome in two defined states will only hint at how it goes from one state to the next. Understanding the process will require a broad attack, including genetic and biochemical analysis, e.g., combining mutational data with structural and kinetic analysis. Emerging single molecule techniques involving detection of conformational changes through fluorescence or direct measurement of forces are also likely to play an important role. Given the central role of translation in biology, it seems a worthwhile cause.

Acknowledgments

Due to limitations on space and the number of references, I apologize for not citing many important original contributions. I thank D.E. Brodersen for generously making the figures used here, and my LMB colleagues and many members of the ribosome community for useful discussions and criticism.

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