Ribosome structure: revisiting the connection between translational accuracy and unconventional decoding

Guillaume Stahl, Gregory P. McCarty and Philip J. Farabaugh

The ribosome is a molecular machine that converts genetic information in the form of RNA, into protein. Recent structural studies reveal a complex set of interactions between the ribosome and its ligands, mRNA and tRNA, that indicate ways in which the ribosome could avoid costly translational errors. Ribosomes must decode each successive codon accurately, and structural data provide a clear indication of how ribosomes limit recruitment of the wrong tRNA (sense errors). In a triplet-based genetic code there are three potential forward reading frames, only one of which encodes the correct protein. Errors in which the ribosome reads a codon out of the normal reading frame (frameshift errors) occur less frequently than sense errors, although it is not clear from structural data how these errors are avoided. Some mRNA sequences, termed programmed-frameshift sites, cause the ribosome to change reading frame. Based on recent work on these sites, this article proposes that the ribosome uses the structure of the codon–anticodon complex formed by the peptidyl-tRNA, especially its wobble interaction, to constrain the incoming aminoacyl-tRNA to the correct reading frame.

Guillaume Stahl
Gregory P. McCarty
Philip J. Farabaugh*
Dept of Biological Sciences and Program in Molecular and Cell Biology, University of Maryland, Baltimore County, Baltimore, MD 21250, USA.
*e-mail: farabaug@umbc.edu

Arguably the most important feature of protein synthesis is the ability to maintain the correct reading frame. When reading an mRNA, a ribosome must correctly interpret each successive tri-nucleotide codon as a particular amino acid. The ribosome must also decode only adjacent, nonoverlapping codons – those lying in a single reading frame. However, mRNA lacks punctuation, internal signals that identify which nucleotide triplets constitute codons (first noted by Crick *et al.* [1]). Therefore, when a ribosome loses track of the correct reading frame it has no way to re-establish this. Although ribosomes do make frameshift errors, these occur at a very low rate, probably much less than 5×10^{-5} per codon, or at least an order of magnitude less frequently than ribosomes incorporate an incorrect amino acid (termed sense errors) [2].

Although we lack an explicit, accepted model for frame maintenance, we do have a more complete understanding about the correction of sense errors. The error-correction machinery distinguishes between correct (cognate) and incorrect (noncognate) aminoacyl-tRNAs (aa-tRNAs) by the structures they form in the decoding sites. Ribosomes increase the accuracy of tRNA recruitment and recognition by a process called kinetic proofreading [3–5]. To amplify discrimination, the process of tRNA selection is divided into two steps, one before and one after GTP hydrolysis, by elongation factor Tu (EF-Tu), which deposits aa-tRNA onto the ribosome. During each step, noncognate tRNA is much more likely to dissociate from the ribosome than is cognate tRNA. Moreover, recent observations show that when bound to the ribosome, cognate complexes formed between aa-tRNA and EF-Tu manipulate the ribosome and improve discrimination [6–8].

The past two years have witnessed an incredible burst of information about the structure of the ribosome and its interactions with ligands. For our purposes, the precise nature of the interaction between the mRNA, tRNAs and the 30S ribosome are most exciting [9–11]. The data give a glimpse of the workings of this amazing molecular machine; in particular, a clearer picture of the nature of the error-correction process. Ribosomes have three tRNA-binding sites, termed aminoacyl (A), peptidyl (P) and exit (E) sites. During translation, aa-tRNAs enter the ribosome and bind to a codon in the A site. After accepting transfer of the growing peptide from the preceding tRNA, they translocate to the P site, donate the peptide to the succeeding tRNA and move to the E site before dissociating from the ribosome. The newly available structures confirm that tRNA base-pairs with the mRNA in the A- and P sites, and show that nucleotides and amino acids in the ribosome directly contact the codon–anticodon complex in each site (Fig. 1).

A description of the interactions in the A site comes from Ogle *et al.* [9], who solved a structure of the 30S subunit complexed with models of the mRNA and A-site tRNA to <3.3 Å. The structure reveals a complex set of direct and indirect interactions between each of the three pairs of bases in the A site and residues of both the 16S rRNA and ribosomal protein S12 (rpS12) (Fig. 1). The contacts between the A site and the first and second base pairs (36–4 and 35–5 in Fig. 1) effectively measure the distance between the phosphoribose backbones by bridging the 2′ OH groups of each ribose. This precludes formation of a non-Watson–Crick pair. The third base pair, or wobble position, does not form a bridging set of contacts. Instead, the ribose 2′ OH of the codon nucleotide makes two contacts, a hydrogen bond and a metal-mediated interaction. A packing interaction between C1054 and the ribose of the anticodon further stabilizes the wobble pair without constraining its geometry as strongly as the geometries of other base pairs are constrained. As a result, a wider variety of interactions are possible,

http://tibs.trends.com 0968-0004/02/$ – see front matter PII: S0968-0004(02)02064-9

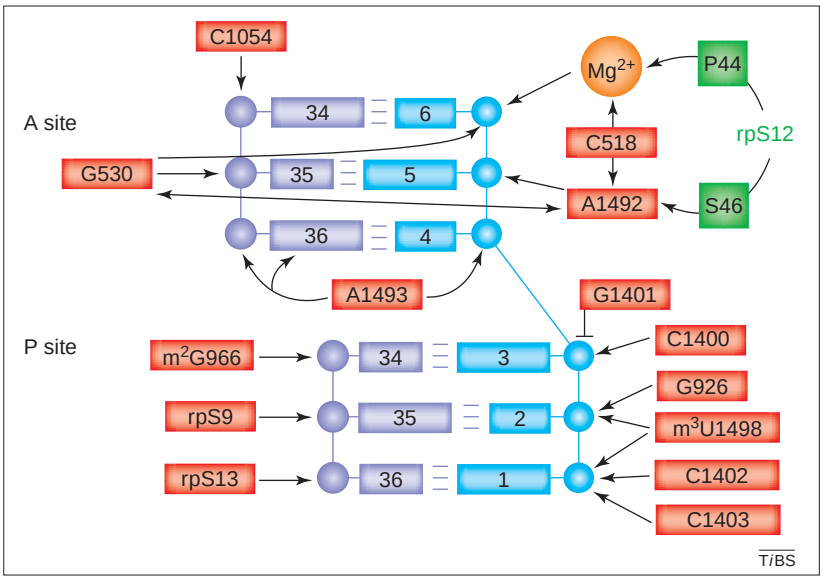

Fig. 1. Interactions between the ribosome and mRNA–tRNA complexes. The nucleotides of the rRNA (red), mRNA (blue) and tRNA (purple) are numbered to represent their position in the RNA chain; the mRNA is numbered 5′–3′ starting at the beginning of the A-site codon. A Mg^{2+} ion (orange) mediates an interaction between the mRNA, C518 of the 16S rRNA and Pro44 of rpS12 (amino acids represented as green squares). Filled circles represent the phosphoribose backbone. Arrows indicate direct contacts without distinguishing between types of interactions. G1401 is shown as blocking further extension of the P-site codon helix.

consistent with the expanded pairing observed at the wobble position.

The nature of the interactions at the P site can be deduced from structures, solved to either 5.5 Å or 7 Å resolution, of the 70S ribosome complexed with a tRNA bound to the P site [10,11], and from a structure, solved to 2.3 Å, of the 30S subunit containing a tRNA-mimic helix in the P site [12]. These structures show evidence of a similar nexus of interactions. Whereas the A-site complex is relatively accessible to solvent on the inner surface of the ribosome, the P-site complex is buried in a cleft created by rRNA and ribosomal proteins. An important feature of the structure is the presence of G1401 at a position that blocks further extension of the P-site codon helix and forces the mRNA to adopt a kinked conformation. Because of this kink, the two tRNA anticodon loops in the A- and P sites come no nearer than 10 Å. Therefore, G1401 effectively defines the end of the codon in the P site and, by forcing the kink, the beginning of the codon in the A site (Fig. 2).

These results explain the role of some of the residues that have been shown genetically to be required to maintain accuracy [13,14]. Some of the essential residues of the 30S subunit directly contact the codon–anticodon helices in the A- and P sites: C1054 and A1493 of 16S rRNA in the A site [9], and C1400 in the P site [10,12]. All other residues implicated in accuracy are located close to, but do not touch, the decoding sites. These include four residues in helix 18, which also contains residues C518 and G530 of the decoding site; seven residues in helix 27, which lies beneath the P site and undergoes a conformational change that regulates the accuracy of the A site [15]; five residues in helix 34, which also includes C1054; six nucleotides in helix 44, a structure that includes elements of both the A- and P sites [16,17]; and multiple residues of rpS12 that are near amino acids Pro44 and Ser46, both of which are elements of the A site. Mutations in the 23S rRNA of the 50S ribosome can also affect accuracy. Some of the required residues lie in helix 69 of the 23S rRNA, an area that directly contacts the 30S subunit at helix 44, which is adjacent to the decoding sites. All these mutational changes might affect accuracy by either stabilizing or destabilizing contacts between the ribosome and the codon–anticodon complexes, either directly or indirectly. Altering the stability of these contacts would affect the efficiency of discrimination during kinetic proofreading: increasing stability would tend to retain noncognate tRNAs in the A site, thus decreasing accuracy, whereas decreasing stability would increase accuracy. A complete list of rRNA mutations, including those that affect accuracy, is available at the website of the Ribosomal Mutation Database Project (http://ribosome.fandm.edu).

How does the ribosome maintain translational frame?

Although interactions between the ribosome and codon–anticodon helix of the A site indicate a model for tRNA discrimination, they do not explain how the reading frame is maintained. One approach to explaining this is to study special circumstances in which the frequency of frameshift errors can be increased by up to 50%. Frame-maintenance can be reduced either by mutations in rRNA or by changes to various other components of the translational apparatus, such as EF-Tu, tRNAs and mRNA sequences. Understanding how the incidence of frameshift can be increased might provide insights into how it is prevented normally.

Theoretically, there are four ways to disrupt reading-frame maintenance:

- Translation assumes that each tRNA recognizes exactly three nucleotides. Occasional expansion or contraction of the codon size could shift the reading frame in the +1 (forward) or –1 (backward) direction, respectively.
- Although recognizing a three-nucleotide codon, the incoming aa-tRNA could bind to three nucleotides that are not in the normal frame.
- After a tRNA pairs with the mRNA, the ribosome must translocate exactly three nucleotides to display the succeeding codon in the A site; translocation of either four or two nucleotides would cause +1 or –1 frameshifting.
- After translocation and before recognition of the next tRNA, only the peptidyl-tRNA (pep-tRNA) is base-paired to the mRNA; at this stage, any slippage of the tRNA on the mRNA could cause a frameshift in either the forward or backward direction.

The extremely low frequency of frameshift errors indicates that errors in the size of the repeating three-nucleotide step are rare. However, how this step size is

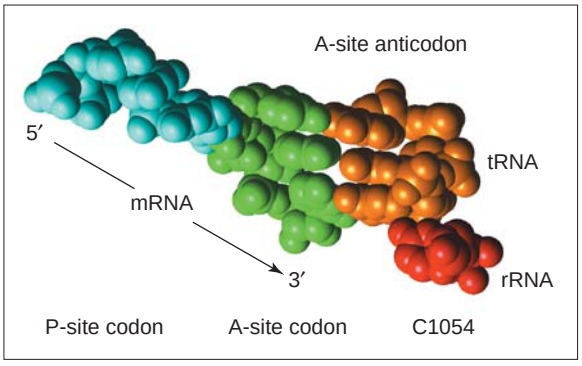

Fig. 2. The structure of the decoding center imposes a three-nucleotide codon. The P-site (pale blue) and A-site (green) codons are connected through a kink in the phosphoribose backbone. The kink constrains the position of the first nucleotide of the A-site codon (top). The anticodon of tRNA entering the A site (orange) must begin pairing with that first base. Nucleotide C1054 (red) of the 16S rRNA inserts immediately below the third, or wobble, nucleotide of the tRNA. This blocks formation of a fourth base-pair. The combination of the kink and C1054 imposes a maximum size of three base-pairs on the codon–anticodon complex. The figure was created in RasMol [33] using coordinates downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/); the identification for the PDB coordinate file is 1IBM.

maintained remains controversial. In one model it is suggested that the size of tRNA anticodons defines the step size by defining both codon length and translocation distance. This view is derived from studies of mutant tRNAs in which expansion of the anticodon to four nucleotides causes a +1 frameshift. In the first characterized example of this, the suppressor *sufD42* alters a $tRNA^{Gly}$ by expanding its anticodon from CCC to CCCC. This allows $tRNA^{Gly}$ to suppress frameshift mutations in structural genes that result from the expansion of GGN Gly codons to GGGN (where N is any nucleotide) [18]. The structures indicate a simple, elegant model, termed the quadruplet translocation model [19]. This proposes that by expanding the anticodon to four nucleotides, the tRNA could base-pair to a four-nucleotide codon in the mRNA and that, during translocation, the tRNA would move four nucleotides into the P site, thus displaying a +1 shifted codon in the A site [19]. It is important to remember that this model is hypothetical and that, for example, there is no solved structure of a frameshift-suppressor tRNA paired with an mRNA.

The quadruplet translocation model assumes that the ribosome has no direct role in defining step size. However, the recent structure of an aa-tRNA–mRNA–30S ribosome complex seems to contradict this assumption [9]. The decoding site explicitly contacts each of the three base-pairs that form in the A- and P sites [9,10], which indicates that the structure of the ribosome implicitly defines the codon. In fact, the ribosome appears to constrain the mRNA so that only three nucleotides of the tRNA can interact with the A-site codon (Fig. 2). A 45° kink in the mRNA on the 5′ side of the A-site codon defines the first nucleotide in the A site. At the other end of the interaction, the ribosome places nucleotide C1054 immediately below the wobble nucleotide of the tRNA. The distance between the kink and C1054 is sufficient to allow codon–anticodon interaction between three pairs of bases, but not four. The model was further called into question by results showing that the nucleotide at the 3′ end of the putative four-nucleotide anticodon is modified to 1-methylguanosine in a major class of frameshift suppressor tRNAs, $tRNA^{Pro}$ suppressors, in *Salmonella typhimurium* [20]. Because methylation blocks base pairing to cytosine, these tRNAs are incapable of a four base-pair interaction with mRNA. Importantly, in the quadruplet translocation model, the modified nucleotide would recognize the first nucleotide in the expanded anticodon. By blocking base pairing, this modification should render the tRNA incapable of distinguishing suppressible sites (e.g. CCCC) from nonsuppressible ones (ACCC, GCCC or UCCC). The fact that the tRNA can distinguish these sites demonstrates that the quadruplet translocation model is invalid for these suppressors and requires that they cause frameshifts by another mechanism, presumably involving triplet recognition. Together with the recent structural data, these results greatly weaken, if not invalidate, the concept that translational step-size is defined explicitly by the tRNA anticodon.

Programmed +1 frameshifts in yeast depend on unconventional P-site decoding

Although the abnormal structure of an expanded anticodon loop appears to prevent the ribosome reading an abnormal four-nucleotide codon, it does cause the ribosome to make frameshift errors. An explanation of this comes from what was thought initially to be an unrelated phenomenon, programmed frameshifting. This is a ubiquitous, although rare, event in which ribosomes are forced to shift reading frame at special sites in mRNAs. The process is stochastic; programmed-frameshift sites increase the probability of a frameshift occurring from the normally low level of random errors ($<5\times10^{-5}$ per codon) to as much as 50%.

According to the current view, programmed frameshifts occur at mRNA sequences that pause the ribosome with the A- and P sites located over special frameshift-stimulating signals. The mechanism of frame disruption varies between sites. In the most common form, termed –1 simultaneous-slippage frameshifting [21], the frameshift signal is a heptanucleotide of the form X-XXY-YYZ, grouped in codons of the upstream normal frame, where XXX is a triplet of any repeating nucleotide, YYY is a triplet repeat of either A or U, and Z varies between species. For example, the heptanucleotide in the Coronavirus Infectious Bronchitis Virus is U-UUA-AAC [22]. In most cases, a downstream secondary structure (a pseudoknot and a hairpin loop in eukaryotes and prokaryotes, respectively) causes the pause over this sequence (reviewed in Ref. [23]). Despite the number and phylogenetic ubiquity of such sites, we do not yet understand how these signals stimulate frameshift errors.

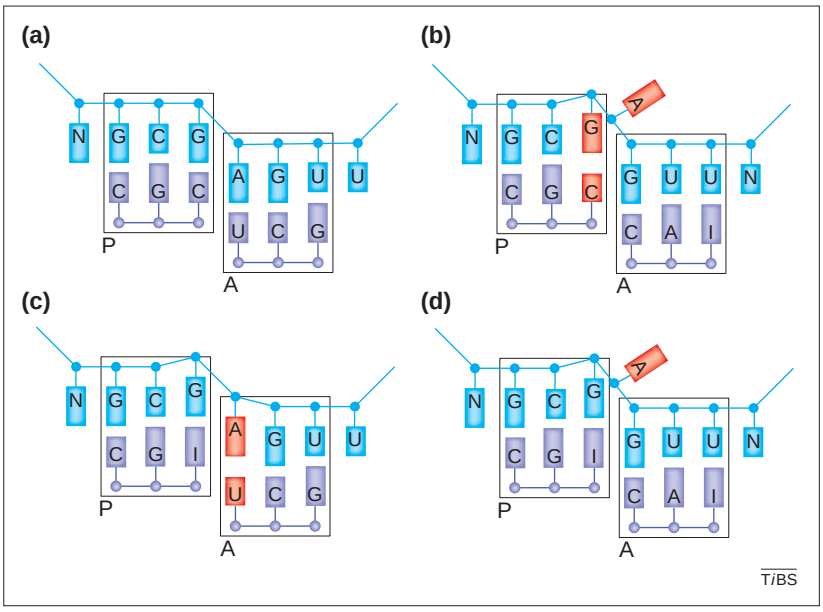

Fig. 3. Unconventional P-site wobble pairs increase the probability of out-of-frame decoding; the possible effect of base mismatches on frame maintenance. The eight nucleotides accessible on the inner surface of the ribosome at the decoding center are shown interacting with P- and A-site tRNAs. Rectangles represent nucleotides; circles represent the phosphoribose backbone; mRNA is shown in blue; tRNAs in purple; A and P represent the two decoding sites; nucleotides that cannot form normal interactions with the ribosome, and that are expected to have an energetic cost, are red. (a) Normal Watson–Crick pairing in both A- and P sites. (b) Out-of-frame binding in the A site. This requires bypassing one nucleotide between the A- and P sites, which could disrupt normal ribosomal interactions with the P-site wobble pair. The cartoon does not indicate a specific structure for the complex. (c) A clash caused by pairing of G–I nucleotides at the wobble position in the P site would, at the minimum, disrupt interaction of an in-frame tRNA at the first nucleotide in the A site, possibly reducing the efficiency of translation. (d) An unconventional P-site wobble pair would reduce the energetic cost of out-of-frame binding relative to in-frame binding.

Recent work on a far less common form of programmed frameshifting provides mechanistic explanations of this process. Programmed +1 frameshifting in *Saccharomyces cerevisiae*, first identified in the Ty family of retrotransposons, also occurs at heptameric sequences, although of a different structure to those discussed above (reviewed in Ref. [23]). The sites consist of two codons of the normal frame plus a seventh base at the 3′ end. The second codon is recognized very slowly in the ribosomal A site because of the low availability of its cognate tRNA. This pauses the ribosome with a pep-tRNA bound to the first codon in the ribosomal P site. Recent evidence indicates that 'unconventional decoding' by this pep-tRNA can directly stimulate frameshift errors by out-of-frame binding of an incoming aa-tRNA [24]. Because of the redundancy of the genetic code, during translation each amino acid can be decoded by one of several tRNAs, termed isoacceptors. Conventional decoding uses an isoacceptor that can fully pair with the codon. For example, although the codons GCG and GCU both encode alanine; the former is read by a cognate tRNA with the anticodon CGC, whereas an isoacceptor with an IGC anticodon decodes the latter (both codons and anticodons are represented in 5′–3′ polarity). The term unconventional decoding refers to reading by tRNAs that have a less than optimal interaction with the mRNA. Reading of GCG by the IGC-tRNA would be an unconventional decoding event because a G–I wobble interaction does not allow a normal Watson–Crick base-pair. In fact, the juxtaposition of the two purines at the wobble position would introduce a clash that destabilizes RNA–RNA helices.

Although conventional, cognate decoding should predominate, the lack of some cognate tRNAs in *S. cerevisiae* allows unconventional decoding by more abundant isoacceptors. Because their structural genes have been deleted, some cognate tRNAs are either present in low concentration or are absent [25]. This unconventional decoding can result in weak pyrimidine–pyrimidine pairs or purine–purine clashes in the wobble position. The presence of such unusual P-site wobble pairs causes frequent, erroneous out-of-frame decoding in the A site and results in frameshifts [24]. Because out-of-frame decoding competes with normal, in-frame decoding [26,27], the occurrence of frameshifts is enhanced by the slow recognition of the next in-frame codon.

An rRNA clamp in the P site might constrain the reading frame in the A site

How might an unconventional wobble pair stimulate frameshift errors? An interaction between the P- and A-site tRNAs had been postulated to stabilize reading of adjacent codons [28]. However, this model is now thought to be unlikely because the kink between the A- and P sites allows the two tRNAs to come no closer than 10 Å [9,10], which makes direct contact difficult and an indirect effect probable. Although it is possible that the effect could propagate through ribosomal components, we propose a simple model in which it propagates through the mRNA (Fig. 3). The kink is the key to this model and it is compatible with simultaneously forming normal cognate pairs in the A- and P sites (Fig. 3a).

In this model, out-of-frame recognition in the A site requires the +1 frame codon to move into the A site, thereby displacing the nucleotide to be skipped (Fig. 3b). This disruption might be incompatible with the normal structure of the wobble pair in the P site (shown in Fig. 3 as forcing breakage of the pair although, alternatively, this might disrupt ribosomal contacts with the codon–anticodon pair). The high-energy cost required to form this structure would reduce the efficiency of out-of-frame recognition. By the same argument, because of the strain of the kink between the two sites, a non-canonical wobble-pair interaction in the P site might disturb formation of a normal codon–anticodon interaction in the A site (Fig. 3c) and reduce the efficiency of in-frame decoding. Such an effect on nonsense suppression has been observed [29,30]. In this case, out-of-frame recognition still requires displacement of the skipped nucleotide (Fig. 3d). However, if the abnormal structure of the P-site wobble pair itself forces disruption of ribosomal interactions, it would eliminate the extra energy cost

relative to in-frame decoding because both in-frame and out-of-frame aa-tRNAs would be precluded from forming their maximum-energy structure. This would reduce the energetic advantage of in-frame decoding and so should indirectly increase the probability of out-of-frame binding.

Experiments using drugs that induce errors demonstrate that minor disruptions to the structure of the ribosome–mRNA–tRNA complex can have profound effects on translational fidelity. Nucleotides A1492 and A1493 of the 16S rRNA undergo an energetically costly rearrangement when cognate tRNA enters the A site, which allows them to interact with the codon–anticodon complex. Paromomycin stabilizes this conformation in the absence of tRNA and so reduces the energy difference between cognate and noncognate tRNA binding; by paying the cost of the rearrangement, paromomycin promotes both sense and frameshift errors [9]. We argue for an opposite effect in which an aberrant wobble-pair interaction in the P site reduces the energetic advantage of in-frame decoding, which indirectly increases the probability of out-of-frame binding. In this model, formation of the correct wobble-pair interaction in the P site is crucial to continued in-frame decoding. This is consistent with the effect of unusual wobble interactions in programmed frameshifting [24], and with the fact that wobble in the P site is constrained by a pincer interaction by two rRNA nucleotides: C1400 and m^2G966 [10]. It is probably significant that of the nucleotides that contact the codon–anticodon complex within the P site, only mutation of C1400 increases the occurrence of frameshifts and other errors [31]. Similarity between the effects of a C1400 mutation and an unusual wobble pair implies that they might increase the probability of out-of-frame recognition in the A site in similar ways. In-frame recognition in the A site could be ensured by holding the P-site wobble-pair tightly between C1400 and m^2G966. Although we lack details of the way these two residues interact with the codon–anticodon complex in the P site, their inability to interact with abnormal, non-Watson–Crick base-pairs could explain why an unusual wobble pair disrupts normal, in-frame decoding.

A possible explanation of the ability of tRNAs with expanded anticodon loops to stimulate frameshifting might be that they, too, interfere with the ability of the P site to restrict recognition of in-frame codons at the A site. Recent data also show that frameshifts induced by +1 suppressor tRNAs occur in competition with normal in-frame decoding of the succeeding codon [20], as occurs in programmed +1 frameshifting. An additional nucleotide in the anticodon loop might disrupt the correct interaction of the P-site rRNA with the codon–anticodon complex, which could increase the probability of out-of-frame recognition despite the fact that the suppressor continues to make three base-pairs with the mRNA. This is plausible because we know that the additional nucleotide in the *sufD42* suppressor form of $tRNA^{Pro}$ slows recognition in the A site, and allows another isoacceptor to read its cognate codon [20]. This misreading does not happen with wild-type tRNA.

Genetic tools for dissecting the translational accuracy system?

The genetic dissection of programmed +1 frameshifting appears to have revealed an important feature of ribosome-mediated error correction. Direct biochemical tests are now necessary to determine whether the inferences drawn from the genetics are correct; that is, that maintaining the reading frame depends crucially on the stability of the pep-tRNA wobble-pair binding in the P site. Of the different forms of programmed frameshifting, it is easiest to hypothesize that programmed +1 frameshifting in yeast results from direct disruption of the translational-accuracy mechanism. For example, an mRNA sequence found at one programmed +1 frameshift site in yeast appears to stimulate errors by base pairing with, and inactivating, helix 18 of the small ribosomal subunit rRNA, which is an essential element of the ribosomal-accuracy mechanism [6,32]. It remains to be seen whether other forms of programmed, unconventional decoding also disrupt error correction by the ribosome. Given the diversity of these decoding events, the ways in which they could undermine accuracy could also be diverse. The diversity of these programmed events is consistent with the results of the structural studies, which show that the ribosome uses an elaborate system to maintain translational accuracy. Therefore, by studying unconventional decoding systems we can dissect the mechanisms of translational accuracy, a process that is crucial to the cell. Recoding systems should continue to provide tools to dissect this still incompletely understood system.

Acknowledgements
This work was supported by grants from the National Institute of General Medical Sciences (GM29480), the Fogarty International Center of the National Institutes of Health (TW02211) and the Swedish Cancer Foundation (3717–B95–01VAA).

References

1 Crick, F. *et al.* (1961) General nature of the genetic code for proteins. *Nature* 192, 1227–1232
2 Kurland, C.G. (1992) Translational accuracy and the fitness of bacteria. *Annu. Rev. Genet.* 26, 29–50
3 Hopfield, J. (1974) Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. *Proc. Natl. Acad. Sci. U. S. A.* 71, 4135–4139
4 Ninio, J. (1975) Kinetic amplification of enzyme discrimination. *Biochimie* 57, 587–595
5 Thompson, R. (1988) EFTu provides an internal kinetic standard for translational accuracy. *Trends Biochem. Sci.* 13, 91–93
6 Powers, T. and Noller, H.F. (1994) The 530 loop of 16S rRNA: a signal to EF-Tu? *Trends Genet.* 10, 27–31
7 Pape, T. *et al.* (1999) Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *EMBO J.* 18, 3800–3807
8 Pape, T. *et al.* (2000) Conformational switch in the decoding region of 16S rRNA during aminoacyl-tRNA selection on the ribosome. *Nat. Struct. Biol.* 7, 104–107
9 Ogle, J. *et al.* (2001) Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* 292, 897–902
10 Yusupov, M.M. *et al.* (2001) Crystal structure of the ribosome at 5.5 Å resolution. *Science* 292, 883–896
11 Yusupova, G. *et al.* (2001) The path of messenger RNA through the ribosome. *Cell* 106, 233–241

12 Carter, A.P. *et al.* (2000) Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* 407, 340–348
13 O'Connor, M. *et al.* (1995) Genetic probes of ribosomal RNA function. *Biochem. Cell Biol.* 73, 859–868
14 Kurland, C. *et al.* (1996) Limitations of translational accuracy. In *Escherichia coli and Salmonella: Cellular and Molecular Biology* (Vol. 1) (Neidhardt, F.C. *et al.*, eds), pp. 979–1004, ASM Press
15 Lodmell, J.S. and Dahlberg, A.E. (1997) A conformational switch in *Escherichia coli* 16S ribosomal RNA during decoding of messenger RNA. *Science* 277, 1262–1267
16 Schluenzen, F. *et al.* (2000) Stucture of functionally activated small ribosomal subunit at 3.3 Å resolution. *Cell* 102, 615–623
17 Wimberly, B.T. *et al.* (2000) Structure of the 30S ribosomal subunit. *Nature* 407, 327–339
18 Riddle, D. and Carbon, J. (1973) Frameshift suppression: a nucleotide addition in the anticodon of a glycine tRNA. *Nat. New Biol.* 242, 230–234
19 Roth, J.R. (1981) Frameshift suppression. *Cell* 24, 601–602
20 Qian, Q. *et al.* (1998) A new model for phenotypic suppression of frameshift mutations by mutant tRNAs. *Mol. Cell* 1, 471–482
21 Jacks, T. *et al.* (1988) Signals for ribosomal frameshifting in the Rous Sarcoma virus *gag–pol* region. *Cell* 55, 447–458
22 Brierley, I. *et al.* (1989) Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. *Cell* 57, 537–547
23 Farabaugh, P.J. (1996) Programmed translational frameshifting. *Microbiol. Rev.* 60, 103–134
24 Sundararajan, A. *et al.* (1999) Near-cognate peptidyl-tRNAs promote +1 programmed translational frameshifting in yeast. *Mol. Cell* 4, 1005–1015
25 Percudani, R. *et al.* (1997) Transfer RNA gene redundancy and translational selection in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 268, 322–330
26 Belcourt, M.F. and Farabaugh, P.J. (1990) Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. *Cell* 62, 339–352
27 Farabaugh, P.J. *et al.* (1993) A novel programmed frameshift expresses the *POL3* gene of retrotransposon Ty3 of yeast: frameshifting without tRNA slippage. *Cell* 74, 93–103
28 Smith, D. and Yarus, M. (1989) tRNA–tRNA interactions within cellular ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 86, 4397–4401
29 Bergemann, K. and Nierhaus, K.H. (1984) Codon–anticodon interaction at the ribosomal P site improves the accuracy of the decoding process. *Biochem. Int.* 8, 121–126
30 Curran, J.F. (1995) Decoding with the A:I wobble pair is inefficient. *Nucleic Acids Res.* 23, 683–688
31 O'Connor, M. *et al.* (1997) Decoding fidelity at the ribosomal A and P sites: influence of mutations in three different regions of the decoding domain in 16S rRNA. *Nucleic Acids Res.* 25, 1185–1193
32 Li, Z. *et al.* (2001) Programmed +1 frameshifting stimulated by complementarity between a downstream mRNA sequence and an error-correcting region of rRNA. *RNA* 7, 275–284
33 Sayle, R.A. and Milner-White, E.J. (1995) RASMOL: biomolecular graphics for all. *Trends Biochem. Sci.* 20, 374

New insights into the mechanism of virus-induced membrane fusion

Sergio G. Peisajovich and Yechiel Shai

Infection by enveloped viruses requires fusion between the viral and cellular membranes, a process mediated by specific viral envelope glycoproteins. Information from studies with whole viruses, as well as protein dissection, has suggested that the fusion glycoprotein (F) from Paramyxoviridae, a family that includes major human pathogens, has two hydrophobic segments, termed fusion peptides. These peptides are directly responsible for the membrane fusion event. The recently determined three-dimensional structure of the pre-fusion conformation of the F protein supported these predictions and enabled the formulation of: (1) a detailed model for the initial interaction between F and the target membrane, (2) a new model for Paramyxovirus-induced membrane fusion that can be extended to other viral families, and (3) a novel strategy for developing better inhibitors of paramyxovirus infection.

The transfer of an enveloped virus genome into the interior of its target cell requires fusion between the viral and endosomal or plasma membranes. The energy barriers associated with this process are overcome with the aid of specific viral envelope proteins [1]. Most viral fusion proteins are integral membrane proteins that form higher-order oligomers [2]. In Paramyxoviridae, a family that includes major human pathogens [3], these proteins are trimers synthesized as inactive precursors that must be cleaved by host proteases for activity. The resulting activated fusion protein comprises an N-terminal and a C-terminal subunit. The latter is anchored to the viral membrane by a transmembrane segment proximal to its C terminus, whereas the N terminus of the C-terminal subunit hosts a region of ~25–35, mainly apolar, residues, termed the fusion peptide. This peptide is thought to play a crucial role during the fusion process [4] (Fig. 1a). After protease activation, but before attachment of the virus to its target cell, fusion proteins are thought to be in a metastable state, also called a pre-fusion conformation. Stimuli derived from the host cell then trigger a cascade of conformational changes that includes the insertion of the N-terminal fusion peptide into the target cell membrane, ultimately leading to the merging of the cell and viral bilayers. Based on the X-ray-determined three-dimensional structure of fragments from several viral fusion proteins [5], it has been postulated that, at the end of the process, some regions of these proteins adopt a stable post-fusion conformation in which a heptad repeat segment, located downstream from the N-terminal fusion peptide, forms a trimeric central coiled coil (Fig. 1a,b). Packed against the grooves of the coiled coil are three helices that are made up of heptad repeat regions located close to the transmembrane domain. The antiparallel nature of this trimeric helical hairpin suggests that, at the end of the fusion process, both the N-terminal fusion peptide and the transmembrane domain are embedded within the same membrane. Despite the present structural knowledge, the actual mechanism of viral-induced

http://tibs.trends.com 0968-0004/02/$ – see front matter PII: S0968-0004(01)02050-3