Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Cell, Vol. 62, 413–423, August 10, 1990, Copyright © 1990 by Cell Press

Ribosome Gymnastics – Degree of Difficulty 9.5, Style 10.0

Review

John F. Atkins,*† Robert B. Weiss,* and Raymond F. Gesteland*
* Howard Hughes Medical Institute
and Department of Human Genetics
University of Utah
Salt Lake City, Utah 84132
† Department of Biochemistry
University College Cork
Ireland

Prolonged and sophisticated investigations into ribosome structure and function are being rewarded with a new understanding of normal decoding. Ribosomal RNA plays a much more active role than previously thought (Dahlberg, 1989; Moazed and Noller, 1989). Sites have been identified for decoding, peptidyl transferase, and termination, though none are yet as firmly established as that for initiation. Recent biochemical data have brought widespread acceptance of the existence of an exit (E) site in addition to the classical A and P sites. Finally, the proposal for a reciprocating motion of the two subunits, involving intermediate hybrid sites during the translocation process, has suggested a comforting rationale for ribosome design. However, just as ribosome functioning is beginning to come more into focus, it has been found that ribosomes are capable of unexpected gymnastic feats that, even classical ribosomologists are coming to accept, will throw light on the great areas of darkness remaining (Dahlberg, 1989). These nonstandard decoding events involve ribosomal hopping, frameshifting, and reading through stop codons, all at unexpectedly high levels using surprising mechanisms. These possibilities mean that the protein sequence cannot always be simply deduced from the sequence of the mature message.

Hopping

During translational elongation, the paired codon and anticodon can sometimes disengage at certain sequences, allowing the mRNA to slip with respect to the ribosome–peptidyl-tRNA complex. The anticodon may then re-pair with a now-nearby similar codon, so that synthesis continues downstream. On a run of 4 identical bases the reengagement may occur at a codon 1 base removed from the original in-frame codon, with a resultant frameshift. "Slipping" of this type is part of the mechanism for many of the examples of −1 or +1 frameshifting described below.

If the shift occurs over a considerable distance without intermediate pairing, however, the ribosome hops down the mRNA. Hopping requires a "takeoff site" codon and a similar sequence acting as a "landing site" immediately 5′ of the codon for the next amino acid on the resumption of synthesis. Hopping was first encountered over short distances by inserting test sequences early in the *lacZ* gene of Escherichia coli (Weiss et al., 1987). For instance, CUU UAG CUA (Leu stop Leu) was decoded with an efficiency of 1% as a single leucine from the 9 nucleotides. Hopping was also detected when the takeoff and landing sites overlapped, as in the sequence GUGUA (Weiss et al., 1987; O'Connor et al., 1989). At about the same time, tRNA mutants were isolated that increased the hopping at certain sites (Falahee et al., 1988; Hughes et al., 1989), and hopping was detected over as many as three stop codons, albeit at decreased efficiency. The mutants have an extra base in a tRNA anticodon (O'Connor et al., 1989) that somehow promotes hopping. One inference to be made from their study is that there may be good reasons why almost all natural tRNAs have seven-membered anticodon loops! Even with these precedents, the discovery of high level, natural, programmed hopping was a surprise.

The 50 nucleotides that separate codon 46 from codon 47 in the mature message of phage T4 topoisomerase subunit gene *60* are bypassed by the translation apparatus with an efficiency approaching 100% (Huang et al., 1988). Several key features required for ribosomal bypass of this coding gap have been defined utilizing variants generated as gene *60–lacZ* fusions (Weiss et al., 1990a). The analogy to low level and tRNA suppressor–mediated hopping is supported by a strict requirement for a matched set of codons at the takeoff and landing sites. As is the case with all high level unusual ribosomal frameshift or readthrough sites, the interesting question is how the message conspires with the translation apparatus to increase the efficiency and scope of these events.

In the gene *60* case, there are at least four distinct elements that contribute significantly to the bypass. Three of these elements are located at the coding gap: the matched codon set defining its borders, a stop codon at the 5′ junction of the gap contained within a short stem–loop structure, and an optimal 50 nucleotide spacing separating the 5′ and 3′ junctions. The fourth, and most surprising, feature is a stringent requirement for a specific amino acid sequence in the nascent peptide translated from the 46 codons preceding the coding gap. This nascent peptide enables the ribosome that has just synthesized it to bypass the coding gap, although its mode of action is undefined. This nascent-chain effect adds another example to an expanding list of interesting translation events mediated through the nascent protein chain, such as signal recognition particle arrest of elongation (Wolin and Walter, 1988), autoregulated instability of β-tubulin mRNA (Yen et al., 1989), and regulation of the carbamoyl-phosphate synthetase A gene, *CPA1*, in Saccharomyces cerevisiae (Werner et al., 1987).

Another example of high level natural hopping could be in the *carA* gene of Pseudomonas aeruginosa, which encodes the small subunit of carbamoyl-phosphate synthetase (Wong and Abdelal, 1990). Two sets of codons that could potentially act as the takeoff and landing sites occur at nucleotides 9 to 15 and 21 to 27 downstream of the start codon. In contrast to the gene *60* case, the untranslated 12 nucleotides do not contain a stop codon. Since this putative example has just been found, the critical features are unknown, but cannot fail to be interesting.

Shifty Sequences and Stimulators for +1 and −1 Frameshifting

The great majority of ribosomal frameshifting events studied are due to a tRNA slipping from pairing with its correct in-frame codon to an overlapping −1 or +1 cognate codon. A string of four or more single base repeats constitutes a "slippery run" prone to frameshifting. In some instances the run is minimal: For instance, in decoding E. coli polypeptide chain release factor 2 (RF2) (Craigen et al., 1985), the run is CUU U (Weiss et al., 1987). A CUU-decoding $tRNA^{Leu}$ slips +1 to a UUU sequence using a G:U pair in the first position. The lack of perfect classical complementarity in re-pairing may mean there are relaxed rules for re-pairing (first position wobble). Shiftiness in this instance does not uniquely depend on the $tRNA^{Leu}$: when the CUU U string is replaced by GUU U or GGG U, then $tRNA^{Val}$ or $tRNA^{Gly}$, respectively, performs high level shifting (Weiss et al., 1987).

As first discovered in retroviruses, the possibility for two adjacent tRNAs to shift in tandem can for some pairs increase the level of shifting higher than the sum of shiftiness at either codon in isolation (Jacks et al., 1988a). The tandem slippery sequences A AAA AAC, U UUU UUA, and G GGA AAC are common in retroviral shift sites (the upstream A, U, or G being essential). The shift in reading frame, which is −1, occurs predominantly at the second codon of the slippery pair (Hizi et al., 1987; Jacks et al., 1988a). Coronaviruses use a combination of the above slippery sequences, namely U UUA AAC, for their frameshifting (Brierley et al., 1987, 1989; Bredenbeek et al., 1990).

The mechanism underlying −1 frameshifting at tandem slippery codons appears to be the most universally conserved of frameshifting signals, given that retroviral shift sites can catalyze efficient −1 shifting when translated in E. coli (Weiss et al., 1989). A single base change in the mouse mammary tumor virus (MMTV) *gag–pro* shift site, from the normal A AAA AAC to A AAA AAG, surprisingly leads from ~2% to 50% −1 frameshifting at this sequence (a 25-fold increase); and the 10-fold decrease between A AAA AAG and A AAA AAA affords an interesting glimpse at how the nuances of codon–anticodon interaction can govern the efficiency of this type of shifting. The high level of shifting at A AAA AAG is, in fact, utilized in the decoding of an E. coli gene, *dnaX*, which encodes two DNA polymerase III subunits (Flower and McHenry, 1990; Blinkowa and Walker, 1990; Tsuchihashi and Kornberg, 1990). Frameshifting is utilized by one bacterial IS1 ("insertion sequence 1") element (Sekine and Ohtsubo, 1989), and by extrapolation from the known sequences, is likely to be utilized by at least some members of the IS3 family as well (Prère et al., 1990). In several of these examples, the shift site is again likely to be A AAA AAG.

Shifty runs alone, however, are not sufficient for high level shifting. Secondary signals programmed in the mRNA augment shifting at the slippery sequence to give high levels of frameshifting. We call these signals "stimulators," and they are very diverse (Figure 1). For the +1 shift for decoding RF2, two stimulators are utilized. One is a Shine–Dalgarno sequence located 3 nucleotides 5′ of

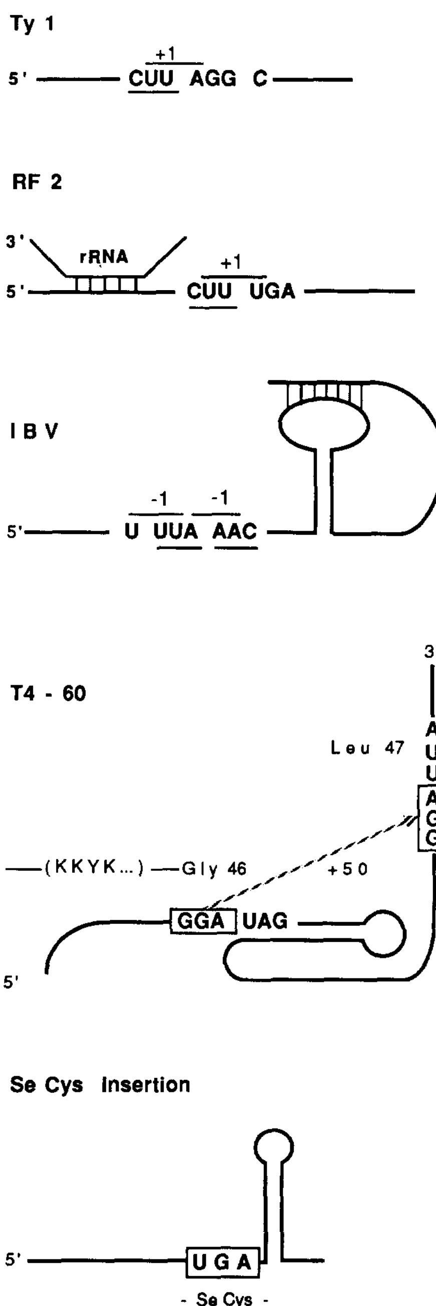

Figure 1. Schematic Comparison of Four Frameshift Models, and a Possible Structure of Nucleotides Essential for Readthrough of UGA with Selenocysteine Incorporation

With IBV, the proposed coaxial structure is not apparent in this simple representation of the pseudoknot. For Se Cys, an alternative structure involving nucleotides 5′ of the UGA may be involved.

the shift site that interacts with the 3′ end of 16S ribosomal RNA of elongating ribosomes (Weiss et al., 1988a). This finding leads to the surprising conclusion that ribosomal RNA is continuously monitoring mRNA sequences during translation. The second stimulator is a UGA terminator at codon 26 flanking the shift site on its 3′ side (Weiss et al., 1987; Curran and Yarus, 1988). The stimulators act independently with substantial activity, but their effects

are synergistic. Use of both stimulators means that RF2 frameshifting utilizes both an interaction normally involved in initiation and one involved in termination, within the middle of a decoding region!

At least in E. coli, a role of 3′ flanking termination codons in promoting frameshifting has been uncovered with constructs made in a *lacZ* reporter system. This role of stop codons has been extensively investigated, and while it may act by causing a long pause in decoding, the alternative of an abortive termination event prior to release factor binding needs to be seriously considered (Weiss et al., 1990b). The 5′ RF2 stimulator, the Shine–Dalgarno sequence, which augments a +1 shift, has the effect of forcing the mRNA in the direction it normally moves (3′ to 5′ [the ribosome normally moves 5′ to 3′ with respect to the message]), while the −1 shift at the retroviral tandem slippery sequence forces the mRNA backward (5′ to 3′). When these two components are put together in an artificial hybrid, the Shine–Dalgarno stimulator for RF2 dampens the retroviral shift. In other words, a stimulator for a +1 shift can act as an inhibitor for a −1 shift (Weiss et al., 1990b).

The stimulator for several of the retroviral shifts, and also the coronoviral and *dnaX* shifts, is 3′ mRNA sequences probably in the form of stem–loop structures. A stem–loop structure can be drawn 7 nucleotides downstream of the actual, or putative, shift site in many retroviruses (Jacks et al., 1988a; Le et al., 1989). In the absence of the stimulator, much less frameshifting occurs at the second codon of the pair, revealing a low level shift at the first codon. Evidence for the involvement of the stimulator loop region in additional pairing to form a pseudoknot has been presented for the coronavirus avian infectious bronchitis virus (IBV) (Brierley et al., 1989). The involvement of a pseudoknot has been inferred for another coronavirus, mouse hepatitis virus (Bredenbeek et al., 1990), for some, but not all, retroviral examples (Brierley et al., 1989; ten Dam et al., 1990), and in a variety of other instances (ten Dam et al., 1990). When the 3′ sequence is deleted, there is a great reduction in coronoviral or MMTV frameshifting.

How pseudoknots affect frameshifting is not clear, but is likely to be more sophisticated than an effect on decreasing or increasing the stability of the stem–loop structure. The question does, however, raise the general issue of how ribosomes open up mRNA secondary structure. Jacks et al. (1987, 1988a) proposed that the stem–loop structures appropriately positioned downstream of the retroviral shift sites cause pausing of ribosomes such that the shifting on the double slippery codons at the decoding site prior to peptide bond formation is facilitated. While the leading ribosome may be caused to pause by certain stem–loop structures, if the following ribosomes are closely spaced they may not encounter the stem–loop structure in the same way. Wolin and Walter (1988) have found that eukaryotic ribosomes can be tightly packed behind the leading ribosome at the stall site they examined in a preprolactin mRNA in vitro.

These results should be interpreted with caution, as all the higher eukaryotic frameshifting studies with altered sequences have been done with a reticulocyte lysate cell-free translation system; there are likely to be differences in the number of ribosomes loaded per message, and perhaps in the tRNA balance, from less specialized cells in vivo. Experiments with tissue culture cells are clearly needed, especially since reticulocyte lysate protein synthesis experients, or studies in yeast cells, showed little or no effect of 3′ sequences on human immunodeficiency virus (HIV) frameshifting (Wilson et al., 1988). Whether this is due to a minor quirk of the in vitro system or is a basic difference of HIV from other retroviruses has not yet been determined. The HIV family of retroviruses is different in many important ways from the other retroviruses in which frameshifting has been studied, and it will be interesting to compare any HIV frameshifting stimulator, when found, with that of the other viruses. Results from tissue culture cells will need to be compared with the findings from infected cells where altered tRNA modification has been reported. The $tRNA^{Phe}$ present in cells infected with a retrovirus that utilizes a U run as the slippery sequence lacks the highly modified Wye base in its anticodon. Similarily, the $tRNA^{Asn}$ decoding AAC in the A AAA AAC sequence lacks the Q base in its anticodon (Hatfield et al., 1989). As the earlier results have shown, these undermodifications are clearly not essential for frameshifting, but to what extent they contribute needs to be assessed.

The in vitro results must not be treated lightly, however. The amino acid sequence of the frameshift junction of in vivo synthesized *gag–pro* fusion polyprotein from MMTV has been determined, and shown to result from a shift at the same site as the in vitro product (Hizi et al., 1987). Even in E. coli cells, frameshifting on retroviral sequences is augmented in a similar way by the same stimulators as in the reticulocyte lysate system (Weiss et al., 1989). This is surprising in view of the divergence of prokaryotic and eukaryotic ribosomes. There appears to be conservation at the ribosomal level of the essential components for this type of shifting. A model for retroviral shifting based on hybrid three-site (A, P, and E) decoding (Moazed and Noller, 1989) has been presented (Weiss et al., 1989). In this variant of the original model, shifting occurs after transpeptidation and perhaps during translocation itself.

For the +1 shifting with the yeast Ty ("transposon yeast") elements (Clare and Farabaugh, 1985; Mellor et al., 1985), the shift site, CUU Agg c, does not at first glance look like a slippery repetitive run used in retroviral shifting (Belcourt and Farabaugh, 1990). However, for the $tRNA^{Leu}$ that decodes the 0 frame CUU, it is a slippery sequence. This tRNA has the anticodon 3′-GAU-5′, with an unmodified uracil in its wobble position, so is able to decode the +1 frame UUA after slipping forward 1 base. The stimulator in this case is a 3′ adjacent rare arginine codon, AGG, decoded by a minor tRNA (see Belcourt and Farabaugh, 1990) that might cause a pause in translation. When the level of the minor $tRNA^{Arg}$ is increased, the level of frameshifting decreases (Xu and Boeke, 1990). However, no other rare codon will act as a stimulator—there must be something special about the interaction of the $tRNA^{Arg}$ with its codon (Belcourt and Farabaugh, 1990).

Thus the complete information for the high level shift

(approaching 50%) in Ty1 is contained within a 7 nucleotide stretch (CUU AGG C) encompassing the shift site, and this is conserved from Ty1 to Ty2. Interestingly, when this consensus sequence is placed within four codons of the translation start site, then shifting drops down almost 40-fold to background levels (Belcourt and Farabaugh, 1990). The reason for this is unknown, but might be explained on the basis of the three-site model for ribosomes with occupancy of the exit (E) site by a noninitiator tRNA affecting A site binding (Rheinberger and Nierhaus, 1986).

Regulation by Frameshifting

E. coli RF2 uniquely causes release at UGA. Before the sequence became known, it was thought that a clever way to regulate the synthesis of RF2 would be to have an in-frame UGA stop codon early in the gene. If there was an adequate level of RF2, termination would ensue and further RF2 would not be synthesized. If RF2 was limiting, then this would allow UGG-decoding $tRNA^{Trp}$ the chance to insert Trp at the UGA and the readthrough would permit decoding of the bulk of the gene to replenish the supply of RF2. As discovered by Craigen et al. (1985), however, this model was only half correct. As introduced above, there is an in-frame UGA terminator at codon 26, but a +1 frameshift is required to decode the main part (downstream) of the gene. The frameshifting can be at high levels (30% or more), which is higher than stop codon readthrough levels (except where selenocysteine is inserted; see below), and this may be one reason for the utilization of frameshifting rather than stop codon readthrough.

Occurrence of Polyproteins Generated by Frameshifting

Many of the examples of frameshifting and readthrough have been found in viruses. This may be due to the compactness of viral genomes, but is also likey to be a reflection of our relatively greater knowledge of the expression of viral genes. The first several examples required the ribosomes to shift frame near the end of a gene, and in the new frame to bypass the normal terminator to produce an elongated protein. The function, if any, of frameshifting in the initial example, the coat lysis hybrid of phage MS2, which is due to a +1 shift (Atkins et al., 1979; Beremand and Blumenthal, 1979), remains unknown. The coat lysis hybrid is not incorporated into the virion, but the possibility that an equivalent frameshift product from the Pseudomonas RNA phage PP7 is incorporated is under investigation (Garde et al., unpublished data). The functions of the elongated products from genes *5.5* and *10* of phage T7, which are due to −1 shifts (Dunn and Studier, 1983), remain unknown. However the gene *10* shift occurs at a moderately high level and utilizes a distant 3′ stimulator (Condron et al., unpublished data).

Much more is known about the retroviral and related examples. The initial findings were with Rous sarçoma virus, where 5% of the ribosomes translating *gag* shift to the −1 frame shortly before the terminator and enter *pol* (Jacks and Varmus, 1985). With HIV the situation is similar except that the level is higher, 12% (Wilson et al., 1988; Jacks et al., 1988b). With MMTV, in contrast, two −1 shifts are required, as the protease gene lies between the *gag* and *pol* genes. A −1 shift at 23% efficiency brings ribosomes to the protease gene, and a second shift at 8% near the end of the protease gene brings ribosomes to the *pol* gene to generate a *gag–pro–pol* fusion at an overall ratio to *gag* similar to that found with the single-shift situation with Rous sarcoma virus (Moore et al., 1987; Jacks et al., 1987). Sequences similar to those responsible for frameshifting in these three retroviruses (see above) are found in the great majority of other sequenced retroviruses, making it likely that frameshifting (all −1) is used by them to make the *gag–pol* fusion polyprotein. (The alternative of stop codon readthrough is seen in murine leukemia virus and a few other retroviruses; see below). The yeast "killer" particle uses a −1 frameshift mechanism similar to that of the retroviruses (Icho and Wickner, 1989; Diamond et al., 1989). A −1 frameshift is used in the replicase gene of two coronaviruses, avian IBV and mouse hepatitis virus, to allow ribosomes to enter a region encoding a further, massive 350 kd of protein. It is likely also to be used by a number of (+)-stranded plant viruses (Miller et al., 1988; Veidt et al., 1988; Xiong and Lommel, 1989), and in at least one of these it is now being confirmed.

There seems to be no significance in the frameshifting in the above examples being −1. Many retrotransposons, retrovirus-like DNA transposable elements that replicate via an RNA intermediate during transposition, use +1 frameshifting. One example is the yeast Ty elements, which utilize +1 frameshifting to generate a coat protein–polymerase fusion at an efficiency of approximately 20%. Virus-like particles have been found for Ty1 and at least one Drosophila retrotransposon. In the Drosophila element 17.6, frameshifting is inferred to occur by analogy with its Ty equivalents, especially Ty3 (Hansen et al., 1988).

With the bacterial IS elements the product of the upstream gene *insA* binds to the terminal inverted repeat sequences of the IS element. A −1 frameshift near the end of the *insA* gene yields an *insA–insB* fusion product that competes with the *insA* product for binding to the terminal sequences and, in addition, has transposase activity. The level of frameshifting appears to determine the level of transposition (see Sekine and Ohtsubo, 1989), but whether the frameshifting level is responsive to stress or other conditions is unknown. Double-stranded DNA phages have also responded to the lure of shiftiness: in phage λ a relatively low level of −1 frameshifting (~2.5%) occurs at a double slippery codon near the end of gene *G* to generate a fusion product termed GT. GT is an essential phage tail component, and translation of the *T* open reading frame except as part of the fusion has not been detected (M. Levin, R. Hendrix, and S. Casjens, unpublished data). Frameshifting is the favored, though not the established, explanation for basal-level synthesis of outer membrane genes coding for major antigenic components of Haemophilus gonorrhoeae, with consequences for influencing the configuration of repeat elements present in these genes (Belland et al., 1989).

Readthrough with the Insertion of a "Standard" Amino Acid

For some time, the genomic RNA from the RNA phages was the only purified natural mRNA available for translation studies, and it is not surprising that the first example of readthrough of a stop codon was found in one of these phages, Qβ. Readthrough of a leaky UGA terminator at the end of the Qβ coat protein gene results in tryptophan being inserted in response to the UGA codon at the relatively low efficiency of ∼3% (Weiner and Weber, 1973). Readthrough results in a considerably elongated product that is incorporated into the virion and is essential for infectivity (Hofstetter et al., 1974). In contrast, UAG readthrough is required for synthesis of a subunit of the pili of toxigenic E. coli (Jalajakumari et al., 1989). A well-known example of UAG readthrough is found in the replicase gene of tobacco mosaic virus (Pelham, 1978). Termination at the leaky UAG stop codon results in the synthesis of a 126 kd protein, whereas readthrough (at an efficiency of 10%) leads to the synthesis of a 183 kd product essential for infectivity. The UAG can be replaced by UAA without affecting infectivity (Ishikawa et al., 1986).

In tobacco mosaic virus and two unrelated viruses, beet necrotic yellow vein virus and turnip yellow mosaic virus, the sequences immediately surrounding the leaky UAG stop codon are conserved, unlike the rest of the nearby sequences. The conserved sequences are CAA UAG CAA $U^{U}_{C}A$. We wondered if the stop codon was hopped over by $tRNA^{Gln}$, rather than read through. However, Skuzeski et al. (unpublished data) have evidence that rather than hopping being involved, there is a special context or stimulator for readthrough. A second group of plant RNA viruses has different sequences flanking the leaky UAG stop codon. This group, which includes maize chlorotic mottle virus (Nutter et al., 1989), has the sequence AAA UAG G.

Readthrough is also used by several (+)-stranded animal viruses of the alphavirus family, such as Sindbis (Strauss et al., 1983); interestingly, other members of the same family, such as Semliki Forest virus (Takkinen, 1986), have a sense codon in place of the stop codon. In Sindbis virus, stop codon readthrough (in the alphaviruses the stop codon utilized is UGA) is involved in expression of one of the replicase constituent proteins. Replacement of the UGA stop codon by either a sense codon or one of the other stop codons has subtle deleterious effects on the virus (Li and Rice, 1989). It is suspected, but not established, that the polyprotein plays some role independent of its processed components.

The first retrovirus found to have a "special" translation mechanism for the generation of its *gag–pol* fusion was murine leukemia virus, where there is in-frame readthrough of a leaky UAG terminator at the end of *gag* (Philipson et al., 1978; Yoshinaka et al., 1985a). A minority of other retroviruses, such as feline leukemia virus (Yoshinaka et al., 1985b), are very likely to use a similar mechanism. The 10% readthrough efficiency of the UAG terminator of the *gag* gene of murine leukemia virus in vivo is dependent on preservation of a strong stem–loop structure containing the UAG in the loop (Jones et al., 1989; see also Panganiban, 1988). It is presumed that the stem–loop structure is required for the readthrough process, and so it is a stimulator in the sense used above, but the possibility has not been excluded that this structure is required solely for some other viral function. ten Dam et al. (1990) have pointed out that the stem–loop containing the stop codon in the loop has not been preserved in feline leukemia virus; instead, one of the most stable potential stem–loop structures occurs just downstream of the UAG codon and is capable of forming a pseudoknot. This putative stem–loop begins 8 nucleotides 3′ of the stop codon, a similar distance from the shift site in those viruses utilizing frameshifting, and a similar structure may also be able to form in murine leukemia virus. An investigation of the requirements for UAG readthrough in the feline leukemia virus context is clearly needed.

As in the case of Sindbis, the leaky UAG of murine leukemia virus can be changed to one of the other terminators and readthrough is still observed (Feng et al., 1989a; Jones et al., 1989). It has been reported that infection with murine leukemia virus induces the synthesis of a minor $tRNA^{Gln}$, and the induction is inhibited by the antiviral compound avarol (Kuchino et al., 1987, 1988). The stimulation has not been seen by others (Feng et al., 1989b). Whatever the resolution of this issue, the minor $tRNA^{Gln}$ is probably not required for readthrough at the end of the *gag* gene.

Readthrough with Insertion of Selenocysteine: mRNA Stimulator, Special tRNA, and Elongation Factor

Recently it has been found that selenium is cotranslationally incorporated in protein in the form of selenocysteine in response to "special" UGA codons. The best studied occurrences of selenocysteine are in formate dehydrogenase of E. coli (Zinoni et al., 1986) and in glutathione peroxidase (Chambers et al., 1986) of several mammalian species. Selenocysteine is highly sensitive to oxidation, but its function is mostly unknown. Preliminary studies have shown that formate dehydrogenase containing cysteine in place of selenocysteine has 4- to 5-fold lower specific activity (Böck and Stadtman, 1988). However, apart from the evolutionary questions raised by the cotranslational insertion of selenium, the main interest raised by this remarkable discovery is in how it occurs, and what is "special" about the UGA codons that encode it.

A minor UGA-decoding seryl-tRNA in a variety of vertebrates has been known of for many years (Diamond et al., 1981). This tRNA can be converted to phosphoseryl-tRNA, which in turn (Lee et al., 1989) can be converted to selenocysteyl-$tRNA^{Ser}$. However, in E. coli the conversion giving selenocysteine on tRNA does not occur by this mechanism (see Leinfelder et al., 1990). Regardless of the details, in both eukaryotes and prokaryotes selenocysteine is the 21st amino acid found to be directly encoded. It will be interesting to see if incorporation of other natural nonstandard amino acids can be engineered by mutational alterations of aminoacyl-tRNA synthetases.

A unique tRNA for selenocysteine incorporation has also been characterized in E. coli. It has the anticodon

3′-ACU-5′, complementary to UGA in the mRNA, and an unusually long acceptor stem (Schön et al., 1989, and references therein). When the "special" UGA triplet decoded by this tRNA is replaced by a UGC cysteine codon (but not by a UCA codon with a second position change), the level of selenocysteine incorporation approaches that found when UGA is present (see Zinoni et al., 1990). The selenocysteine-inserting tRNA can therefore compete effectively with $tRNA^{Cys}$, as well as with RF2, when the mRNA context is "special."

The mRNA signals that specify incorporation of selenocysteine at the "special" UGA codon in formate dehydrogenase are partially known (Zinoni et al., 1990). The 27 nucleotides 3′ of the UGA are crucial, and the efficiency of the process is influenced by the 12 nucleotides downstream (i.e., up to 39 nucleotides 3′ of the UGA) and 9 bases upstream of the UGA. A stem–loop structure with 39 bases can be drawn immediately downstream of the UGA in formate dehydrogenase mRNA, and a similarily positioned stem–loop can be drawn immediately 3′ of the "special" UGA in human glutathione peroxidase mRNA. Whether 5′ nucleotides in formate dehydrogenase mRNA are involved in an alternate stem–loop also awaits testing.

Decoding of the "special" UGA in E. coli formate dehydrogenase mRNA also requires a unique elongation factor Tu–like protein, SELB, the product of the *selB* gene (see Forchhammer et al., 1990). SELB is considerably larger than EFTu (68 vs. 43 kd), but it may have additional functions such as recognition of the aminoacyl residue, specific recognition of the mRNA context around the selenocysteine-specific UGA codon, and/or competition for the binding of RF2 (see Forchhammer et al., 1990). The protein interacts with guanosine nucleotides and selenocysteyl-tRNA.

It is intriguing to think that there may be families of elongation factors for specific purposes, perhaps analogous to multiple RNA polymerase sigma factors. The eukaryotic analog of EFTu is EF-1. Recently, studies on yeast mutants that promoted frameshifting revealed a new EF-1-like protein that is considerably larger than EF-1 (Wilson and Culbertson, 1988). This protein plays a role in coordinating translation with global cellular events such as progression through the cell cycle (Kikuchi et al., 1988; see Culbertson et al., 1990). Surprisingly, there may even be families of different types of ribosomes in special circumstances, such as specific developmental stages in Plasmodium (Waters et al., 1989).

Frameshifting and Readthrough versus Splicing

The simple expedient of having promoters or translational start sites of different strengths can ensure a set ratio of two products. However, on occasion it is advantageous to have large amounts of one product (e.g., for structural purposes) and proportionally small amounts of another (e.g., for catalytic purposes), but to have the latter made as part of a fusion protein with the structural component at the 5′ end of the polyprotein. Most of the time, the ribosomes terminate at the end of the structural gene. However, by having a small proportion of messages with the stop codon spliced out (alternative splicing) or by its equivalent, circumventing the stop codon at the translational level, a small amount of the fusion polypeptide could be made.

Frameshifting and readthrough avoid one potentially deleterious consequence of splicing and also offer some potentially beneficial possibilities not provided by splicing. There is one consideration peculiar to (+)-stranded RNA viruses. Since they use genomic (+)-stranded RNA as a template both for protein synthesis and for replication, they need to avoid packaging spliced RNA that would lead to the accumulation of defective viruses. Many of them avoid RNA splicing completely, but others restrict splicing to the generation of spliced RNAs that do not contain the packaging site. Splicing is used, for example, to generate a subgenomic RNA that contains the leader region fused to the *env* gene, but lacking the packaging site in the *gag* gene, which encodes the structural core ("coat") proteins.

Interest in nonstandard decoding increased sharply with the finding that frameshifting or readthrough, but not splicing, is utilized to produce the *gag–pol* fusion polyproteins of retroviruses. In the retroviruses, there is no ribosome initiation at the beginning of the *pol* gene, and the *gag–pol* fusion is the only source of the catalytic *pol* products, reverse transcriptase and endonuclease. One reason for generating the *gag–pol* fusion may be to ensure the packaging of the polymerase by virtue of its attachment to the core *gag* proteins. A second may be to ensure also that the reverse transcriptase component of the *pol* product is inactive, by virtue of being part of a fusion polyprotein (Witte and Baltimore, 1978; Felsenstein and Goff, 1988), until the viral RNA is sequestered by the core proteins. The ratio of *gag–pol* product to *gag* is rather critical, giving rise to hopes that compounds affecting the process of readthrough or frameshifting may be more detrimental to viral decoding than the translation of any putative cellular genes that utilize either process.

Interestingly, a minority of retrotransposons and viruses such as hepatitis B virus (Chang et al., 1989) and cauliflower mosaic virus (Schultze et al., 1990), which are not retroviruses or retrotransposons but which use reverse transcriptase, do not utilize frameshifting or readthrough to generate their *pol* product. A comparison of their life cycles with those of the retroviruses and the majority of retrotransposons is helpful in discerning the reasons for readthrough or frameshifting (Chang et al., 1989; Schultze et al., 1990). A minor alternative to splicing, readthrough, and frameshifting is posttranscriptional editing of some molecules of a particular mRNA to generate an in-frame termination codon in the coding region. An example where only a single base is altered is apolipoprotein B, where the modification is tissue specific and subject to hormonal modulation.

Nonproduct Roles

When frameshifting or readthrough brings ribosomes to a region downstream of the gene terminator, it may not be the protein product per se that is important, but rather the consequences of ribosome movement. Translation of bacterial biosynthetic operon leader peptide genes provides a precedent for the role of ribosome movement itself being important. There is no role for the peptide product—only

the act of its synthesis is important. An equivalent role for frameshifting has not been established, but has been proposed. The −1 frameshift product of the replicase gene of the RNA phage MS2 detected in vitro (Atkins et al., 1979) has not yet been seen in vivo, but if made, the shifted ribosomes may have a crucial role in influencing the progress of phage replicase (Dayhuff et al., 1986).

Similarily, it may be that a function of ribosomes that shift during decoding of the mRNA from the *D* gene of the single-stranded DNA phage φX174 is to regulate lysis expression by unmasking mRNA structure and permitting reinitiation (Buckley and Hayashi, 1987). (However, even though frameshifting was proposed to be involved in the normal mode of expression of phage MS2 lysis [Kastelein et al., 1982], this was later shown to be incorrect for elaborate reasons [Berkhout et al., 1987].) Though complicated and only partially understood, the nonproduct role of translation of the leader peptide gene of the tryptophanase operon, which encodes an enzyme for the degradation of tryptophan, is likely to be intriguing (Gollnick and Yanofsky, 1990). It may well be that ribosomes downstream of the terminator in some instances will have a role in influencing mRNA half-life by disrupting secondary structure and thus the rate of degradation by RNAases. In certain cases, such a device could be used to tie the timing of degradation to the number of times the message has been translated.

Truncated Proteins

When shifted ribosomes encounter a stop codon in the new frame before bypassing the terminator in the original frame, they will synthesize a truncated product that will have some amino acids at its carboxy-terminal end not present in the 0 frame product. At an early stage this was suggested as the explanation for some plant viral products, but has not been substantiated. However, this is the explanation for the decoding of a 52 kd subunit of E. coli DNA polymerase III from the gene *dnaX*, where the 0 frame encodes the 71 kd polymerase subunit. The −1 frameshift event occurs two-thirds of the way through the gene transcript at a 50% level, and the shifted ribosomes terminate early in the new frame to yield the 52 kd protein. Both products are present at high levels in the polymerase complex. Both subunits share a binding site for ATP (or dATP), but only the larger subunit has a DNA-dependent ATPase activity, presumably due to a DNA binding site present in its carboxy-terminal domain (see Tsuchihashi and Kornberg, 1990). Why the two different subunits are utilized and why frameshifting is involved remain intriguing questions. It has been proposed that the longer product is associated with the highly processive leading-strand half of the polymerase, while the shorter, frameshifting-derived product may be associated with the lagging-strand half (see Flower and McHenry, 1990).

Shifting at "Hungry" and Noncognate Codons

In vitro protein synthesis experiments with an unperturbed mix of normal tRNAs have shown that E. coli $tRNA_3^{Ser}$ (anticodon 3′-[U]CG-5′) and $tRNA_3^{Thr}$ (anticodon 3′-[U]GG-5′), at an efficiency of a few percent, read the first 2 bases of GCA alanine and CCG proline codons, respectively, to cause −1 frameshifting (Atkins et al., 1979; Dayhuff et al., 1986). This type of frameshifting is not a general property of tRNAs but, at least at a high level, may be unique to $tRNA_3^{Ser}$ and $tRNA_3^{Thr}$. Anticodon replacement experiments have shown that the shifting ability is a special property of the anticodon and not a peculiarity of the rest of the tRNA (Bruce et al., 1986). Increasing the ratio of $tRNA_3^{Ser}$ or $tRNA_3^{Thr}$ in proportion to the levels of the tRNAs that normally decode the GCA alanine and CCG proline codons increases the level of frameshifting. This noncognate type of mechanism has been proposed to explain the frameshifting seen with φX174.

The alternative way to perturb the balance of aminoacylated tRNAs is to cause amino acid starvation (Weiss and Gallant, 1983). In some instances this also causes frameshifting, but in the cases analyzed it is due to cognate reading of an overlapping triplet codon rather than to noncognate reading of an in-frame doublet codon (Weiss et al., 1988b). There is currently no evidence that frameshifting promoted by amino acid starvation is utilized in vivo, but it is not very different from the Ty case where a combination of rare codon and minor tRNA is the stimulator for frameshifting at a slippery codon. Before the mechanism of Ty frameshifting was discovered, frameshifting promoted by tandem rare AGG arginine codons had been investigated in E. coli. AGG AGG placed in a gene expressed from an efficient promoter off a high copy number plasmid showed +1 frameshifting at this sequence at an efficiency of up to 50% (Spanjaard and van Duin, 1988). The shift occurs at the rare codons, but is dependent on extreme expression levels that may result in sequestration of the $tRNA^{Arg}$ at the first of the two codons so that the tRNA may be limiting even for the first codon and more so for the second codon.

Normal Frame Maintenance

An estimate of the "background" level of frameshifting came from studies of the leakiness of frameshift mutants (Atkins et al., 1972; Kurland, 1979). However, some frameshift mutants are leaky at much higher levels than others, owing to the chance occurrence of nearby sequences prone to shiftiness (Fox and Weiss-Brummer, 1980; Atkins et al., 1983). A more revealing study has examined the level of frameshifting in a long natural sequence that is free of stop codons in one or another of the alternative frames. The value obtained for an aggregate of over 90 codons in each alternative frame was, at most, a few percent. Interestingly, the level of shifting was not higher when frameshifting from the alternative frame to the wild-type 0 frame was monitored (Weiss et al., 1990b). This result argues against a recently proposed (Trifonov, 1987) intrinsic framing mechanism within the coding sequence.

One reason for isolating mutants that promote frameshifting (frameshift mutant suppressors) was to try to define the components responsible for frame maintenance. The first mutant sequenced had a tRNA anticodon enlarged by 1 base and caused a 4 base translocation (see Riddle and Carbon, 1973). However, several frameshift suppressors first isolated (Riyasaty and Atkins, 1968) have

the normal 7 base anticodon loops (with their changes elsewhere in the tRNA) and yet cause both doublet and triplet reading (O'Mahony et al., 1989). The tRNA mutants do not permit a simple answer to the question of frame maintenance (Tuohy et al., 1990; see Culbertson et al., 1990). Perhaps the most telling experiment is that reported by Spirin (1987), where under certain conditions tRNA was translocated in mRNA-free ribosomes, implicating, as expected, tRNA as a principal component of normal mRNA movement. However, as has been pinpointed by the suppressor studies, many other components of the translation apparatus, such as EF-Tu and rRNA, influence frame maintenance.

Codon–anticodon interaction involving only 3 bp is insufficiently strong in the absence of ribosomes to permit decoding. This poses a problem for considering the evolutionary origin of decoding, which necessarily took place before the advent of ribosomes with their numerous protein constituents. One possibility is that the original codon–anticodon interaction involved 5 bp, which would confer the necessary stability. Woese (1970) and Crick et al. (1976) proposed a model for how this may happen without disastrous consequences in subsequently changing to triplet codon–anticodon pairing on ribosomes. In their reciprocating ratchet model, the tRNA paired with 5 codon bases but only a 3 base codon was decoded. A modified form of this model (Weiss, 1984) seemed an attractive explanation for several of the anomalous results obtained with the hungry codon and noncognate frameshifting studies described in the last section, but further work has shown that this model is unlikely (Bruce et al., 1986; Weiss et al., 1988b). Since then, a different type of explanation for the dilemma of the origin of decoding has been advanced. It has been proposed that ribosomal RNA, without protein involvement, can stabilize codon–anticodon interaction by coaxial stacking of ribosomal RNA (Noller et al., 1986). Such stacking could, by strengthening codon–anticodon interaction, also help in maintaining the reading frame.

High levels of frameshifting programmed by signals in the mRNA are a far cry from the once widely held view (see Whitfield et al., 1966) that decoding is invariably sequentially triplet. Even now, the increasingly common recurrence of the retroviral type −1 frameshifting might lead one to think that there is a narrowly limited number of mechanisms and circumstances where efficient ribosomal frameshifting occurs. However, the variety of new examples of single-base frameshifting, as well as hopping and high level readthrough, makes it likely that nature has many tricks in store. The stimulatory effect of RNA–RNA interactions, often as stem–loop structures, in enhancing an unusual translation event by elongating ribosomes is an interesting and new feature. A tempting thought is that the diversity revealed is a reflection of the varied mechanisms used to cause pausing, but there is clearly much more involved. The novelty and intricacy of the unfolding insights into these phenomena reinforce the view that translational elongation and termination are proving to be no exceptions to the rich versatility being revealed in nearly every aspect of gene expression.

Acknowledgments

We thank Diane Dunn, Norma Wills, and, at Trinity College Dublin, Shahla Thompson, for their major contributions.

References

Atkins, J. F., Elseviers, D., and Gorini, L. (1972). Low activity of β-galactosidase in frameshift mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. USA *69*, 1192–1195.

Atkins, J. F., Gesteland, R. F., Reid, B. R., and Anderson, C. W. (1979). Normal tRNAs promote ribosomal frameshifting. Cell *18*, 1119–1131.

Atkins, J. F., Nichols, B. P., and Thompson, S. (1983). The nucleotide sequence of the first externally suppressible −1 frameshift mutant, and of some nearby leaky frameshift mutants. EMBO J. 2, 1345–1350.

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*, 000–000.

Belland, R. J., Morrison, S. G., van der Ley, P., and Swanson, J. (1989). Expression and phase variation of gonococcal P.II genes in *Escherichia coli* involves ribosomal frameshifting and slipped-strand mispairing. Mol. Microbiol. *3*, 777–786.

Beremand, M. N., and Blumenthal, T. (1979). Overlapping genes in RNA phage: a new protein implicated in lysis. Cell *18*, 257–266.

Berkhout, B., Schmidt, B. F., van Strien, A., van Boom, J., van Westrenen, J., and van Duin, J. (1987). Lysis gene of bacteriophage MS2 is activated by translation termination of the overlapping coat gene. J. Mol. Biol. *195*, 517–524.

Blinkowa, A. L., and Walker, J. R. (1990). Programmed ribosomal frameshifting generates the *Escherichia coli* DNA polymerase III γ subunit from within the τ subunit reading frame. Nucl. Acids Res. *18*, 1725–1729.

Böck, A., and Stadtman, T. (1988). Selenocysteine, a highly specific component of certain enzymes, is incorporated by a UGA-directed cotranslational mechanism. BioFactors *1*, 245–250.

Bredenbeek, P. J., Pachuk, C. J., Noten, A. F. H., Charité, J., Luytjes, W., Weiss, S. R., and Spaan, W. J. M. (1990). The primary structure and expression of the second open reading frame of the polymerase gene of the coronavirus MHV-A59; a highly conserved polymerase is expressed by an efficient ribosomal frameshifting mechanism. Nucl. Acids Res. *18*, 1825–1832.

Brierley, I., Boursnell, M. E. G., Binns, M. M., Bilimoria, B., Blok, V. C., Brown, T. D. K., and Inglis, S. C. (1987). An efficient ribosomal frameshifting signal in the polymerase-encoding region of the coronavirus IBV. EMBO J. *6*, 3779–3785.

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.

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.

Buckley, K. J., and Hayashi, M. (1987). Role of premature translational termination in the regulation of expression of the φX174 lysis gene. J. Mol. Biol. *198*, 599–607.

Chambers, I., Frampton, J., Goldfarb, P., Affara, N., McBain, W., and Harrison, P. R. (1986). The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the "termination" codon, TGA. EMBO J. *5*, 1221–1227.

Chang, L.-J., Pryciak, P., Ganem, D., and Varmus, H. E. (1989). Biosynthesis of the reverse transcriptase of hepatitis B viruses involves *de novo* translational initiation not ribosomal frameshifting. Nature *337*, 364–368.

Clare, J., and Farabaugh, P. (1985). Nucleotide sequence of a yeast Ty element: evidence for an unusual mechanism of gene expression. Proc. Natl. Acad. Sci. USA *82*, 2829–2833.

Craigen, W. J., Cook, R. G., Tate, W. P., and Caskey, C. T. (1985). Bacterial peptide chain release factors: conserved primary structure and

possible frameshift regulation of release factor 2. Proc. Natl. Acad. Sci. USA *82*, 3616–3620.

Crick, F. H. C., Brenner, S., Klug, A., and Pieczenik, G. (1976). A speculation on the origin of protein synthesis. Origins of Life *7*, 389–397.

Culbertson, M. R., Leeds, P., Sandbaken, M. G., and Wilson, P. G. (1990). Frameshift suppression. In Ribosomes: Structure and Function, W. Hill, P. Moore, R. Garrett, J. Warner, A. Dahlberg, and D. Schlessinger, eds. (Washington, D.C.: American Society for Microbiology), in press.

Curran, J. F., and Yarus, M. (1988). Use of tRNA suppressors to probe regulation of *Escherichia coli* release factor 2. J. Mol. Biol. *203*, 75–83.

Dahlberg, A. E. (1989). The functional role of ribosomal RNA in protein synthesis. Cell *57*, 525–529.

Dayhuff, T. J., Atkins, J. F., and Gesteland, R. F. (1986). Characterization of ribosomal frameshift events by protein sequence analysis. J. Biol. Chem. *261*, 7491–7500.

Diamond, A., Dudock, B., and Hatfield, D. (1981). Structure and properties of a bovine liver UGA suppressor serine tRNA with a tryptophan anticodon. Cell *25*, 497–506.

Diamond, M. E., Dowhanick, J. J., Nemeroff, M. E., Pietras, D. F., Tu, C., and Bruenn, J. A. (1989). Overlapping genes in a yeast double-stranded RNA virus. J. Virol. *63*, 3983–3990.

Dunn, J. J., and Studier, F. W. (1983). Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. J. Mol. Biol. *166*, 477–535.

Falahee, M. B., Weiss, R. B., O'Connor, M., Doonan, S., Gesteland, R. F., and Atkins, J. F. (1988). Mutants of translational components that alter reading frame by two steps forward or one step back. J. Biol. Chem. *263*, 18099–18103.

Felsenstein, K. M., and Goff, S. P. (1988). Expression of the *gag–pol* fusion protein of Moloney murine leukemia virus without *gag* protein does not induce virion formation or proteolytic processing. J. Virol. *62*, 2179–2182.

Feng, Y.-X., Levin, J. G., Hatfield, D. L., Schaefer, T. S., Gorelick, R. J., and Rein, A. (1989a). Suppression of UAA and UGA termination codons in mutant murine leukemia viruses. J. Virol. *63*, 2870–2873.

Feng, Y.-X., Hatfield, D. L., Rein, A., and Levin, J. G. (1989b). Translational readthrough of the murine leukemia virus *gag* gene amber codon does not require virus-induced alteration of tRNA. J. Virol. *63*, 2405–2410.

Flower, A. M., and McHenry, C. S. (1990). The γ subunit of DNA polymerase III holoenzyme of *Escherichia coli* is produced by ribosomal frameshifting. Proc. Natl. Acad. Sci. USA *87*, 3713–3717.

Forchhammer, K., Rücknagel, K.-P., and Böck, A. (1990). Purification and biochemical characterization of SELB, a translation factor involved in selenoprotein synthesis. J. Biol. Chem. *265*, 9346–9350.

Fox, T. D., and Weiss-Brummer, B. (1980). Leaky +1 and −1 frameshift mutations at the same site in a yeast mitochondrial gene. Nature *288*, 60–63.

Gollnick, P., and Yanofsky, C. (1990). $tRNA^{Trp}$ translation of leader peptide codon 12 and other factors that regulate expression of the tryptophanase operon. J. Bacteriol. *172*, 3100–3107.

Hansen, L. J., Chalker, D. L., and Sandmeyer, S. B. (1988). Ty3, a yeast retrotransposon associated with tRNA genes, has homology to animal retroviruses. Mol. Cell. Biol. *8*, 5245–5256.

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 of HIV, HTLV-1, and BLV. Virology *173*, 736–742.

Hizi, A., Henderson, L. E., Copeland, T. D., Sowder, R. C., Hixson, C. V., and Oroszlan, S. (1987). Characterization of mouse mammary tumor virus *gag–pro* gene products and the ribosomal frameshift site by protein sequencing. Proc. Natl. Acad. Sci. USA *84*, 7041–7045.

Hofstetter, H., Monstein, H.-J., and Weissman, C. (1974). The read-through protein A_1 is essential for the formation of viable Qβ particles. Biochim. Biophys. Acta *374*, 238–251.

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.

Hughes, D., Thompson, S., O'Connor, M., Tuohy, T., Nichols, B. P., and Atkins, J. F. (1989). Genetic characterization of frameshift suppressors with new decoding properties. J. Bacteriol. *171*, 1028–1034.

Icho, T., and Wickner, R. B. (1989). The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. J. Biol. Chem. *264*, 6716–6723.

Ishikawa, M., Meshi, T., Motoyoshi, F., Takamatsu, N., and Okada, Y. (1986). *In vitro* mutagenesis of the putative replicase genes of tobacco mosaic virus. Nucl. Acids Res. *14*, 8291–8305.

Jacks, T., and Varmus, H. E. (1985). Expression of the Rous sarcoma virus *pol* gene by ribosomal frameshifting. Science *230*, 1237–1242.

Jacks, T., Townsley, K., Varmus, H. E., and Majors, J. (1987). Two efficient ribosomal frameshifting events are required for synthesis of mouse mammary tumor virus *gag*-related polyproteins. Proc. Natl. Acad. Sci. USA *84*, 4298–4302.

Jacks, T., Madhani, H. D., Masiarz, F. R., and Varmus, H. E. (1988a). Signals for ribosomal frameshifting in the Rous sarcoma virus *gag–pol* region. Cell *55*, 447–458.

Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J., and Varmus, H. E. (1988b). Characterization of ribosomal frameshifting in HIV-1 *gag–pol* expression. Nature *331*, 280–283.

Jalajakumari, M. B., Thomas, C. J., Halter, R., and Manning, P. A. (1989). Genes for biosynthesis and assembly of CS3 pili of CFA/II enterotoxigenic *Escherichia coli*: novel regulation of pilus production by bypassing an amber codon. Mol. Microbiol. *3*, 1685–1695.

Jones, D. S., Nemoto, F., Kuchino, Y., Masuda, M., Yoshikura, H., and Nishimura, S. (1989). The effect of specific mutations at and around the *gag–pol* gene junction of Moloney murine leukaemia virus. Nucl. Acids Res. *15*, 5933–5945.

Kastelein, R. A., Remaut, E., Fiers, W., and van Duin, J. (1982). Lysis gene expression of RNA phage MS2 depends on a frameshift during translation of the overlapping coat protein gene. Nature *295*, 35–41.

Kikuchi, Y., Shimatake, H., and Kikuchi, A. (1988). A yeast gene required for the G_1–to-S transition encodes a protein containing an A-kinase target site and GTPase domain. EMBO J. *7*, 1175–1182.

Kuchino, Y., Beier, H., Akita, N., and Nishimura, S. (1987). Natural UAG suppressor glutamine tRNA is elevated in mouse cells infected with Moloney murine leukemia virus. Proc. Natl. Acad. Sci. USA *84*, 2668–2672.

Kuchino, Y., Nishimura, S., Schröder, H. C., Rottmann, M., and Müller, W. E. G. (1988). Selective inhibition of formation of suppressor glutamine tRNA in Moloney murine leukemia virus-infected NIH-3T3 cells by avarol. Virology *65*, 518–526.

Kurland, C. G. (1979). Reading frame errors on ribosomes. In Nonsense Mutations and tRNA Suppressors, J. E. Celis and J. D. Smith, eds. (New York: Academic Press), pp. 97–108.

Le, S.-Y., Chen, J.-H., and Maizel, J. V. (1989). Thermodynamic stability and statistical significance of potential stem–loop structures situated at the frameshift sites of retroviruses. Nucl. Acids Res. *17*, 6143–6152.

Lee, B. J., Worland, P. J., Davis, J. N., Stadtman, T. C., and Hatfield, D. L. (1989). Identification of a selenocysteyl-$tRNA^{Ser}$ in mammalian cells that recognizes the nonsense codon, UGA. J. Biol. Chem. *264*, 9724–9727.

Leinfelder, W., Forchhammer, K., Veprek, B., Zehelein, E., and Böck, A. (1990). *In vitro* synthesis of selenocysteinyl-$tRNA_{UCA}$ from seryl-$tRNA_{UCA}$: involvement and characterization of the *selD* gene product. Proc. Natl. Acad. Sci. USA *87*, 543–547.

Li, G., and Rice, C. (1989). Mutagenesis of the in-frame opal termination codon preceding nsP4 of sindbis virus: studies of translational readthrough and its effect on virus replication. J. Virol. *63*, 1326–1337.

Mellor, J., Fulton, S. M., Dobson, M. J., Wilson, W., Kingsman, S. M., and Kingsman, A. J. (1985). A retrovirus-like strategy for expression of a fusion protein encoded by yeast transposon Ty1. Nature *313*, 243–246.

Miller, W. A., Waterhouse, P. M., and Gerlach, W. L. (1988). Sequence

and organization of barley yellow dwarf virus genomic RNA. Nucl. Acids Res. *16*, 6097–6111.

Moazed, D., and Noller, H. F. (1989). Intermediate states in the movement of transfer RNA in the ribosome. Nature *342*, 142–148.

Moore, R., Dixon, M., Smith, R., Peters, G., and Dickson, C. (1987). Complete nucleotide sequence of a milk-transmitted mouse mammary tumor virus: two frameshift events are required for translation of *gag* and *pol*. J. Virol. *61*, 480–490.

Noller, H. F., Asire, M., Barta, A., Douthwaite, S., Goldstein, T., Gutell, R. R., Moazed, D., Normanly, J., Prince, J. B., Stern, S., Triman, K., Turner, S., VanStolk, B., Wheaton, V., Weiser, B., and Woese, C. R. (1986). Studies on the structure and function of ribosomal RNA. In Structure, Function and Genetics of Ribosomes, B. Hardesty and G. Kramer, eds. (New York: Springer-Verlag), pp. 143–163.

Nutter, R. C., Scheets, K., Panganiban, L. C., and Lommel, S. A. (1989). The complete nucleotide sequence of the maize chlorotic mottle virus genome. Nucl. Acids Res. *17*, 3163–3177.

O'Connor, M., Gesteland, R. F., and Atkins, J. F. (1989). tRNA hopping: enhancement by an expanded anticodon. EMBO J. *8*, 4315–4323.

O'Mahony, D. J., Mims, B. H., Thompson, S., Murgola, E. J., and Atkins, J. F. (1989). Glycine tRNA mutants with normal anticodon loop size cause −1 frameshifting. Proc. Natl. Acad. Sci. USA *86*, 7979–7983.

Panganiban, A. T. (1988). Retroviral *gag* gene amber codon suppression is caused by an intrinsic *cis*-acting component of the viral mRNA. J. Virol. *62*, 3574–3580.

Pelham, H. R. B. (1978). Leaky UAG termination codon in tobacco mosaic virus RNA. Nature *272*, 469–471.

Philipson, L., Andersson, P., Olshevsky, U., Weinberg, R., Baltimore, D., and Gesteland, R. (1978). Translation of MuLV and MSV RNAs in nuclease-treated reticulocyte extracts: enhancement of the *gag–pol* polypeptide with yeast suppressor tRNA. Cell *13*, 189–199.

Prère, M.-F., Chandler, M., and Fayet, O. (1990). Transposition in *Shigella*: isolation and analysis of IS911, a new member of the IS3 group of insertion sequences. J. Bacteriol. *172*, 4090–4099.

Rheinberger, H.-J., and Nierhaus, K. H. (1986). Allosteric interactions between the ribosomal transfer RNA-binding sites A and E. J. Biol. Chem. *261*, 9133–9139.

Riddle, D. L., and Carbon, J. C. (1973). Frameshift suppression: a nucleotide addition in the anticodon of a glycine transfer RNA. Nature New Biol. *242*, 230–234.

Riyasaty, S., and Atkins, J. F. (1968). External suppression of a frameshift mutant in *Salmonella*. J. Mol. Biol. *34*, 541–557.

Schön, A., Böck, A., Ott, G., Sprinzl, M., and Söll, D. (1989). The selenocysteine-inserting opal suppressor serine tRNA from *E. coli* is highly unusual in structure and modification. Nucl. Acids Res. *17*, 7159–7165.

Schultze, M., Hohn, T., and Jiricny, J. (1990). The reverse transcriptase gene of cauliflower mosaic virus is translated separately from the capsid gene. EMBO J. *9*, 1177–1185.

Sekine, Y., and Ohtsubo, E. (1989). Frameshifting is required for production of the transposase encoded by insertion sequence 1. Proc. Natl. Acad. Sci. USA *86*, 4609–4613.

Spanjaard, R. A., and van Duin, J. (1988). Translation of the sequence AGG-AGG yields 50% ribosomal frameshift. Proc. Natl. Acad. Sci. USA *85*, 7967–7971.

Spirin, A. S. (1987). Structural dynamic aspects of protein synthesis on ribosomes. Biochimie *69*, 949–956.

Strauss, E. G., Rice, C. M., and Strauss, J. H. (1983). Sequence coding for the alphavirus nonstructural proteins is interrupted by an opal termination codon. Proc. Natl. Acad. Sci. USA *80*, 5271–5275.

Takkinen, K. (1986). Complete nucleotide sequence of the nonstructural protein genes of Semliki Forest virus. Nucl. Acids Res. *14*, 5667–5682.

ten Dam, E. B., Pleij, C. W. A., and Bosch, L. (1990). RNA pseudoknots: translational frameshifting and readthrough on viral RNAs. Virus Genes *4*, in press.

Trifonov, E. N. (1987). Translation framing code and frame-monitoring mechanism as suggested by the analysis of mRNA and 16S rRNA nucleotide sequences. J. Mol. Biol. *194*, 643–652.

Tsuchihashi, Z., and Kornberg, A. (1990). Translational frameshifting generates the γ subunit of DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA *87*, 2516–2520.

Tuohy, T. M. F., Thompson, S., Hughes, D., Gesteland, R. F., and Atkins, J. F. (1990). The role of EF-Tu and other translation components in determining translocation step size. Biochim. Biophys. Acta, in press.

Veidt, I., Lot, H., Leiser, M., Scheidecker, D., Guilley, H., Richards, K., and Jonard, G. (1988). Nucleotide sequence of beet western yellows virus RNA. Nucl. Acids Res. *16*, 9917–9932.

Waters, A. P., Syin, C., and McCutchan, T. F. (1989). Developmental regulation of stage-specific ribosome populations in *Plasmodium*. Nature *342*, 438–440.

Weiner, A. M., and Weber, K. (1973). A single UGA codon functions as a natural termination signal in the coliphage Qβ coat protein cistron. J. Mol. Biol. *80*, 837–855.

Weiss, R. B. (1984). Molecular model of ribosome frameshifting. Proc. Natl. Acad. Sci. USA *81*, 5797–5801.

Weiss, R. B., and Gallant, J. (1983). Mechanism of ribosomal frameshifting during translation of the genetic code. Nature *302*, 389–393.

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.

Weiss, R. B., Dunn, D. M., Dahlberg, A. E., Atkins, J. F., and Gesteland, R. F. (1988a). Reading frame switch caused by base-pair formation between the 3′ end of 16S rRNA and the mRNA during elongation of protein synthesis in *Escherichia coli*. EMBO J. *7*, 1503–1507.

Weiss, R. B., Lindsley, D., Falahee, B., and Gallant, J. (1988b). On the mechanism of ribosomal frameshifting at hungry codons. J. Mol. Biol. *203*, 403–410.

Weiss, R. B., Dunn, D. M., Shuh, M., Atkins, J. F., and Gesteland, R. F. (1989). *E. coli* ribosomes re-phase on retroviral frameshift signals at rates ranging from 2 to 50 percent. New Biol. *1*, 159–169.

Weiss, R. B., Huang, W. M., and Dunn, D. M. (1990a). 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. (1990b). Ribosomal frameshifting from −2 to +50 nucleotides. Prog. Nucl. Acids Res. Mol. Biol. *39*, 159–183.

Werner, M., Feller, A., Messenguy, F., and Piérard, A. (1987). The leader peptide of yeast gene *CPA1* is essential for the translational repression of its expression. Cell *49*, 805–813.

Whitfield, H. J., Martin, R. G., and Ames, B. N. (1966). Classification of aminotransferase (*C* gene) mutants in the histidine operon. J. Mol. Biol. *21*, 335–355.

Wilson, P. G., and Culbertson, M. R. (1988). *suf12* suppressor protein of yeast. A fusion protein related to the EF-1 family of elongation factors. J. Mol. Biol. *199*, 559–573.

Wilson, W., Braddock, M., Adams, S. E., Rathjen, P. D., Kingsman, S. M., and Kingsman, A. J. (1988). HIV expression strategies: ribosomal frameshifting is directed by a short sequence in both mammalian and yeast systems. Cell *55*, 1159–1169.

Witte, O. N., and Baltimore, D. (1978). Relationship of retrovirus polyprotein cleavages to virion maturation studied with temperature-sensitive murine leukemia virus mutants. J. Virol. *26*, 750–761.

Woese, C. (1970). Molecular mechanics of translation: a reciprocating ratchet mechanism. Nature *226*, 817–820.

Wolin, S. L., and Walter, P. (1988). Ribosome pausing and stacking during translation of a eukaryotic mRNA. EMBO J. *7*, 3559–3569.

Wong, S. C., and Abdelal, A. T. (1990). Unorthodox expression of an enzyme: evidence for an untranslated region within *carA* from *Pseudomonas aeruginosa*. J. Bacteriol. *172*, 630–642.

Xiong, Z., and Lommel, S. A. (1989). The complete nucleotide sequence and genome organization of red clover necrotic mosaic virus RNA-1. Virology *171*, 543–554.

Xu, H., and Boeke, J. D. (1990). Inhibition of Ty1 transposition by mating pheromone in yeast. Proc. Natl. Acad. Sci. USA *87*, in press.

Yen, T. J., Machlin, P. S., and Cleveland, D. W. (1988). Autoregulated instability of β-tubulin mRNAs by recognition of the nascent amino terminus of β-tubulin. Nature *334*, 580–585.

Yoshinaka, Y., Katoh, I., Copeland, T. D., and Oroszlan, S. (1985a). Murine leukemia virus protease is encoded by the *gag–pol* gene and is synthesized through suppression of an amber termination codon. Proc. Natl. Acad. Sci. USA *82*, 1618–1622.

Yoshinaka, Y., Katoh, I., Copeland, T. D., and Oroszlan, S. (1985b). Translational readthrough of an amber termination codon during synthesis of feline leukemia virus protease. J. Virol. *55*, 870–873.

Zinoni, F., Birkmann, A., Stadtman, T. C., and Böck, A. (1986). Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA *83*, 4650–4654.

Zinoni, F., Heider, J., and Böck, A. (1990). Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. Proc. Natl. Acad. Sci. USA *87*, 4660–4664.

Notes Added in Proof

Recent work on IBV frameshifting has shown that the structure of the stems and the minimal size of the two loops are important, but there is no apparent sequence requirement within the loops (Inglis et al., In Post-Transcriptional Control of Gene Expression, J. E. G. McCarthy, M. F. Tuite, and A. N. Brown, eds. [Berlin: Springer-Verlag], in press, 1990). For a discussion of frameshifting versus splicing, see also Wickner (FASEB J. *3*, 2257–2265, 1989).

This work was supported by the Howard Hughes Medical Institute and National Institutes of Health grant 12295C-02.