Suppression and the code: Beyond codons and anticodons

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Summary. Specificity and accuracy in the decoding of genetic information during mRNA-programmed, ribosome-dependent polypeptide synthesis (translation) involves more than just hydrogen bonding between two anti-parallel trinucleotides, the mRNA codon and the tRNA anticodon. Other macromolecules are also involved, and translational suppression has been and continues to be an appropriate and effective way to identify them, as well as other parts of mRNA and tRNA, and to elucidate the structural determinants of their functions and interactions. Experimental results are presented that bear upon codon context effects, the role of tRNA structural features in aminoacyl-tRNA selection and in codon selection (reading-frame maintenance), determinants of tRNA identity, elongation factor suppressor mutants, and termination codon recognition by the ribosomal RNA of the small subunit. The examples presented illustrate the complexity of the decoding process and the interconnectedness of translational macromolecules in achieving specificity and accuracy in polypeptide synthesis.
Key words. Translational suppression; codon context effects; tRNA structure; tRNA identity; elongation factors; ribosomal RNA.

Introduction

Of hydrogen bondage

The opening words of an apocryphal 'gospel of molecular biology' read as follows:

In the beginning, there were antiparallel polynucleotides;
And the Force of Hydrogen Bonding was with them;
And they came together, as it was written,
According to the ancient scribe, Erwin of Columbia.
Kings and Potentates, and some postdocs and grad students
came from near and far,
following the star
to Cold Spring Harbor,
to bear witness to this wonder;
And they gazed upon it
and saw that it was all very good!

The implicit point of interest here is the central role of hydrogen bonding in the association of two polynucleotides [79], one might even say in the 'recognition' of one polynucleotide by the other.

Caveat adaptor

The dating of this bit of dubious hagio-scientific history to 1953 or so allows one to understand the origins and reasonableness of the 'adaptor' hypothesis, formulated roughly two years later (see Crick [9]), and the privileged role perceived for hydrogen bonding in the decoding of genetic information during mRNA-programmed, ribosome-dependent polypeptide synthesis (translation). At the same time, however, that understanding allows one to be aware of the basis for the ultraconservative view of the adaptor hypothesis, the view that has prevailed to this day in virtually every textbook, namely that all there is to mRNA codon recognition is hydrogen bonding between antiparallel trinucleotides. That awareness can, in turn, allow one to appreciate how such a belief could have become canonized by the 'wobble hypothesis' [10]. Although that name focused our attention on the latitude of base-pairing with the third nucleotide of a codon, the hypothesis clearly stated that the base-pairing with the first two nucleotides follows the strict rules applicable to DNA. Both aspects of the hypothesis, however, were based on the underlying assumption that codon recognition can be explained entirely by base-pairing between two antiparallel trinucleotides, the mRNA codon and the 'anticodon' of a transfer RNA (tRNA), as the adaptor had come to be called. In the last 24 years, however, it has become evident that that interaction alone cannot account for the speed and accuracy of translation [1, 5, 18, 19, 31, 34, 54, 70, 72, 76]. From the work discussed below, it should become evident that the same conclusion applies to the specificity of decoding.

Where do we go from here?

The first point of this article is to convince the reader that, to understand specificity and accuracy in translation, one must look beyond codons and anticodons. The second point is that translational suppression has been and still is a most effective tool for identifying all of the molecules and factors involved in the specificity and accuracy of translation and for analyzing their functions and interactions (I am referring not to the initiation stage of polypeptide synthesis, but only to elongation and termination). This is possible because in translational suppression, a cellular defect caused by a mutation in some gene is corrected by a mistake in the translation of the mRNA of that mutant gene. Such a 'mistake upon a mistake' can be caused by a mutant form of any translational macromolecule, as long as, and to the extent that, that molecule is involved in the specificity or accuracy of translation [81].

When I was a little boy growing up in Brooklyn, New York, it was impressed upon me that success in learning

required mastery of three fundamentals, the 'three Rs', namely 'Readin,', 'Ritin', and 'Rithmetic'! Nowadays, for the decoding of genetic information, we can acknowledge an analogous set of fundamentals, the '3Rs of translation', namely, m*R*NA, t*R*NA, and r*R*NA (ribosomal RNA). Consistent with the view that 'the translation apparatus is an RNA machine'[33], the suppression studies I discuss below concentrate on essential aspects of those three intimately related, fundamental informational macromolecules, but include also aspects of a few 'friends of the family', insofar as they are involved in the decoding of genetic information.

Beyond codons

Concerning codons, it is necessary to consider the contribution of nucleotides immediately preceding and following a codon that is being translated. With the use of missense and nonsense suppression, codon context has been shown to include the participation of at least three nucleotides upstream and three downstream of the codon (for recent data and a survey of earlier findings, see Buckingham et al.[6] and the paper by R. H. Buckingham in this review). But it is reasonable to ask whether mRNA nucleotides farther away than three in either direction can influence translation of a codon. One possible cause of context effects is the interaction of two tRNAs on the ribosome, and experimental evidence for it has been provided[68]. The current view of *three* tRNA binding sites on the ribosome[46], and the theoretical possibility of a fourth (an entry or 'pre-A' site), forces the consideration of 6–9 nucleotides in either direction. Furthermore, apart from tRNA-tRNA interactions, the apparent inclusion 'in' the ribosome of three or more times as many mRNA nucleotides[27] makes it necessary to consider the possible influence of nucleotides much farther away than just the immediate vicinity of the translated codon. Although previous experimental work and statistical analyses of codon context effects were focused on the nucleotides immediately adjacent to the given codon – the codon's 'neighborhood' – I propose here the 'extended neighborhood' hypothesis, analogous to Yarus' notion of the 'extended anticodon' of a tRNA[82]. The hypothesis states that nucleotides farther up- or down-stream of a given codon than three to six (or so) can influence the translation of that codon. With translational suppression as the signal, this hypothesis is testable by genetic procedures, both classical and modern, both in vivo and in vitro.

Beyond anticodons

tRNA: Structural features outside of the anticodon are involved in decoding

Several studies have clearly demonstrated the involvement of nucleotides in the anticodon loop and stem. Here I give highlights of some of those studies and also present work that indicates a role for regions as far from the anticodon as the amino acid acceptor stem, work that suggests that one may think of the 'extended anticodon' as the entire tRNA molecule!

Several suppression studies have shown clearly that there are decoding determinants outside the anticodon. One class of such studies essentially amounted to in vivo 'anticodon transplants', from one kind of tRNA to another and from one isoacceptor to another. Examples of the former are found among missense suppressor tRNAs, such as mutant glycine tRNAs whose anticodons are complementary to the codons for Arg, Asp, Cys, Glu, Lys, Ser, and Trp[48]. Studies with some of those suppressors have revealed non-canonical decoding properties that are not evident with the wild-type tRNAs containing those same anticodons, namely, first position wobble and second position 'wiggle'[52], and altered fidelity in the maintenance of the correct translational reading frame[77]. A particularly interesting case of in vivo anticodon transplant involved two glycine isoacceptor tRNA species from *Salmonella*, gly1 and gly2[48]. The anticodon of wild-type gly2 is 5′-U*CC-3′ (where U* is an unidentified modification of uridine) and this tRNA species reads the mRNA codons GGA and GGG. On the other hand, wild-type tRNA gly1 has 5′-CCC-3′ as its anticodon and reads only GGG[52]. Neither tRNA species produces detectable frameshifting. However, the mutational placing, if you will, of the gly1 anticodon (5′-CCC-3′) into the gly2 'body' led to two dramatically new properties: significant frameshifting and gross misreading of GGA[57].

In the examples mentioned above, it is not possible to pinpoint the precise extra-anticodon sites or structural features that bring about the new properties. Other suppression studies, however, have led to the identification of relatively simple non-anticodon mutational changes that can be correlated directly with particular new phenotypes. A search for missense suppressors led to a class caused by insertion of a nucleotide into the anticodon loop, a type of mutant that revealed the possibility of 'anticodon shift' in decoding[55, 62]. Single base changes that point to the involvement of the anticodon stem and loop and the D-stem have been discovered or produced in studies of frameshift and nonsense suppression[22, 26, 84]. Finally, a recent study of suppressors of −1 frameshift mutations has drawn attention to the TFC stem and loop (T, 5-methyl-uridine, formerly ribothymidine; F, formerly ψ, pseudouridine) and the amino acid acceptor stem[57]. The potentially special importance of the acceptor stem mutant from that study is emphasized by the existence of another acceptor stem mutant, a lysine tRNA missense suppressor[20]. That mutant tRNA resulted from a C70 to U base substitution in the acceptor stem, 4 nucleotides from the terminal pCpC-pA_{OH} sequence, and causes missense suppression by being misacylated with alanine (refs 53, 58, 63; F. T. Pagel and E. J. Murgola, unpublished results). The more recent and striking finding is that this missense suppressor can

correct + 1 frameshift mutations[77]. Furthermore, evidence has been provided that the misacylation with Ala is not required for the in vivo frameshifting activity[58]. Hence the U70 mutation itself causes the frameshifting.

In all three cited frameshift suppressor studies, the mutant tRNAs not only shift frame but also continue to read a triplet codon to a significant extent[26, 57, 77]. In each study, the authors suggested that alternate conformational states of the suppressor tRNA could be responsible for the two decoding properties. One study provided physical evidence to that effect[26]. Finally, in two of the studies[57, 77], the authors suggest that the acceptor stem mutants may reflect interaction of the tRNA with an as-yet-unidentified domain of the ribosome, perhaps specifically ribosomal RNA. Interestingly, recent reports from H. F. Noller's laboratory indicate that the acceptor end of tRNA is in intimate contact with specific sites in 23S rRNA, the large RNA of the large ribosomal subunit of *Escherichia coli*, indeed, in contact with *different* 23S sites in the different ribosome binding sites for tRNA (A, P, and E)[42–44].

These studies and similar ones have supported and fleshed out earlier notions of specificity and accuracy in translation, namely a) that the ribosome is sensitive to the entire tRNA molecule, not just the anticodon[18, 19, 34, 72], and b) that tRNA selection and mRNA movement are coupled to each other[30]. Recent stimulating discussions of these points can be found in Kurland et al.[33].

tRNA identity and the code: I know mine and mine know me (John 10:14)

An important consideration for decoding is the question of what determines the specificity of aminoacylation of a tRNA. The amino acid attached to a particular tRNA must 'match' the decoding specificity of that tRNA. Determinants of this specificity are expected to reside both in the aminoacyl tRNA synthetases (AARSs) and in the tRNAs. For the study of this general question, translational suppression has been especially helpful and revealing. Although some work has been done with synthetase mutants[60], most of the published information is about factors that determine 'tRNA identity'. That term, now in common use, describes the features of a tRNA molecule that make it recognizable by one AARS (the 'cognate' one) and that prevent its recognition by all other AARSs[56, 66, 83].

tRNA identity is currently a hot topic in studies on translation. The recent reference to it as 'the second genetic code'[13] was unfortunate, since that expression is simplistic, equivocal, and rather confusing[66]. But it did rightly give prominence to the importance of this topic for gene expression. Although the search for tRNA identity has been going on for more than 20 years, most of the exciting progress has been made in the last few years[56, 66, 83]. Furthermore, translational suppression has played a major role in that search and will continue to do so[14, 23, 40, 56, 66, 83]. From the studies just cited, several generalizations can be drawn. First, tRNA identity is defined by a relatively small number of elements. Secondly, the anitcodon contains one or more identity elements in more than half of the tRNAs examined and those tRNAs can be subdivided according to whether cognate recognition remains normal or is diminished or abolished. Finally, in addition to the positive elements in a tRNA, the 'determinants', which dictate interaction with the correct or cognate synthetase, there are negative elements, 'deterrents' or 'anti-determinants', which contribute to tRNA identity by blocking recognition by noncognate synthetases[56, 66, 83].

When it comes to the investigation of elements outside of the anticodon, however, there is a serious deficiency so far in the studies utilizing translational suppression. Except for the unique case of the missense suppressor, *lysT*(U70), an acceptor stem mutant in which a mutationally produced G–U base pair allows misacylation with alanine (refs 20, 53, 63; F. T. Pagel and E. J. Murgola, unpublished data), all of the suppressor tRNAs, whether selected for or synthesized[56], begin with an anticodon alteration: as amber suppressors, they contain a mutant anticodon, 5′-CUA-3′, which is complementary to the chain-termination codon 5′-UAG-3′ in mRNA. This allows one to examine in vivo the consequences for aminoacylation of any subsequent mutations in a given tRNA. One tests for suppression of UAG mutations corresponding to amino acid positions at which the functionality, for the whole protein, of all or most amino acids is known[23, 40]. This procedure, however, begs the question of whether the additional mutations produce their effects on tRNA identity on their own or in concert with the anticodon change (that is, the amber anticodon). Nevertheless, the solution to this is at hand: a method for uncovering identity determinants outside of the anticodon by isolating tRNA mutations that allow maintenance of the normal decoding specificity while altering tRNA identity. This is precisely one of the mechanisms proposed for missense suppression[4, 80, 81] and is exemplified by *lysT*(U70), the misacylated lysine tRNA[20, 53, 63], as mentioned above. For many tRNAs, appropriate missense mutations exist (in *E. coli*) or can be generated at functionally well-characterized positions in a protein gene[48]. With a particular tRNA gene cloned in a plasmid and an appropriate method for mutagenizing the plasmid (in vivo or in vitro), straightforward in vivo selections for missense suppressors should lead to misacylated tRNAs bearing mutations at one position or another outside of the anticodon.

Peptide chain elongation proteins

The elongation stage of polypeptide synthesis, in which a nascent protein is extended one amino acid at a time on an mRNA-programmed ribosome, can be conveniently divided into three steps: a) aminoacyl-tRNA selection

and binding; b) peptide bond formation; and c) translocation (see Hershey[21] for overview of elongation). Steps a and c exhibit a high degree of specificity, for the selection of the correct aminoacyl-tRNA and of the correct reading frame, respectively, and, in the organisms studied, each requires the participation of at least one protein factor. In *E. coli*, elongation factor EF-Tu accomplishes the codon-specific binding of a particular aminoacyl-tRNA to the ribosome and EF-G is considered responsible for the accurate translocation of peptidyl-tRNA from the A (aminoacyl-tRNA) site to the P (peptidyl-tRNA) site on the ribosome. The accuracy of tRNA selection is ensured by a proof-reading mechanism[31,33] and the same is likely to be true for translocation[32]. In any case, the specificity and accuracy required in each step leads to the thought that one ought to be able to obtain translational suppressor mutations in the genes for EF-Tu and EF-G.

At first thought, one might consider it reasonable to search for EF-Tu suppressors of missense (or nonsense) mutations and for EF-G suppressors of frameshift mutations. It is known, however, that these two factors bind to the ribosome at overlapping sites, that they both protect bases in a universally conserved loop of *E. coli* 23S rRNA (the RNA of the large ribosomal subunit), and that they both interact with tRNA[41,45,65]. Furthermore, ample evidence exists for the coupling of errors in tRNA selection with errors in codon selection, that is, reading frame maintenance[30,32,33]. Therefore, it need not be surprising that EF-Tu mutants have been found that suppress frameshift mutations[25]. Hughes et al.[25] suggest two general mechanisms by which the mutant Tu can achieve the suppression, one by faulty positioning of the incoming aminoacyl-tRNA, the other by 'error coupling' due to an increase in missense errors. These Tu mutants, and others, also exhibit suppression of nonsense mutations[24,25,78]. Finally, the suppression activities of the Tu mutants are highly specific and dependent on context[24,25,78]. It is clearly important to isolate more suppressor mutant forms of Tu, particularly as missense suppressors. Indeed, one Tu mutant has been shown to increase missense errors in vitro[73]. Also necessary is a search for EF-G suppressors of frameshift mutations and perhaps also of missense and nonsense mutations. The characterization of a recently identified EF-G mutant provided the first direct evidence that the accuracy of mRNA movement is influenced by EF-G function[33,64]. Analyses of such novel suppressors and related mutants should go a long way toward achieving an understanding of the roles of EF-Tu and EF-G in the specificity and accuracy of polypeptide elongation. It is comforting that similar mutants are being found in eucaryotic cells[11].

Two rather unorthodox recent findings should be mentioned before closing this section. The first is related to a kind of 'natural' suppression of a termination codon that results in the insertion of selenocysteine at a specific residue of formate dehydrogenase[36] in bacteria and of glutathione peroxidase[35] in mammals (see discussion by F. Caron in this issue). In all of the cases, a special tRNA that reads UGA is first acylated with serine; the serine is then converted in at least two steps to selenocysteine while it is still attached to the novel tRNA. Further details come from the *E. coli* studies which demonstrate that a) the novel tRNA, acylated with serine, does not participate in polypeptide synthesis, and that b) the selenocysteyl-tRNA, which reads UGA, and inserts selenocysteine at that codon, participates in polypeptide synthesis only with the assistance of a novel translation factor, the product of the *selB* gene[16]. The SelB protein bears significant sequence homology to *E. coli* elongation factor EF-Tu and binds guanine nucleotides. It specifically interacts with the novel UGA-reading tRNA when that tRNA is acylated with selenocysteine but not when it is acylated with serine[16]. The fact that the insertion of selenocysteine is so context-specific (it happens at the *internal* UGA in *specific* mRNAs) and so efficient leads one to speculate, as do Forchhammer et al.[16] that the SelB protein plays a role in the context specificity and in competition with the normal peptide chain termination mechanism. The authors point to the larger size of SelB (68K) compared with EF-Tu (43K) as consistent with additional functions. The 'extra' portion of SelB, however, need not be the source of the context specificity since, as discussed above (the preceding paragraph), EF-Tu mutants exist whose actions are highly context dependent[24,25,78]. Even in the region of significant homology between SelB and EF-Tu, there are enough differences to account for an extremely high context specificity for SelB. On the other hand, the 'extra' part of SelB could be involved in a novel competition with the UGA-dependent peptide chain termination mechanism (in conjunction with the context-specific placing of selenocysteyl-tRNA at UGA). It could, as Forchhammer et al.[16] suggest, interact with release factor 2. As an alternative, however, I suggest that the 'extra' part of SelB interacts with 16S ribosomal RNA, thereby interfering in some way with the antiparallel base-pairing that now seems certain to occur between the UGA codon and a 5′-UCA-3′ triplet in 16S rRNA as the initial step in UGA-dependent peptide chain termination[49,51] (and see below, next section).

The second unorthodox finding that seems appropriate for mention in this section, although it did not arise from studies with translational suppressors, concerns the number of GTP and EF-Tu molecules that participate in peptide bond formation. Examining steady state translation in an accurate and fast in vitro translation system prepared from *E. coli*, Ehrenberg et al.[15] observed that *two* EF-Tu·GTP cycles are required to make *one* peptide bond. They found furthermore that there are *two* complexes of EF-Tu·GTP bound to one molecule of aminoacyl-tRNA under their conditions. On the basis of their data, they suggest that aminoacyl-tRNA enters the ribosomal A-site in a pentameric complex together with two EF-Tus and two GTPs. It has not escaped the authors'

notice that the two EF-Tu·GTP cycles 'could be associated with two separate ribosomal binding states prior to peptide bond formation'. In other words, the possibility can again be suggested of a 'pre-A' site for tRNA binding to the ribosome, and we can end this paragraph with a reference to translational suppression after all. I suggest that, if a pre-A site exists, evidence for its existence should be able to be provided by combining site-directed mutagenesis of an appropriate gene sequence with the use of specific well-characterized suppressor tRNAs.

rRNA and termination codon recognition: The beginning of the end

The termination of ribosome-dependent polypeptide synthesis requires at least one termination codon (usually UGA, UAA, or UAG), one or two proteins called release factors (RFs), and both subunits of the ribosome[7,8]. In *E. coli* there are two codon-specific RFs. Both act at UAA, but RF1 is specific for UAG and RF2 for UGA. The precise function and mode of action of RFs are not known but it is clear that they act in a codon-specific way and lead to peptidyl-tRNA hydrolysis[7,8]. Evidence, however, for a direct involvement of the ribosome in the *specificity* of termination was lacking until recently. About two years ago, Murgola et al.[51] reported the discovery and initial characterization of a novel codon-specific translational suppressor in *E. coli*, a mutant 16S ribosomal RNA that caused the suppression of UGA mutations but not of UAA, UAG, or related missense mutations. The mutant, referred to phenotypically as ΔC1054, resulted from the deletion of a cytidine residue at position 1054, in the 3′ major domain of 16S rRNA, the RNA of the small ribosomal subunit (30S) of *E. coli*. The analysis of that mutant established the direct involvement of 16S rRNA in the specificity of peptide chain termination but it did not elucidate the precise manner of involvement or mechanism of action.

The question of whether a termination codon is 'recognized' by a protein-RNA interaction (RF-mRNA) or by an RNA-RNA interaction (rRNA-mRNA) has never been resolved[7,8,71,74]. In the early 1970s, it was suggested that peptide chain termination might require base-pairing of each termination codon with 3′-terminal nucleotides of the *E. coli* 16S rRNA[67]. That suggestion was rendered unlikely, if not totally untenable, by subsequent comparative sequence analyses of 16S-like RNAs (see Steege and Söll[71] for review) and also by experiments performed by W. P. Tate and colleagues, who examined termination using 16S RNA whose 3′ end was either modified or base-paired with a complementary oligonucleotide[74,75]. So, despite the results of an early study that provided evidence that termination codon function behaved as if it depended on an RNA-RNA base-pairing interaction[69], the assumption has remained strong for the last 15 years or so that RFs directly recognize and interact with the codons. Recent experimental results indicate otherwise.

Based on their analysis of the ΔC1054 mutant, Murgola et al.[49–51] proposed base-pairing between rRNA and mRNA as a plausible model for the initial stage of UGA-dependent peptide chain termination, namely, recognition of the termination codon, and suggested that a similar mechanism operates for termination at UAA and UAG codons. Specifically, the model proposed that UGA-dependent termination requires anti-parallel base-pairing between the mRNA UGA codon and a particular complementary triplet in 16S rRNA. For the rRNA triplet, the model suggested one or both of the two evolutionarily conserved tandem 5′-UCA-3′ sequences comprising nucleotides 1199 to 1204 in the 3′ major domain (see Murgola et al.[49], fig. 1). Such base-pairing would prevent any tRNA from misreading UGA and allow subsequent hydrolysis of peptidyl-tRNA. It was suggested, therefore, that the deletion of residue C1054, which is located on the opposite side of helix 34[3], the same helix that contains the 5′-UCA-3′ triplets, renders one or both triplets inaccessible for base-pairing with the UGA codon, possibly by altering the conformation of the 16S rRNA in that region, and so allows misreading of UGA and readthrough by some normal tRNA. The model was supported by several observations[49–51,69].

The main prediction of the rRNA-mRNA base-pairing model of peptide chain termination[49–51] is that mutations in the appropriate 16S rRNA triplet (the one complementary to the particular termination codon) away from strict complementarity to the codon should lead to a decrease in or the elimination of termination at that codon. Such an effect should be manifested by suppression of one or another corresponding nonsense mutation. In the case of UGA termination, therefore, mutations in one or both 5′-UCA-3′ triplets, that is, the first at 1199 to 1201, and the second at 1202 to 1204, should lead to UGA-specific suppression. The question of lethality, however, was also a consideration. If only one of the two triplets is used for termination, then only mutations in that triplet should display UGA suppression. But such mutations, when present in a 16S gene cloned in a high copy, high expression plasmid, could be detrimental, or even lethal, to the cell. If, on the other hand, both triplets can function in UGA suppression and one is sufficient, then mutations in one or the other, when present in high copy, should not be lethal unless present simultaneously in the cloned gene.

The latter expectations were observed with site-directed base substitutions made at positions 1199, 1200, 1202, and 1203[49,50] (and Göringer, H. U., O'Connor, M., Hijazi, K. A., Dahlberg, A. E., and Murgola, E. J., unpublished data). When the mutations in one triplet or the other were present in the high copy number plasmid pKK3535, the strains containing them grew more slowly than the cells containing the wild-type plasmid. Such growth effects were reduced or eliminated when the plasmids were introduced into a strain containing a chromosomal *pcnB* mutation[49] (and O'Connor, M., and

Dahlberg, A. E., pers. comm.). With each set of mutants, it was found that the double mutant and each single exhibited suppression of UGA, but not UAA or UAG, mutations and that each double mutant was lethal in high copy while the corresponding singles were not[49] (and Göringer, H. U., O'Connor, M., Hijazi, K. A., Dahlberg, A. E., and Murgola, E. J., unpublished data). These results fulfill the main prediction of the base-pairing model, the complementary codon-specific involvement of residues 1199 to 1204 in UGA termination, and indicate further that at least one operative triplet is essential but that either one can serve reasonably well. Selections for rRNA suppressors of UAA and/or UAG, as well as site-directed mutagenesis of conserved 5′-UUA-3′ and 5′-CUA-3′ triplets, should provide evidence that the helix 34 region is, as proposed, a 'domain for termination' at all three codons[49, 50]. Although the model makes no commitment as to the involvement of RFs in termination, several testable suggestions can be made[49].

Three other points should be mentioned before closing this section. First, implied in the base-pairing model is the notion that the ribosome scans the mRNA during elongation so as to be in a position to form hydrogen bonds with a termination codon when it is encountered. Therefore, a second prediction of the model is that the rRNA triplet, for example 5′-UCA-3′, can misread (mispair with) a related sense codon, such as UGG, resulting in chain termination at the sense codon[49]. Since the level of such misreading may be too low to be easily detected, use has been made of site-directed mutations and an in vivo UGG-suppression system to provide preliminary evidence for rRNA-dependent premature termination[49]. Second, analysis of the original UGA suppressor mutant, ΔC1054, indicated that the suppressor exhibits a defect in termination at only two of the four UGA mutations tested in trpA[51]. If the failure to suppress the two other mutations truly reflects a continued ability to terminate at those sites, ΔC1054 and other rRNA termination mutants should introduce a new dimension into studies of codon context effects in translation[6]. Finally, the involvement of 16S rRNA in peptide chain termination described in this section completes the demonstration of direct base-pairing interactions between rRNA and mRNA in all three stages of polypeptide synthesis[12].

Summary and outlook: E pluribus unum

Although herculean inroads were made in the 1950s and 1960s into the understanding of the genetic code and mechanisms of decoding[85], things are now seen to be so much more complex than was apparent 25 or so years ago. In particular, despite the attractive simplicity, the initial heuristic satisfaction, and the investigative usefulness of Crick's 'codon-anticodon pairing' hypothesis[10], it is clear now that a) there is more to codon recognition than hydrogen-bonding between antiparallel trinucleotides and that b) codon-reading properties are not simply predictable from codon and anticodon sequences, based on hydrogen-bonding 'rules' deduced from non-tRNA polymers.

The 'body' of a tRNA is involved in the specificity of codon recognition, as I discussed above, and codon-reading differences are demonstrable even for synonymous codons that are read by the same tRNA species[54, 70, 76]. Hence, it is not clear what one learns by examining and comparing only anticodons, whether it be for speculations about coding specificities of present-day tRNAs or for conjectures about the evolutionary origin of the genetic code. It is also imperative that the investigator should not be content with a tRNA gene sequence but should vigorously attempt to isolate the tRNA itself for sequence analysis and determination of the nature and extent of nucleoside modification at specific residues. Observations have been made of significant, even drastic effects of nucleoside modifications on tRNA structure, function, and interactions, including decoding properties and amino acid specificity[2, 26, 28, 47, 61]. Furthermore, the ability to determine experimentally a tRNA's identity (that is, its aminocylation specificity) could lead to the discovery of an unexpected determinant of decoding specificity, as in the case of a yeast mitochondrial tRNA that reads a leucine codon but inserts threonine into the growing polypeptide chain[37]. What some construe as 'errors' may in reality be adjustable 'translational alternatives' that serve to regulate specific cell functions[59].

In this paper, I have also briefly referred to aspects of codon context effects, some of which are so dramatically a part of the decoding scenario (see, for example, refs 6, 16, 35, 36 and R. H. Buckingham's paper in this issue) that one may consider the genetic code as not completely known until one knows and understands those determinants[17]. Finally, I discussed the specific participation of elongation factors and the ribosome, going into some detail about part of what is more and more being discovered to be the intricately multifaceted involvement of ribosomal RNA in all three stages of decoding[12]. Clearly, in addition to tRNAs, all of these molecules must be considered as possible contributors in speculations about the origins and evolution of 'codon assignments'.

One of my favorite cartoons depicts a cave man and a cave woman sitting in front of their cave. One is saying to the other: 'I miss the good old days when all we had to worry about was codons and anticodons!' Certainly, the study of decoding has become very complex, but the attempt to elucidate the mechanisms involved has become challenging and exciting. Furthermore, translational suppression will continue to be a powerful tool in this venture. The knowledge gained not only satisfies our basic desire to know what makes things tick but also, for example, helps us to understand the molecular basis of diseases caused by defects in protein synthesis and to produce efficiently and accurately medically and industrially important proteins through biotechnology.

The translational complexities that I have discussed in this paper should not be construed as *consequences* of the genetic code. Rather, the reverse is the reality. The genetic code, I submit, is not a fixed, monolithic, uni-directional entity that exists on its own. It is, for one thing, a mental construct that enables us to understand specificity and accuracy in mRNA-programmed polypeptide synthesis. It denotes a multifaceted, highly organized informational network that derives its very existence from the intricate interconnectedness of all of its component molecular determinants. Those determinants are many; they include codons and anticodons but go far beyond them to encompass other macromolecules and interactions. From those 'many' we derive the 'one' [it is also very 'good', generally 'true', and obliquely 'beautiful'[29]], the artfully orchestrated molecular symphony we call THE GENETIC CODE.

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