Alternative Readings of the Genetic Code

Meeting Review

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The ribosome is a molecular machine that has evolved to translate the message of the RNA transcript faithfully and efficiently into a protein product. The ribosome must maximize the conflicting demands of speed and accuracy: to the extent that the translation rate increases, accuracy suffers, and vice versa. Nonetheless, the ribosome remains very accurate, making an estimated 5×10^{-4} mistakes per amino acid incorporated (Kurland, 1992). Some genes have evolved mechanisms that allow them to manipulate the ribosome, causing changes in the way the ribosome decodes the message. Some of these sites cause apparently 100% of elongating ribosomes to read in a noncanonical fashion. A recent meeting at Parknasilla, County Kerry, Ireland (Alternate Readings of the Genetic Code, a Human Frontier Science Program Organization/European Molecular Biology Organization/International Union of Biochemistry and Molecular Biology Workshop, May 18–23, 1993) addressed the relationship between the translational machinery and genes that program alternate modes of translation. The meeting underscored the sometimes bewildering complexity of phenomenology of noncanonical translation, but at the end one was left with an impression of a field tending toward simplification rather than confusion.

General Comments

Translation elongation is a relatively accurate process, though a missense error rate of 5×10^{-4} per codon results in no more than 78% of proteins of 500 amino acids being accurately decoded (Kurland, 1992). Since the rate of processivity errors, those leading to premature termination, is estimated to be 3×10^{-4}, only about 86% of proteins of 500 amino acids would not be truncated. These errors result either from spontaneous dissociation of peptidyl-tRNA from the ribosome or from translational frameshifting leading to premature termination at out-of-frame stop codons (Kurland, 1992); release factor–dependent termination at sense codons is very rare (Jørgenson et al., 1993). The low level of misincorporation is achieved by kinetic proofreading during elongation (Hopfield, 1974; Ninio, 1974). Elongation factor Tu (EF-Tu), responsible for bringing aminoacyl-tRNA to the ribosomal A site, imposes two timing steps during tRNA selection, one before and one after GTP hydrolysis. These steps allow for the dissociation of noncognate tRNAs. Since elongation is regulated kinetically, very small changes in, for example, equilibrium dissociation constants can translate into large changes in outcome.

Some genes include sites that cause altered readings of the code that are superficially similar to random errors — nonsense codons are misread as sense, or the reading

frame shifts — but is the difference between these events and random errors one of extent, the probability of error, or of kind? Since the programed changes in elongation are phenomenologically diverse, there is no one answer for this question. The events run the gamut from truly programed, for example incorporation of selenocysteine at special UGA codons, which depends on a specially encoded analog of EF-Tu (reviewed by Böck et al., 1991), to those that are nearly indistinguishable from random error sites. However, even the most clearly programed events depend on features of translation responsible for random elongation errors. Programed frameshifts therefore provide tools to understand how translational accuracy is maintained, since they help to identify the steps in elongation that are most prone to producing errors, and they provide the opportunity to determine how genes evolve to manipulate the mechanism of translational fidelity.

Termination Codons

Translational termination can be thought of as a programed alternative coding event. Translation elongation reads successive codons until a termination codon is encountered. The efficiency of termination depends on competition between two processes, normal decoding by a near-cognate or cognate tRNA and termination. E. Murgola (University of Texas) suggested that mutations affecting the translational machinery that increase the probability of decoding or decrease the probability of termination skew this competition toward readthrough. The effect can be general, increasing mistranslation of sense and nonsense codons, or specific, increasing decoding only of nonsense codons or perhaps only of one nonsense codon. Y. Nakamura (University of Tokyo) described one kind of nonsense-specific suppressor that increases misreading of all three types of nonsense mutations. A mutation termed tos, a null mutation of the probable gene for release factor 3 (RF3), stimulates readthrough of nonsense codons. RF3 stimulates the activity of the other codon-specific RFs. Presumably, reduced RF1 and RF2 activity causes increased readthrough by skewing the competition between termination and readthrough. A mutation changing G_{1093} of Escherichia coli 23S rRNA to A, described by Murgola, affects termination only at UGA codons. G_{1093} is located in the highly conserved domain II of rRNA, involved in binding ribosomal protein L11, a protein that both stimulates RF1 activity (UAG-specific) and inhibits RF2 (UGA-specific). Murgola suggested that were the mutation to stimulate L11 activity, it would cause readthrough of UGA indirectly by reducing RF2 activity. Consistent with this interpretation, preliminary data suggest that the same mutation reduces readthrough of UAG codons, as would happen if UAG-specific RF1 activity were increased. Another way to cause readthrough is to stabilize noncognate decoding of nonsense codons. Mutations of 23S rRNA, described by M. O'Connor (Brown University), stimulate read-through of nonsense codons and induce −1 frameshifting. The mutations are located in regions associated

with tRNA interactions and elongation factor binding, suggesting that the mutations stabilize binding of noncognate tRNAs, overcoming discrimination dependent on the small subunit.

W. Tate (University of Otago, New Zealand) addressed the issue of how RFs recognize termination codons. He has proposed that RF recognizes a tetranucleotide signal consisting of a termination codon and a 3' fourth nucleotide (Brown et al., 1990). This proposal is based on comparing sequences around terminators, revealing a strong bias both in termination codon usage and in the identity of the fourth nucleotide. This bias probably reflects differences in the rate of recognition of the tetranucleotides by RF. In a model peptide release assay, Tate showed that rabbit RF recognizes the UGAX series in the order UGAG = UGAA >> UGAC > UGAU, similar to their occurrence at rabbit terminators. Second, he showed that cross-linking between RF and the UGA codon was affected by the fourth base in the order UGAA = UGAG > UGAU > UGAC. This ranking is similar to the first. However, with other codon families, cross-linking is not correlated with efficiency of peptide release. So the proximity of the codon may not directly reflect efficiency of decoding.

Programed Termination Readthrough

Some genes have evolved sites that program readthrough of termination codons. These sites may direct suppression by stabilizing binding of near-cognate tRNAs or by interfering with recognition by RF. These sites vary from a few codons to a large region encompassing an RNA pseudoknot. T. Tuohy (University of Utah) showed that Sindbis virus requires only one or possibly two codons downstream of the termination codon: UGA CUA ACC. This context contrasts with previous results with tobacco mosaic virus, which requires UAG CAR YYA (R = A or G; Y = T or C) (Skuzeski et al., 1991). The role of the sequence distal to the stop codon is not clear. Tuohy suggests that the requirement for CUR reflects a requirement for the CUA/CUG-decoding tRNA^{Leu} distal to the readthrough. It is not clear how the identity of the tRNA distal to the stop codon could affect the rate of acceptance of a near-cognate tRNA at the UGA codon, though it perhaps points toward a pre-A site allowing preselection of the next cognate tRNA, which might influence near-cognate decoding in the A site.

Termination suppression in murine leukemia virus (MLV) is more complicated. A region of 57 nt including a pseudoknot is necessary for efficient readthrough of all three termination codons (Feng et al., 1992; Wills et al., 1991). J. Levin (National Institutes of Health [NIH]) and N. Wills (University of Utah) presented recent work showing that both the single-stranded and double-stranded regions of the pseudoknot are required to stimulate readthrough. It is not clear as yet whether this context stabilizes noncognate decoding or reduces RF efficiency. The fact that the region works with all three stop codons and, as shown by Levin, at least four tRNAs argues against a specific interaction with the tRNA, though even a nonspecific interaction could push the reaction toward readthrough.

Leftward Frameshifting

A large portion of the meeting at Parknasilla concerned translational frameshifting, which can be divided into "leftward" or negative (e.g., −1) frameshifting and "rightward" or positive (e.g., +1) frameshifting. The vast majority of the discussion of −1 frameshifting concerned so-called retroviral, or simultaneous slippage, frameshifting. In retroviral −1 frameshifting the change of frames occurs on a "slippery" heptanucleotide of the form X XXY YYZ. Two tRNAs bound to nucleotides 2 to 7 of this site (XXY YYZ) simultaneously slip onto nucleotides 1 to 6 (XXX YYY). A secondary structure, commonly a pseudoknot, stimulates the event at least partially by causing a translational pause, as has recently been demonstrated biochemically (Tu et al., 1992). Such frameshift sites have been found in retroviruses, coronaviruses, retrotransposons, bacterial transposons, and a prokaryotic gene. The plasticity of the frameshift mechanism explains the ubiquity of these independently evolved systems. The frameshift depends neither on particular tRNAs nor on the primary sequence of the pseudoknot. The mechanism apparently requires only a slippery heptanucleotide and a stable pseudoknot spaced between 4 and 8 nt apart (ten Dam et al., 1990).

The canonical secondary structure stimulator is that of the coronavirus infectious bronchitis virus (IBV) (Brierley et al., 1989). S. Inglis (Cambridge University) described evidence that this pseudoknot transiently stalls the ribosome during elongation; similar results have been obtained for the pseudoknot of the L1 dsRNA virus of yeast (Tu et al., 1992). Inglis also found that a stem–loop predicted to have a greater free energy than the IBV pseudoknot promotes less frameshifting. He suggested that the particular tertiary structure of the pseudoknot, rather than simply its stability, was required to stimulate frameshifting efficiently; perhaps a helicase associated with the elongating ribosome has more difficulty with a pseudoknot than with a simple stem–loop. However, many examples exist of simultaneous slippage sites that are stimulated by stem–loops. Such structures were described for human T-lymphotropic virus type 2 (HTLVII) (A. Honigman, Hebrew University, Jerusalem), transposons IS150 (B. Rak, Universität Freiburg) and IS911 (O. Fayet, Centre du Recherche en Biochimie et Génétique Cellulaire du CNRS, Toulouse), red clover necrotic mosaic virus (S. Lommel, North Carolina State University), and barley yellow dwarf virus (A. Miller, Iowa State University). The stem–loops are much more sensitive to changes in stability. For example, Honigman showed that decreasing the stability of a stem–loop by replacing four G–C base pairs with A–U base pairs eliminated stimulation of frameshifting. In an unusual experiment, H. Hauser (Gesellschaft für biotechnologische Forschung, Braunschweig) described replacing the stem–loop of HTLVII with the iron response element (IRE) of the ferritin H chain gene. In high concentrations of iron, an IRE-binding protein (IRE-BP) binds to and stabilizes the stem–loop. Hauser presented evidence that the stem–loop could replace the HTLVII stem–loop and that binding of IRE-BP stimulated frameshifting. This result underscores the importance of increased stability to frameshift stimulation.
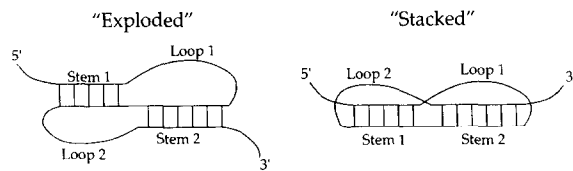
Figure 1. Secondary Structure of Pseudoknots

The stability of pseudoknots is also important. Structure-destabilizing mutations decrease −1 frameshifting, though mutations targeting the two stems of the pseudoknot (see Figure 1) need not be equivalent. E. ten Dam (Leiden University) presented data showing that mutations that destabilize stem 1 could have a greater effect than those affecting stem 2. He suggested that, since stem 1 is in closer contact with the ribosome, it may be more critical in impeding ribosome movement. L. Shen (University of California, Berkeley) showed deduced structures, based on two-dimensional nuclear magnetic resonance analysis, for two similar pseudoknots that differ in their ability to stimulate frameshifting (see Figure 2). The less efficient stimulator of frameshifting has a considerably less compact structure. Several predicted base pairs in fact do not form, particularly at the junction of the two helices.

Why would a pseudoknot more efficiently stimulate frameshifting? Clearly the ability of a structure to stimulate frameshifting is directly related to how difficult it is to unravel. Whereas a ribosome-associated helicase could easily unwind the local secondary structure of a stem–loop, a pseudoknot presents more of a problem. First, the structure is more constrained, since successive segments

Figure 3. Structure of Pseudoknot

The pseudoknot has four parts. Stem 1 lies on top and to the right, stem 2 lies underneath and to the left, loop 1 is at the left, and loop 2 is at the right. The free 5′ end is top right, and the free 3′ end is bottom left. Loop 1 lies in the minor (shallow) groove of the structure (behind stem 2), and loop 2 lies in the major (deep) groove (behind stem 1). Photograph kindly provided by J. Wyatt (University of California, Berkeley).

twice switch from what would be the Watson to the Crick strand of a true duplex. Second, as pointed out by K. Pleij (Leiden University) in his talk, the loops lie above the major and minor grooves of the two stems (see Figure 3). The loops could interfere sterically with the helicase, slowing down the unwinding of the structure.

How does the pseudoknot stimulate frameshifting? Other than by inducing a pause, we do not know. Does it do it on its own, that is, without a protein cofactor? This is also not clear, though evidence presented by ten Dam suggests that it does. In an in vitro frameshift assay, he added a 67 nt competitor RNA corresponding to the pseudoknot of the frameshift site of simian retrovirus 1, hoping to titrate any pseudoknot-binding factor. The addition had no effect, meaning that either there is no pseudoknot-binding factor or the factor is tightly bound to the ribosome and not titratable. Though it is not clear that a factor should be necessary, a genetic approach might resolve the question. Two speakers described attempts to identify trans-acting regulators of −1 frameshifting in yeast. S. Lee (University of California, San Francisco) isolated six recessive mutations that increase frameshifting from 50% to 100%. R. Wickner (NIH) described mutations of eight genes that also increase −1 frameshifting on the L-A dsRNA virus frameshift site from 2- to 5-fold, though they have varying effects on other slippery heptanucleotides.
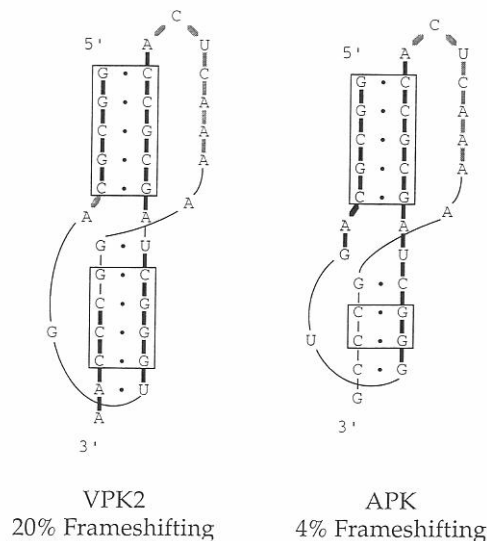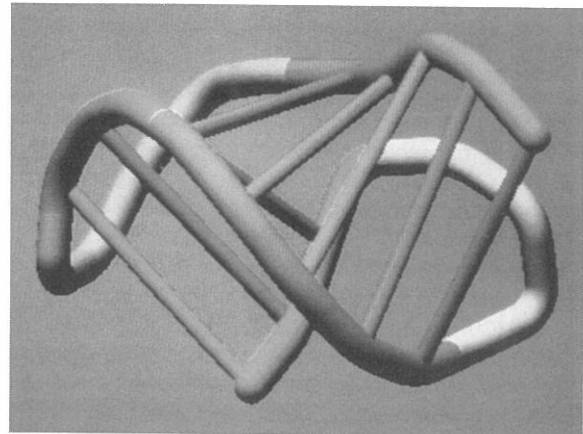

VPK2
20% Frameshifting

APK
4% Frameshifting

Figure 2. Frameshift Stimulation by Pseudoknots Correlates with Structural Stability

The structure deduced for two pseudoknots by two-dimensional nuclear magnetic resonance (nuclear Overhauser effect spectroscopy) (L. Shen, J. S. Lucia, Jr., and I. Tinoco, unpublished data). Apparent hydrogen-bonded base pairs are indicated by dots, base stacking by closed bars, and non–A form stacking by stippled bars. Boxed base pairs are stacked.

Rightward Frameshifting

Rightward, or +1, frameshifting is mechanistically distinct from −1 simultaneous slippage frameshifting. First, all known +1 frameshifts occur with a single peptidyl-tRNA engaging the ribosome in the P site. Second, the event is stimulated by the absence of a tRNA in the ribosomal A site. The canonical example of such a site is from the E. coli RF2 gene prfB. A +1 frameshift occurs at a slippery

codon stimulated by the adjacent in-frame termination codon; during slow recognition of the codon by RF2, an autogenous control loop, the peptidyl-tRNA slips +1. This is stimulated by a Shine–Dalgarno interaction between 16S rRNA and a sequence upstream of the shift site that may stimulate frameshifting by decreasing the $t_{1/2}$ of slipping, applying a force to push the ribosome in the direction intended for the frameshift (Atkins et al., 1990; Weiss et al., 1990a).

J. Curran (Wake Forest University) has constructed a set of 32 codons replacing the normal CUU slippery codon of prfB (Curran, 1993). The results of this study show that the 32 codons tested vary by more than 1000-fold in their ability to promote frameshifting. Some codons are very active (the normal CUU, CCC, UUU, GUU, and CCU), some show low activity (UGU, CAU, and AUU), and the rest are nearly inactive. Curran explains this behavior as caused by the strength of interaction of the cognate tRNAs with the overlapping +1 frame codon; those with higher stability tend to cause more frameshifting, and those with lesser stability cause less.

One common feature of all frameshifts described has been that they involve tRNA slippage. Curran's data are the most complete example published showing that this is true; to the extent that the probability of slipping is decreased, by reducing base pairing in the shifted frame, frameshift expression decreases. I presented my laboratory's data on a frameshift system in a yeast retrotransposon, which does not abide by this rule; the data have since been published (Farabaugh et al., 1993). In this case frameshifting occurs at a sequence GCG AGU U (shown in codons of the unshifted frame), which expresses Ala-Val by decoding the underlined codons. The shift depends on a peptidyl-tRNA^{Ala} bound to GCG in the P site and the low availability of the tRNA^{Ser} that decodes AGU. What is odd about this site is that the peptidyl-tRNA^{Ala} cannot slip onto the overlapping +1 frame codon CGA (it would make no base pairs). We tested whether other tRNAs unlikely to slip could also stimulate frameshifting, replacing GCG with all 64 codons. Our data show that the codons CUU, GCG, and CCG, and to a lesser extent AAU, AGG, and GGG, stimulate +1 frameshifting. Interestingly, several of these, GCG, CCG, and AAU, are not predicted to slip +1 easily, and several codons predicted to slip easily, UUU, AAA, and CCC, do not promote frameshifting. The conclusion from these data is that +1 frameshifting does depend on "special" tRNAs. Clearly, the ability to slip between codons in alternate reading frames stimulates frameshifting. However, at least in yeast, this is not sufficient. tRNAs that promote frameshifting, including the slippery ones, must have a second feature that allows them to promote frameshifting. One possibility is that they stabilize decoding in the new shifted reading frame. The nature of the special status of these tRNAs remains to be determined.

Hopping

The third kind of discontinuous translation elongation is translational hopping. The original example of a hop, recounted by R. Weiss (University of Utah), is from the bacteriophage T4 gene 60, in which a segment of 50 nt encoded

in the mRNA is skipped by 100% of the ribosomes (Huang et al., 1988; Weiss et al., 1990b). The hop occurs by dissociation of a tRNA from a "takeoff" codon followed by its rebinding to an identical "landing" codon. The hop also requires a nascent peptide sequence encoded upstream of the hop site and a stem–loop structure in the RNA that includes the takeoff codon. H. Engelberg-Kulka (Hebrew University, Jerusalem) described her characterization of a hop in the E. coli trpR gene. This hop is unlike the gene 60 hop, since it does not require identical takeoff and landing codons or a specific upstream peptide sequence, though it does require a nonspecific sequence of 10 upstream codons (Benhar and Engelberg-Kulka, 1993). This hop will require further work to clarify the mechanism by which it occurs. For example, how does the ribosome determine the length of the hop without repairing of a tRNA at the landing site? Another potential hop, in the carboxymethyl cellulase gene of Prevotella ruminicola, was described by D. Wilson (Cornell University). Preliminary data suggest that as much as a 500 nt region of the gene is skipped during translation. The existence of programed hops and the occurrence of short variably efficient hops in a variety of in vitro constructs (Weiss et al., 1987) suggest that translational hops may be a more general phenomenon.

Selenocysteine

Incorporation of selenocysteine (Sec) depends on a special tRNA^{Sec}, a specifically encoded analog of EF-Tu, and a special mRNA structure. Sec is incorporated cotranslationally at special UGA codons ubiquitously in organisms from prokaryotes to eukaryotes. D. Hatfield (NIH) has suggested that the conserved nature and ubiquity of this process qualify Sec as a universal 21st amino acid and an alternative meaning for the UGA code word (Hatfield and Diamond, 1993); the need for a special translation factor specific to a unique tRNA^{Sec} and a special codon is analogous to the need for special translation factors unique to the initiator tRNA^{Met}.

In E. coli there are four genes required for Sec incorporation, selA, selB, selC, and selD (Böck et al., 1991). The selC product is tRNA^{Sec}, a tRNA charged with serine. The tRNA-bound serine is modified to selenocysteine by the combined action of selA and selD. The selB product is the specialized EF-Tu. The cis sites required are a UGA codon, decoded by selenocysteyl-tRNA^{Sec}, and an immediately distal stem–loop, required for efficient decoding of the UGA as Sec. A. Böck (Universität München) showed that the secondary structure is a binding site for SelB. A. Herzog (Universität München) selected mutations in selB that restore expression to a gene carrying a defective stem–loop. The suppressing mutation maps to a unique C-terminal domain, downstream of an N-terminal domain homologous to EF-Tu. This suggests that the unique domain interacts with at least the loop portion of the secondary structure, although Herzog did not present evidence of allele specificity, which would have ruled out a more indirect interaction. In addition, mutations of selB that allow recognition of seryl-tRNA^{Sec} map both in the EF-Tu-like domain and clustered in the unique domain. Thus, the unique domain of SelB probably recognizes the site of insertion and

discriminates between seryl- and selenocysteyl-tRNA^{Sec}. Both R. Wilting (Universität München) and A. Krol (CNRS, Strasbourg) demonstrated that tRNA^{Sec} is very unlike normal elongators: the acceptor stem is 8 bp rather than 7, the D arm consists of a 6 bp stem and a tetraloop rather than a 4 bp stem with a 7 or 8 member loop, and the T stem is 4 bp rather than 5. In addition, several invariant residues are changed, allowing two unusual tertiary interactions (A_8–U_{14}–A_{21} and C_{16}–C_{59}). These changes explain why the tRNA is not recognized by EF-Tu and is specifically recognized by a dedicated analog.

The Sec system in eukaryotes is both similar to and distinct from the prokaryotic system. Sec is cotranslationally incorporated using a special tRNA^{Sec}, dependent on a stem–loop structure that is not located immediately downstream of the UGA decoded as Sec but rather in the 3' noncoding region of the mRNA. Some genes, such as type 1 5' deiodinase, described by M. Berry (Harvard Medical School), include one UGA codon read as Sec. Both rat and human selenoprotein P, described by K. Hill (Vanderbilt University School of Medicine), include ten UGA codons (eight at conserved positions). Hill noted that, since the selenoprotein P genes each have only two conserved stem–loops in the 3' noncoding region, there must not be a one-to-one functional connection between stem–loops and insertion sites. This implies that there is not a tertiary structure of the mRNA that juxtaposes a stem–loop with an insertion site, mimicking the bacterial system, and that the system must be less structurally constrained. Consistent with this conclusion, Berry showed evidence suggesting that UGA codons inserted at random into the 5' deiodinase gene were efficiently decoded as Sec. It remains to be seen whether there is any constraint on recognition of UGA codons by tRNA^{Sec} in selenoprotein genes in eukaryotes. With a permissive system like this, it might be possible to insert selenocysteines at will in eukaryotes, which could be useful in protein engineering applications.

tRNA Structure and Codon Recognition

The mechanism of selenocysteine incorporation emphasizes the importance of tRNA structure. Clearly, the very abnormal structure of tRNA^{Sec} allows the cell to dedicate it to a specific purpose. But are the other elongator tRNAs really generic, or do they have special characteristics, revealed when placed into the unusual circumstances of an alternative coding site? Or rather, what features of tRNA sequences reduce random misreading? Some tRNAs have been recognized as unusually prone to misreading. For example, the tRNA$^{Ser}_{2}$, which decodes AGU and AGC, promotes unusual levels of −1 frameshifting, which occurs by doublet decoding involving the middle and wobble nucleotides of the anticodon (Bruce et al., 1986). Some features of tRNAs, when present, reduce miscoding. Modification of the nucleotide immediately 3' of the wobble base, G_{37}, to 1-methylguanosine reduces frameshift errors. A mutation in trmD, responsible for the modification, increases frameshifting by tRNA^{Pro}, possibly by allowing quadruplet decoding of CCCN (Björk et al., 1989). Modification of A_{37} prevents misreading by tRNA^{Phe} in E. coli

(Wilson and Roe, 1989). Similarly, undermodification of tRNAs in infected cells, particularly the absence of queuine (Q) and wybutoxine, has been proposed to stimulate −1 frameshifting in retroviruses (Hatfield et al., 1989). Data presented by I. Brierley (Cambridge University) question the interpretation that these changes in modification increase frameshifting. Brierley tested whether the lack of Q modification affected frameshifting on the IBV −1 frameshift site in E. coli. A null mutation of the tgt gene, responsible for Q modification, had no effect on frameshifting. However, he found that elimination of 2-thiouracil in a trmE mutant background does increase frameshifting about 3-fold, presumably by decreasing the codon–anticodon affinity of a tRNA^{Lys} for the codon AAA, present in IBV slippery heptanucleotide. Though these data tend to question the role of Q modification in frameshifting, they emphasize the importance of modification in modulating frameshifting.

C. Claesson (University of Göteborg) described a primary structure feature that allows tRNA^{Gly} to recognize all four members of the Gly codon family, GGX. Previously, U. Lagerkvist's laboratory had shown that a mycoplasma tRNA^{Gly} with the anticodon UCC decoded all four Gly codons and that this depended on a sequence outside the anticodon (Lustig et al., 1989). The feature required is a C at position 32, 2 nt 5' of the anticodon. When this nucleotide is U, decoding is restricted to Watson–Crick pairing, and when it is C, all four wobble nucleotides are recognized. The wobble U is unmodified, and it had been thought that this lack of modification explained its ability to decode the full codon family. The fact that C_{32} allows indiscriminate decoding even by a tRNA with the anticodon CCC suggests that, as proposed by Lagerkvist, decoding occurs by two out of three decoding.

Rules of the Game

Returning to the questions posed early in this review, is there a difference in kind between programed alternate coding events and random errors? The data do not clearly answer this question. R. Gesteland (University of Utah) made a plea to not consider programed events as errors, to avoid having the field marginalized by the pejorative connotation of "error." On one level, they clearly are not simply elongation errors. We see from the examples presented at the meeting that genes have evolved elaborate mechanisms to achieve noncanonical decoding. But the events themselves in many cases are similar to random errors, except for their probability. The Sindbis and tobacco mosaic virus readthrough sites and the +1 frameshift sites are very simple, requiring the juxtaposition of only two or three codons. The simplest interpretation is that these sites manipulate elongation by making subtle changes in the dynamics of the ribosome, which, in the context of kinetic proofreading, result in relatively large increases in noncanonical reading. The phenomena of −1 simultaneous slippage, MLV termination readthrough, and translational hopping are more difficult to classify as amplified errors. They are more elaborate than the simple systems, requiring larger primary and secondary structure contexts. However, the mechanisms of these events are

still not well enough characterized to conclude that they are different in kind from the simpler systems. For example, in simultaneous slippage frameshifting, does the downstream secondary structure merely cause a translational pause, or does it in some way push the ribosome into the −1 reading frame? The selenocysteine incorporation system makes the clearest distinction between a programed event and an error, using a dedictated EF-Tu analog and special tRNA to achieve decoding of UGA. But even here the system seems to have evolved to maximize the ability of EF-Tu to compete with RF for termination codons. So to varying extents, programed alternate coding events appear to resemble normal translational errors. They therefore are the best tools to use to understand the mechanisms underlying maintenance of translational accuracy. The challenge in studying these events will be to get past their phenomenology in order to characterize their underlying mechanism.

References

Atkins, J. F., Weiss, R. B., and Gesteland, R. F. (1990). Ribosome gymnastics—degree of difficulty 9.5, style 10.0. Cell 62, 413–423.

Benhar, I., and Engelberg-Kulka, H. (1993). Frameshifting in the expression of the E. coli trpR gene occurs by the bypassing of a segment of its coding sequence. Cell 72, 121–130.

Björk, G. R., Wikström, P. M., and Byström, A. S. (1989). Prevention of translational frameshifting by the modified nucleoside 1-methylguanosine. Science 244, 986–989.

Böck, A., Forchhammer, K., Heider, J., and Baron, C. (1991). Selenoprotein synthesis: an expansion of the genetic code. Trends Biochem. Sci. 16, 463–467.

Brierley, I., Digard, P., and Inglis, S. C. (1989). Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. Cell 57, 537–547.

Brown, C. M., Stockwell, P. A., Trotman, C. N. A., and Tate, W. P. (1990). Sequence analysis suggests that tetra-nucleotides signal the termination of protein synthesis in eukaryotes. Nucl. Acids Res. 18, 6339–6345.

Bruce, A. G., Atkins, J. F., and Gesteland, R. F. (1986). tRNA anticodon replacement experiments show that ribosomal frameshifting can be caused by doublet decoding. Proc. Natl. Acad. Sci. USA 83, 5062–5066.

Curran, J. F. (1993). Analysis of effects of tRNA:message stability on frameshift frequency at the Escherichia coli RF2 programmed frameshift site. Nucl. Acids Res. 21, 1837–1843.

Farabaugh, P. J., Zhao, H., and Vimaladithan, A. (1993). A novel programed frameshift expresses the POL3 gene of retrotransposon Ty3 of yeast: frameshifting without tRNA slippage. Cell 74, 93–103.

Feng, Y.-X., Yuan, H., Rein, A., and Levin, J. G. (1992). Bipartite signal for read-through suppression in murine leukemia virus mRNA: an eight-nucleotide purine-rich sequence immediately downstream of the gag termination codon followed by an RNA pseudoknot. J. Virol. 66, 5127–5132.

Hatfield, D., and Diamond, A. (1993). UGA: a split personality in the universal genetic code. Trends Genet. 9, 69–70.

Hatfield, D., Feng, Y.-X., Lee, B. J., Rein, A., Levin, J. G., and Oroszlan, S. (1989). Chromatographic analysis of the aminoacyl-tRNAs which are required for translation of codons at and around the ribosomal frameshift sites. Virology 173, 736–742.

Hopfield, J. (1974). Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. Proc. Natl. Acad. Sci. USA 71, 4135–4139.

Huang, W. M., Ao, S.-Z., Casjens, S., Orlandi, R., Zeikus, R., Weiss, R., Winge, D., and Fang, M. (1988). A persistent untranslated sequence within bacteriophage T4 DNA topoisomerase gene 60. Science 239, 1005–1012.

Jørgenson, F., Adamski, F. M., Tate, W. P., and Kurland, C. G. (1993). Release factor-dependent false stops are infrequent in Escherichia coli. J. Mol. Biol. 230, 41–50.

Kurland, C. G. (1992). Translational accuracy and the fitness of bacteria. Annu. Rev. Genet. 26, 29–50.

Lustig, F., Borén, T., Guindy, Y. S., Elias, P., Samuelsson, T., Gehrke, C. W., Kuo, K. C., and Lagerkvist, U. (1989). Codon discrimination and anticodon structural context. Proc. Natl. Acad. Sci. USA 86, 6873–6877.

Ninio, J. (1974). A semiquantitative treatment of missense and nonsense suppression in the strA and ram ribosomal mutants of Escherichia coli. Evaluation of some molecular parameters of translation in vitro. J. Mol. Biol. 84, 297–313.

Skuzeski, J. M., Nichols, L. M., Gesteland, R. F., and Atkins, J. F. (1991). The signal for a leaky UAG stop codon in several plant viruses includes the two downstream codons. J. Mol. Biol. 218, 365–373.

ten Dam, E., Pleij, C., and Bosch, L. (1990). RNA pseudoknots: translational frameshifting and readthrough of viral RNAs. Virus Genes 4, 121–136.

Tu, C., Tzeng, T.-H., and Bruenn, J. A. (1992). Ribosomal movement impeded at a pseudoknot required for frameshifting. Proc. Natl. Acad. Sci. USA 89, 8636–8640.

Weiss, R., Dunn, D., Atkins, J., and Gesteland, R. (1990a). Ribosomal frameshifting from −2 to +50 nucleotides. Prog. Nucl. Acids Res. Mol. Biol. 39, 159–183.

Weiss, R. B., Huang, W. M., and Dunn, D. M. (1990b). A nascent peptide is required for ribosomal bypass of the coding gap in bacteriophage T4 gene 60. Cell 62, 117–126.

Weiss, R. B., Dunn, D. M., Atkins, J. F., and Gesteland, R. F. (1987). Slippery runs, shifty stops, backward steps, and forward hops: −2, −1, +1, +2, +5, and +6 ribosomal frameshifting. Cold Spring Harbor Symp. Quant. Biol. 52, 687–693.

Wills, N. M., Gesteland, R. F., and Atkins, J. F. (1991). Evidence that a downstream pseudoknot is required for translational read-through of the Moloney murine leukemia virus gag stop codon. Proc. Natl. Acad. Sci. USA 88, 6991–6995.

Wilson, R. K., and Roe, B. A. (1989). Presence of the hypermodified nucleotide N^6-(Δ^2-isopentenyl)-2-methylthioadenosine prevents codon misreading by Escherichia coli phenylalanyl-transfer RNA. Proc. Natl. Acad. Sci. USA 86, 409–413.