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PROGRAMMED TRANSLATIONAL FRAMESHIFTING

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ABSTRACT

Errors that alter the reading frame occur extremely rarely during translation, yet some genes have evolved sequences that efficiently induce frameshifting. These sequences, termed programmed frameshift sites, manipulate the translational apparatus to promote non-canonical decoding.

Frameshifts are mechanistically diverse. Most cause a −1 shift of frames; the first such site was discovered in a metazoan retrovirus, but they are now known to be dispersed quite widely among evolutionarily diverse species. +1 frameshift sites are much less common, but again dispersed widely. The rarest form are the translational hop sites which program the ribosome to bypass a region of several dozen nucleotides. Each of these types of events are stimulated by distinct mechanisms. All of the events share a common phenomenology in which the programmed frameshift site causes the ribosome to pause during elongation so that the kinetically unfavorable alternative decoding event can occur. During this pause most frameshifts occur because one or more ribosome-bound tRNAs slip between cognate or near-cognate codons. However, even this generalization is not entirely consistent, since some frameshifts occur without slippage. Because of their similarity to rarer translational errors, programmed frameshift sites provide a tool with which to probe the mechanism of frame maintenance.

INTRODUCTION

Transfer of genetic information from DNA to proteins must be faithful. Cells have evolved mechanisms to recognize and eliminate errors in information transfer at each step of that pathway—transcription, processing, and translation. Because of the multiple error-correction mechanisms, errors in information

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transfer are very rare. However, some genes have evolved that modify translation so as to express protein products by mechanisms that closely resemble spontaneous errors. In some genes, translation efficiently continues through in-frame termination codons (termed programmed readthrough). In others, the ribosome changes the frame in which it reads the mRNA, shifting either in the leftward, or upstream direction (termed programmed −1 frameshifting) or in the rightward or downstream direction (termed programmed +1 frameshifting). The ribosome is even capable of bypassing a short stretch of nucleotide sequence by what is termed a programmed translational hop. Each of these events superficially resembles a spontaneous translational error. For example, programmed readthrough resembles a missense error since a noncognate tRNA reads the in-frame termination codon. It is by no means clear that this resemblence reflects a mechanistic similarity between the spontaneous and programmed events, though evidence is beginning to accumulate to link the +1 frameshift events to their spontaneous counterparts.

Frameshifting is conceptually a simple process. At a particular step in the cycle of translational elongation the ribosome shifts its reading frame from the one it initiated translating into a new reading frame. The consequence of this event is that the ribosome will continue reading in the new frame until it encounters a termination codon. Since this event occurs during elongation, the protein product expressed will partly be encoded in the normal frame upstream of the frameshift, and partly in the shifted frame downstream of it. Spontaneous events of this sort invariably lead to premature termination of translation since a termination codon will normally be encountered in the shifted frame in the first few dozen codons. It is difficult to estimate the frequency of spontaneous frameshifts, as discussed by Kurland (53). The common means that has been used to estimate the frequency of frameshift errors is to measure the frequency of phenotypic suppression of frameshift mutations. The estimates vary, but a frequency of 3×10^{-5} per codon is appropriate (52). The problem with this assay is that any frameshift mutation introduces an in-frame nonsense codon that can stimulate the frequency of frameshifting, overestimating the frequency of spontaneous frameshifting (see below).

In some genes the frequency of frameshifting is much higher, approaching 100%. These efficient frameshifts are termed programmed frameshifts since invariably important structural features of the frameshift sites predispose the ribosome toward the shift in frames, and thus program the change. As a consequence of the event, the protein product is not directly encoded in the DNA as a single open-reading-frame (ORF), but in two overlapping reading frames. Since these events are nearly always much less than 100% efficient, frameshifting also allows for the expression of two primary translational products from a single mRNA that share the N-terminal sequence encoded upstream of the

shift, and differ in the sequence encoded downstream of the shift. This review focuses on the mechanism of programmed translational frameshifting and its connection to translational errors. Excellent reviews exist that deal with the issue of spontaneous translational errors (51–53, 75), and on the phenomenology of programmed translational frameshifting (2, 30).

FUNCTIONS OF PROGRAMMED FRAMESHIFTS

Spontaneous frameshifting occurs at a very low frequency, and increasing that frequency requires that the normal rules of translational elongation be altered. The change can be structural—increasing the size of the anticodon loop of particular tRNAs, or changing the structure of the elongation factor that delivers aminoacyl-tRNA to the ribosomal A site. The change can also be physiological—starving cells for specific amino acids can stimulate frameshifting at codons that specify those amino acids. In each case, frameshifting results because the competition between canonical, in-frame decoding and frameshifting is shifted in the direction of the noncanonical event. In some cases, the competition is shifted because the likelihood of the normal decoding event is decreased, whereas in others the likelihood of the abnormal frameshift event is increased. The changes described are global since they alter the translational machinery or its function on all genes. At programmed frameshift sites it is local changes to mRNA sequences that stimulate frameshift efficiency. They do so also by reducing the efficiency of normal decoding, or by increasing the efficiency of frameshift decoding, and often by a combination of both effects. Just as suppressor mutations identify elements of the translational apparatus necessary for frame maintenance, programmed frameshift sites may also identify other required elements.

Programmed frameshifts appear in genes from a variety of organisms—from bacteria, to lower eukaryotes (the yeast *Saccharomyces cerevisiae*), to animal and plant viruses—and in a variety of genes. These programmed frameshifts have likely evolved multiple times, converging on a common set of solutions, since they appear in genes with little or no evolutionary relatedness: the genes for peptide release factor 2 and a subunit of DNA polymerase III in *E. coli*; the gene for ornithine decarboxylase antizyme in mammalian cells; and genes encoding transposition enzymes in insertion sequences in bacteria, retrotransposons in yeast, and various viruses in higher eukaryotes. The mechanisms of the frameshifts occurring on these sites are diverse, though they can be divided into two general types, −1 and +1 frameshifts. However, in addition to these relatively simple events some programmed changes of frame are much more bizarre, involving very efficient translational hops where the ribosome bypasses several dozen nucleotides in the mRNA. Finally, recent evidence has provided

an example of a unique type of repositioning of the ribosome in which translation transfers from one mRNA onto another during elongation (46, 95). Each of these events employs a frameshifting mechanism to accomplish a specific end. These ends can be catagorized into three general types: morphogenesis, autogenous control, and alternative enzymatic activities.

Programmed Frameshifting and Morphogenesis

In eukaryotic viruses and retrotransposons programmed frameshifting accomplishes a morphogenetic purpose. Frameshifting in these elements occurs between the genes for structural and enzymatic products involved in the process of reverse transcription. This process does not occur free in the cytoplasm, but in a virus-encoded protein particle, the nucleocapsid. In retroviruses, the *gag* gene encodes the nucleocapsid as well as other structural components, while the *pol* gene encodes the enzymatic activities necessary for replication, including a serine protease that cleaves the *gag* and *pol*-encoded polyproteins to release the individual virus proteins. Expression of the *pol* product is unusual since it is produced as a translational fusion to the product of the upstream *gag* gene. This translational fusion occurs either by programmed readthrough of an in-frame termination codon (UAG), or by programmed translational frameshifting. The *gag* encoded portion of this protein can assemble with *gag* monomers to form the nucleocapsid particle. Because of the topology of the attachment to the *pol* protein, the enzymatic activities are placed within the particle where they can then catalyze the reactions necessary for reverse transcription of the packaged viral mRNA. Thus, the fusion between the two products brought about by frameshifting leads to targeted insertion of the enzymatic activities into the nucleocapsid particle.

Programmed Frameshifting and Genetic Control

Frameshifting occurs only when the ribosome pauses in translation over a special sequence that can induce the shift in reading frames. The mechanisms leading to this pause include blockage of ribosomal movement by a RNA pseudoknot, slow decoding of an in-frame sense codon, and slow recognition of an in-frame termination codon by peptide release factor (RF) (see below). Since frameshift efficiency appears to vary directly with the duration of the pause, these codons are targets for physiological regulation of frameshifting. Two systems have evolved to modulate gene expression in this way. The *prfB* gene uses autogenous control to regulate expression of peptide release factor 2 (RF2) (15–17, 25). The concentration of RF2 directly regulates the efficiency of frameshifting to express the factor. The gene encoding ornithine decarboxylase (ODCase) antizyme is regulated by feedback control. ODCase is the first and rate-limiting step in synthesis of polyamines (putrescine, spermidine,

and spermine) (76). Antizyme both inhibits the enzymatic activity of ODCase (35, 66) and targets it for proteolysis (71, 91). Expression of the antizyme is regulated translationally by programmed frameshifting, with the efficiency of frameshifting increasing in vitro as the concentration of any of the polyamines is increased (63, 82).

Production of Alternative Enzymatic Activities

A frameshifting system allows the expression of two proteins that share a common N-terminal region, but that differ at their C terminus. One gene has evolved a frameshift system allowing expression of two protein products that appear to have very different enzymatic activities. An *E. coli* gene, *dnaX*, encodes two factors associated with the DNA polymerase III holoenzyme. One of these factors, γ, appears to be an N-terminal fragment of the other, τ. Original experiments suggested that γ was generated by proteolytic degradation of τ, but now it is clear that γ is synthesized as a result of translational frameshifting at a site in the middle of the gene encoding τ, *dnaX*. Frameshifting ribosomes encounter a termination codon only two codons after the shift, so frameshifting allows the expression of a truncated form of the protein. The canonical product of the *dnaX* gene appears to confer extreme processivity on DNA polymerase III, whereas the presence of the truncated γ protein results in a much less processive form. These two activities would be appropriate for leading and lagging strand synthesis, respectively.

MECHANISMS OF PROGRAMMED FRAMESHIFTING

The first studies that showed that translational frameshifting is possible, and that began to identify the rules governing it, concerned fortuitous efficient frameshift sites located within structural genes. Particular *E. coli* tRNAs added in excess to in vitro translation reactions could stimulate high frequencies of apparent frameshift errors (1, 20). The two tRNAs, the AGY-decoding $tRNA^{Ser}_{GCU}$, and ACC-decoding $tRNA^{Thr}_{GGU}$, both induce frameshifting by decoding a doublet codon (10). Because they recognize only two base pairs they shift the reading frame in the -1 direction. It is perhaps surprising that these two tRNAs show marked propensity to induce frameshifting, whereas the other approximately 43 tRNAs in *E. coli* do not. Clearly, these tRNAs must have some structural feature that allows them to induce the unlikely event of frameshifting. Detailed mutagenesis on tRNA showed that certain features of the anticodon loop were both necessary and sufficient to induce the shift (10). Three things about frameshifting induced by these tRNAs are significant for frameshifting in general. First, frameshifting depends on the special structure of the tRNAs; apparently, something about the structure predisposes the ribosome to make

the mistake of doublet decoding, which causes the shift in frames. We will repeatedly see that structural differences among the elements of the translational machinery can significantly increase the probability of frameshifting. Second, frameshifting only occurs when these tRNAs are present in excess. Thus doublet decoding by these tRNAs must compete inefficiently with normal triplet decoding. Again, a common motif in frameshift systems is that frameshifting only occurs when the kinetic difference between cognate in-frame and out-of-frame decoding is narrowed. Third, these tRNAs induce frameshifting at discrete sites. Not all of the potential doublet codons are recognized, so there must be a hierarchy among these sites, with some allowing much more efficient frameshifting.

A second example, fortuitous frameshifting induced by starvation for particular amino acids, underscores these same points, and most particularly the last. Gallant and coworkers have identified both −1 and +1 frameshifts that can be induced at particular sites by starvation for the amino acids tryptophan or lysine (33, 48, 58, 77, 106, 107, 110). Starvation of *E. coli* strains for either of these amino acids phenotypically suppresses certain bacteriophage T4 *rII* mutants. Suppression occurs on a small minority of all Lys and Trp codons (only four of 15 tested), suggesting that special mRNA sequences stimulate frameshifting. The rules of +1 frameshifting derived from analysis of sites induced by lysine-starvation indicate that frameshifting depends on the identity of three codons: the last zero-frame codon decoded before the shift, the "hungry" lysine codon, and the first +1 frame codon decoded (a Ser codon overlapping the hungry Lys codon) (reviewed in Ref. 30). One possible explanation of the event is that the last zero-frame tRNA (GCC-decoding $tRNA^{Ala}_{GGC}$) causes out-of-frame binding of incoming seryl-$tRNA^{Ser}_{GCU}$ at the +1 frame AGC codon that overlaps the zero-frame AAG Lys codon. −1 frameshifting at hungry Lys codons has different rules, depending on only the four nucleotides upstream of the hungry codon. −1 frameshifting depends on the ability of a peptidyl-tRNA to slip −1 on that sequence (48, 58). The mechanisms of −1 and +1 programmed frameshifts are also unrelated; however, in both types of events by far the predominant cause of frameshifting is tRNA slippage; only two examples of programmed +1 frameshifting occurring by a different mechanism have been reported (31, 63).

Both the in vitro and in vivo examples of fortuitous frameshifting underscore the idea that frameshifting occurs in competition with the more kinetically favorable, canonical decoding events. A universal aspect of programmed frameshift sites is that they cause aberrant decoding by making the probabilities of canonical and noncanonical decoding much more similar than they are at other sites; this results either from decreasing the likelihood of the canonical event, or increasing the likelihood of the noncanonical event, and often from both.

The Simultaneous Slippage Mechanism of −1 Frameshifting

Programmed frameshifts of the −1 class are by far the most prevalent, with the vast majority conforming to a single mechanistic model. These frameshift sites were first identified in retroviruses (29, 38, 40–43, 59, 65, 70, 72), and coronaviruses (7, 22, 23, 27, 37). They have subsequently been found in a wide variety of animal and plant viruses (6, 34, 44, 47, 61, 67, 68, 80, 87, 104, 111, 113), retrotransposons (19, 62, 79, 83), a virus-like element in yeast (24, 39, 98), a bacterial gene (5, 32, 93), bacteriophage genes (13, 14, 26, 57), and bacterial insertion sequences (28, 50, 78, 81, 84, 89, 102, 103). In many cases, these sites have only been identified by analogy to other, extensively characterized sites. However, a comparison of these sites produces a clear picture of a generic mechanism of −1 frameshifting, though some sites, especially those in bacteria, have special features.

Sequence comparisons and mutagenesis of a large number of −1 frameshift sites have identified a common structural motif. The sites consist of a heptameric sequence of the form X-XXY-YYZ, shown as codons in the upstream zero frame. For example, the retrovirus Rous sarcoma virus (RSV) has the sequence A-AAU-UUA, while the coronavirus infectious bronchitis virus has the sequence U-UUA-AAC. ten Dam et al (90) catalogued 38 of these sites but found only 11 such sequences: A-AAA-AAC, A-AAU-UUA, A-AAU-UUU, G-GGA-AAC, G-GGC-CCC, G-GGU-UUA, G-GGU-UUU, U-UUA-AAC, U-UUA-AAU, U-UUU-UUA, and G-GAU-UUA. Of these, all but the last conform to the X-XXY-YYZ motif, although many sequences that would conform do not appear in the list. Mutagenesis studies emphasize the importance of the repetitive nature of these sites (8, 40). Changes to the first three nucleotides of the site, such as changing A-AAU-UUA to C-AAU-UUA, A-CAU-UUA, A-ACU-UUA, reduced frameshifting about fivefold. Changes of this type to the next three nucleotides essentially eliminated frameshifting. In addition, introducing GGG or CCC repeats into the YYY position severely reduced frameshifting (8, 24).

Peptide sequencing of the frameshift product showed that this heptameric sequence is in fact the site of frameshifting, with the YYZ codon as the last one decoded in the zero frame (40). The requirement for the repetitive structure involving two adjacent codons suggested a model for the frameshift to Jacks et al (40). The model as later refined by Weiss et al (109) is presented in Figure 1. The model proposes that two tRNAs bound to the XXY and YYZ codons simultaneously slip −1 onto the overlapping XXX and YYY codons. Because the site allows −1 slippage, the sequence is termed a slippery heptamer. The ability of the tRNAs each to form at least two base pairs after slippage is essential for the frameshift to occur. Mutations that disrupt frameshifting presumably do so because they reduce the possibility of base pairing after slippage.

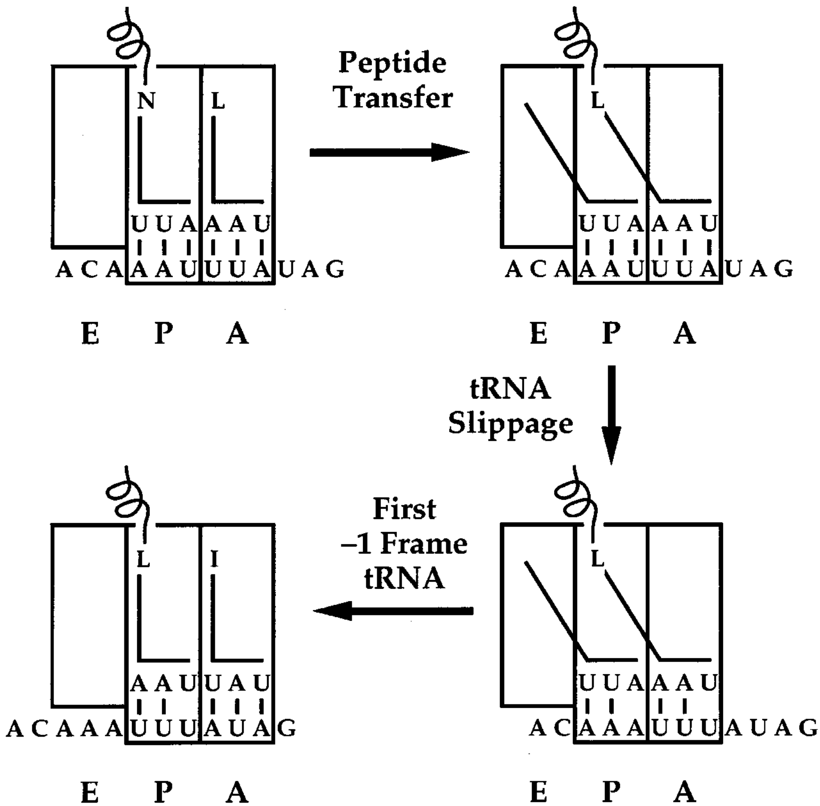

Figure 1 The proposed mechanism of −1 simultaneous slippage frameshifting, after Weiss et al (116). The rectangles represent the three decoding sites of the ribosomes; tRNAs are represented as Ls with the amino acid (in single letter code) and anticodon shown. The RNA sequence is derived from RSV frameshift site.

Simultaneous slippage does not occur efficiently at all slippery heptamers, probably because the ribosome does not pause long enough on the site for slippage to take place. A downstream secondary structure, usually a pseudoknot (90), stimulates frameshifting at least partly by pausing the ribosome with the slippery heptamer in the decoding sites (88, 94). Pseudoknots occur averaging 6 nt downstream of the heptamer (90). This distance is critical; changing the spacing by as little as 2 nt in either direction eliminates stimulation (8). It is not clear whether stimulation results simply from pausing the ribosome in the correct position. Some pseudoknots can pause the ribosome but cannot induce frameshifting (88). This implies that the pseudoknot may have a second function in frameshifting.

Nearly all efficient simultaneous slippage frameshift sites include such a pseudoknot. However, a few replace it with a simple hairpin loop, and some sites appear to include no structure at all distal to the frameshift site (90). It is difficult to conclude, however, that these sites do not include more complex structures, perhaps involving interactions with distant sequences. Moreover, since the role of the pseudoknot is to increase the efficiency of frameshifting at the slippery heptanucleotide, the degree of dependence on such a structure may vary with the intrinsic slipperiness of the site. Thus sites with no structure, or a hairpin replacing the pseuoknot, may have heptamers on which slippage is much more likely.

Prokaryotic Sites Diverge from the Eukaryotic Simultaneous Slippage Mechanism

Eukaryotic −1 frameshift sites are remarkably consistent in structure and function despite the different RNA structures used to stimulate frameshifting. It appears that the mechanism of simultaneous slippage can describe all of the −1 programmed frameshifts in eukaryotes. However, −1 programmed frameshifting in prokaryotes differs in significant ways from the eukaryotic paradigm. Such sites occur within a bacterial gene, the *dnaX* gene of *E. coli* (5, 32, 93), at the 3′ end of gene *10* of bacteriophage T7 (13, 14, 26), and within the overlap between the lambda phage tail genes, G and T (57), as well as within many insertion sequences from a variety of bacterial species (28, 50, 78, 81, 84, 89, 102, 103). None of these events is precisely like the eukaryotic paradigm, and some diverge very significantly.

The *dnaX* event exemplifies the many aspects of bacterial −1 frameshifting. The frameshift occurs on the slippery heptamer, A-AAA-AAG. About 50% of the ribosomes encountering this site shift frames. Part of the reason for this efficiency is that the tRNA that reads AAG binds very weakly in the normal frame as a result of modification of the wobble base (92). Frameshifting is further stimulated by two other elements: a downstream hairpin and an upstream Shine-Dalgarno interaction site. The hairpin probably performs the same role as the pseudoknot, stalling the ribosome over the slippery heptamer, though there is in fact no documented example of a pseudoknot frameshift stimulator in a prokaryotic site. The reason that pseudoknots appear not to be required may be that prokaryotic sites have evolved an alternative method to achieve high efficiency. During the translational pause on the *dnaX* site the 16S rRNA can base pair with an upstream Shine-Dalgarno site to further stimulate frameshifting. In initiation, the 16S rRNA pairs with a Shine-Dalgarno site situated about 7 nt upstream of the initiator. The spacing at the *dnaX* frameshift site is 10 nt; changing the spacing to 16 or 7 nt eliminated stimulation, and changing it to 3 nt actually inhibited frameshifting (56). These results suggest that the effect of the

interaction is to stress the ribosome, causing it to shift to the left on the mRNA, shifting the tRNAs bound to the decoding sites on the slippery heptamer. The smaller spacing would have the opposite effect, tending to cause slippage in the rightward, or +1 direction, and therefore inhibiting −1 frameshifting.

The *dnaX* frameshift site achieves high-efficiency frameshifting by combining the stimulatory effects of both a downstream secondary structure and an upstream rRNA interaction. Other highly efficient sites have used the same mechanism. For example, the insertion sequence IS*911* frameshift site includes both a downstream secondary structure, a "rabbit ear" hairpin (11), and a upstream Shine-Dalgarno interaction (O Fayet, M-F Prère, P Polard, J Atkins, & M Chandler, personal communication). As in *dnaX*, these combine with the extremely slippery A-AAA-AAG heptamer. The same heptamer occurs in seven other insertion sequences (IS*2*, IS*150*, IS*222*, IS*861*, IS*895*, IS*904*, and IS*1133*). In each of these cases, a potential Shine-Dalgarno interaction is located from 7 to 19 nt upstream of the heptamer, and in all but two cases a potential hairpin exists 6 to 8 nt downstream. Each of these sites likely employs a mechanism similar to that of *dnaX* to stimulate frameshifting, though most of these are only putative frameshift sites, and the role of these sequence elements has only been demonstrated for IS*150* (102).

Insertion sequence frameshift sites are a very heterogeneous group, with very few conforming to this structure exactly. Of 33 insertion sequences, 27 had potential slippery heptamers, though only 19 of these were of the X-XXY-YYZ type; among these were 8 variations (A-AAA-AAG, A-AAA-AAA, A-AAA-AAC, G-GGA-AAG, A-AAA-GGG, G-GGA-AAA, U-UUA-AAG, and A-AAG-GGG). The noncanonical sites were all within one base of the prototype (including *G*-AAA-AAA, *G*-UUU-UUU, *A*-UUU-UUU, U-UU*C*-UUC, and U-UU*U*-AAA). All of the six sites lacking a heptamer included instead a potential slippery tetramer, three with the sequence A-AAG, and one each with A-AAA, U-UUU, and G-AAG. These four base shift sites are physiologically relevant since frameshifting in IS*1*, which includes the sequence A-AAA-AAC, apparently requires only the first four nucleotides, A-AAA (85). IS*1*, and potentially the six other IS elements lacking a heptamer, presumably employ a single tRNA slippage mechanism rather than the simultaneous slippage mechanism. The IS*1* frameshift is extremely inefficient, possibly because it uses this alternative mechanism. However, its inefficiency could result from its lack both of an upstream Shine-Dalgarno interaction, and a downstream stimulatory secondary structure (85). Of the 26 heptamer-containing IS frameshift sites, 23 include a potential upstream Shine-Dalgarno interaction site, and 21 may have a downstream hairpin; of the tetramer sites other than IS*1*, four of six may have Shine-Dalgarno sites, and all but one may have a downstream hairpin. The involvement of these sites in stimulating frameshifting is, of course, still

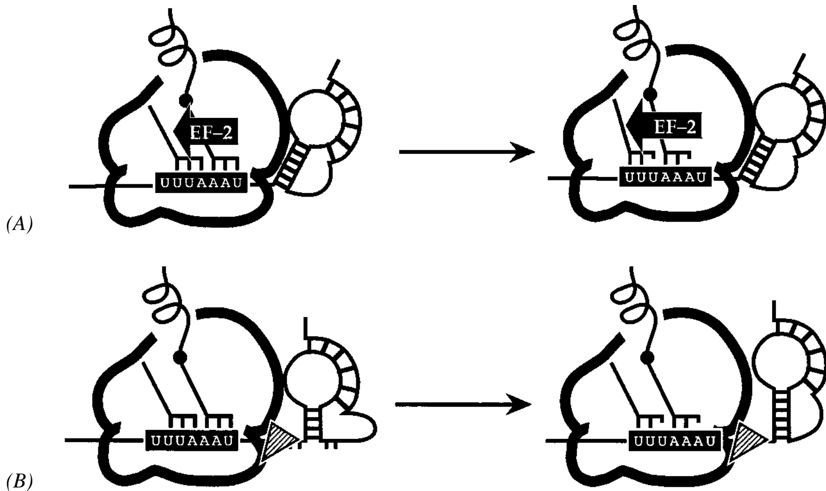

Figure 2 Possible frameshift stimulatory mechanisms. (*A*) On the ribosome stalled by a pseudoknot EF-2 (arrow) could push the two bound tRNAs to the left, causing slippage. (*B*) Alternatively, a ribosome-associated helicase (triangle) may partially unwind the pseudoknot; reforming base pairs may pull the mRNA to the right, causing tRNA slippage.

hypothetical, but their presence suggests that these sites may resemble the *dnaX* site in mechanism, if not in efficiency.

How is it that prokaryotic −1 frameshift sites lacking pseudoknots can achieve extremely high efficiencies? Clearly, part of the answer may be the addition of the interaction with the 16S rRNA. If that interaction compensates for the presence of hairpins rather than pseudoknots, it also implies that the mechanistic role of the Shine-Dalgarno interaction and the pseudoknot may be similar. Since the evidence argues that the rRNA interaction literally pushes the ribosome into the shifted frame, the pseudoknot may have the same role. This would be the second role beyond pausing the ribosome suggested by the data of Somogyi et al (88). Two potential explanations for such a role for the pseudoknot have been proposed (30). In the model of Weiss et al (109), frameshifting occurs after peptide transfer with the two mRNA-bound tRNAs occupying the hybrid sites (E/P and P/A) proposed by Moazed & Noller (69). The next step in the elongation cycle is for EF-G (EF-2 in prokaryotes) to catalyze translocation. Since the effect of EF-G is to cause the tRNA · mRNA complex to slip to the left three nucleotides, and that effect probably involves an interaction with the mRNA-bound tRNA, it is possible that the role of EF-G/EF-2 in −1 frameshifting is to promote slippage of the tRNA −1 with respect to the mRNA when the pseudoknot impedes free slippage of the mRNA (Figure 2*A*).

An alternative hypothesis suggests that the ribosome partially unwinds the pseudoknot as the slippery heptamer occupies the decoding sites (Figure 2*B*). A putative ribosome-associated helicase must unwind secondary structures as the ribosome progresses through a gene. Hypothetically, the ability of the helicase to unwind pseudoknots might be much less than its ability to unwind hairpins, leading to only partial unwinding of the stem nearest the frameshift site. Data from Tu et al (94) are consistent with this hypothesis (30). Reforming one or more of those base pairs might pull the mRNA in the direction of the helicase, i.e. to the right. The tRNAs bound to the decoding sites being held rigidly by the ribosome would tend to slip in the leftward direction on the mRNA during this movement. Either of these models could explain how the pseudoknot might directly promote tRNA slippage. There exists no experimental evidence to confirm or contradict either of these models.

+1 Frameshifting by tRNA Slippage

Programmed frameshift sites that shift reading in the rightward, or +1 direction occur less commonly than do −1 frameshift sites; however, they are as widely dispersed evolutionarily since they occur in bacteria (15), yeast (3, 31), and mammalian cells (63). The *prfB* gene exemplifies the major features of +1 frameshifting. The shift occurs when a peptidyl-tRNA is bound to the ribosomal P site and the A site is empty, and requires a translational pause with the ribosome positioned over the frameshift site. In *prfB*, the A site codon is a UGA terminator recognized by peptide release factor 2 (RF2), the protein product of *prfB*. When RF2 is limiting, recognition of the UGA is slow, and consequently frameshifting occurs; when RF2 is abundant, termination occurs instead of frameshifting. This produces an autogenous regulatory loop controlling levels of RF2 by regulating frameshifting (15, 17, 25). The shift in frames appears to require that during the translational pause caused by slow recognition of the UGA, peptidyl-$tRNA^{Leu}_{GAG}$ slips from CUU onto a +1 overlapping UUU codon. Curran (18) used mutant variants of the *prfB* site to demonstrate that the efficiency of frameshifting varies directly with the stability of the peptidyl-tRNA in the shifted frame, with the CUU codon giving the highest efficiency of those tested.

As with the bacterial −1 frameshifts, an upstream Shine-Dalgarno interaction stimulates frameshifting on *prfB*. The site is only 3 nt upstream of the CUU slip codon, much closer than in −1 frameshift sites. Mutagenesis of the site and the complementary sequence in 16S rRNA demonstrated that the interaction stimulates frameshifting (105). The mechanism resembles that of the *dnaX* −1 site, except that spacing the interaction site much closer than is optimal strains the ribosome and causes slippage in the rightward, or +1 direction (17, 105). This interaction is of course unique to bacterial systems since a Shine-Dalgarno interaction is not known to occur with eukaryotic ribosomes.

The *prfB* frameshift mechanism shares with the +1 frameshift site from the Ty1 retrotransposon of the yeast *Saccharomyces cerevisiae* the requirement that a slowly recognized codon occupy the A site during the frameshift, and that the peptidyl-tRNA be able to shift reading into the new reading frame (3). The Ty1 frameshift site consists of a seven nucleotide sequence, CUU-AGG-C (shown in codons of the upstream *TYA* gene). Frameshifting occurs when the CUU-bound peptidyl-$tRNA^{Leu}_{UAG}$ slips from CUU to the overlapping UUA codon during the slow decoding of the AGG codon. The event appears to be entirely stochastic, and it requires no other stimulatory sequences.

+1 Frameshifting Independent of tRNA Slippage

Two other programmed +1 frameshifts apparently diverge from this mechanism. Frameshifting between the *GAG3* and *POL3* genes of the yeast retrotransposon Ty3 occurs on the seven nucleotide sequence GCG-AGU-U (31). Again, frameshifting occurs because of slow decoding of the AGU codon, with reading of the +1 frame GUU codon. Since the tRNA predicted to decode GCG could not base pair with the overlapping CGA codon, frameshifting must not involve peptidyl-tRNA slippage (31). The frameshift mechanism used in expressing the mammalian ornithine decarboxylase antizyme appears to be similar (63). Frameshifting occurs on the sequence UCC-UGA-U with decoding of the UCC followed by the GAU codons. Although the UCC decoding $tRNA^{Leu}_{IGA}$ could slip +1 onto CCU and still retain two of three base pairs, mutations that would preclude slippage had little effect on frameshifting in a rabbit reticulocyte cell-free assay. This result suggested that slippage was not necessary (63). Surprisingly, when the same site was studied in vivo in a heterologous yeast system, these same mutations virtually eliminated frameshifting, and frameshifting occurred predominantly by −2 slippage, from CCU to CUC (64). Thus, we have examples of both slippage-induced (Ty1, and antizyme in yeast) and slippage-independent (Ty3, and antizyme in mammalian extract) programmed +1 frameshifts. Detailed mutagenesis of the Ty3 site, replacing the GCG P site codon with all 63 other codons, showed that in yeast +1 frameshifting does not depend on the ability of the tRNA to slip, and that both slippage-dependent and slippage-independent frameshifts can be efficient (101).

Recent work on the Ty3 frameshift sites showed that a low concentration of the first +1 frame tRNA drastically reduced frameshifting (74). This result suggests that frameshifting may occur only when the first +1 frame tRNA transiently binds in the A site out of the normal reading frame. In slippage-dependent frameshifting, the peptidyl-tRNA stimulates the efficiency of frameshifting by slipping +1 during this transient binding, in effect correcting the reading frame of the incoming aminoacyl-tRNA, and insuring its acceptance by the ribosome. In the slippage-independent events, the structure of the tRNA occupying the P site codon may stabilize out-of-frame binding of aminoacyl-

tRNA, allowing it to be accepted by the ribosome. Since the frameshift in either case would occur while two tRNAs occupy the decoding sites, +1 frameshifting may in fact resemble −1 frameshifting more closely than previously believed.

Translational Hopping

Arguably, the most unusual example of programmed changes in reading frame is the 50 nt translational hop that occurs during expression of the bacteriophage T4 topoisomerase gene, gene *60* (36, 108). Hopping occurs when a peptidyl-$tRNA^{Gly}$ dissociates from a GGA codon and reassociates with a second GGA codon 50 nt downstream (108). Three features stimulate the event: an in-frame UAG termination codon, a hairpin loop immediately following the GGA codon, and a 14-amino acid region of the primary polypeptide encoded upstream of the hop site. The terminator probably pauses the ribosome with the GGA in the P site. The other two elements promote detachment and/or reassociation of the peptidyl-tRNA by unknown mechanisms. Herbst et al (36) showed that the hairpin probably interacts with ribosomal protein L9, and they suggested that a dynamic change in mRNA secondary structure, possibly modulated by L9, promotes the hop. Whether this is the case remains unclear.

The fundamental requirements predicted by the gene *60* system for any programmed hop include matching take-off and landing codons, and a translational pause that would, as in frameshifting, allow the kinetically unlikely event to occur. A second putative translational hop in the gene encoding the tryptophan repressor of *E. coli*, the *trpR* gene, fails to conform to this model (4). Frameshifting is proposed to occur though there are no matching codons, and no evidence for a pause-inducing structure or codon. Understanding the mechanism underlying this event will require that these aspects be explained.

Two other translational hops more closely resemble the gene *60* paradigm. A translational hop occurs when bovine placental lactogen is overexpressed in *E. coli* (45). The hop occurs by movement between identical UUG codons and past an intervening poorly decoded AGG (Arg) codon. This event occurs only when the gene is heavily overexpressed, possibly because overexpression lengthens the pause at AGG (45). The *plaA* gene of *Prevotella loescheii* includes a discontinuity in the mRNA that ribosomes appear able to bypass by hopping (60). The exact nature of the hop is not known. A proposal of a similar four-codon hop in the *carA* gene of *Pseudomonas aeruginosa* (112) has since been demonstrated to be erroneous (54, 96)

A Specialized Translational Hop Targets Some Proteins for Degradation

All of the events described above involve changes of reading frame while the ribosome translates particular mRNAs. Recently, another type of event was

described which strongly resembles programmed frameshifts and hops, yet involves the movement of ribosomes between two distinct RNA templates (46, 95). An unusual RNA molecule found in various bacterial species, termed 10Sa RNA (9, 73, 97, 100), has the unusual capacity to be charged by alanyl-tRNA synthetase based on a portion of the RNA that mimics a $tRNA^{Ala}$ (49, 99) and binds to 70S ribosomes (99). Recent evidence shows that 10Sa encodes an 11-amino acid oligopeptide used to tag proteins for degradation (46, 95).

What is amazing about this RNA is that the tag is attached to nascent proteins on mRNAs lacking termination codons, presumably as a result of partial RNA degradation (46). Apparently, the $tRNA^{Ala}$ mimicry extends to translational elongation such that ribosomes carrying peptidyl-tRNAs that are paused at the end of 3′-truncated mRNAs can accept the alanyl-10Sa molecule as a tRNA, transferring the nascent polypeptide to the small RNA. After translocation to the P site, a segment of the RNA then mimics an mRNA allowing incorporation of the 11-amino acid tag from a short ORF encoded by the RNA. Termination occurs as normal at an inframe nonsense codon, and the polypeptide produced is degraded by a protease which recognizes the C-terminal tag.

This system strongly resembles the programmed translational events discussed above, though not mechanistically since peptide transfer resembles a normal in-frame elongation step. The resemblance is in the way that a change in competition for the A site biases these ribosomes to use alanyl-10Sa as a mimic of an aminoacyl-tRNA. Clearly, incorporation of this molecule at random positions by normally translating ribosomes would be counterproductive. In fact, the mimic is unlikely to compete effectively against either aminoacyl-tRNA at sense codons, or peptide release factor at termination codons. The fact that ribosomes paused at the end of truncated mRNAs lack any codon in the A site allows for the alternative event, incorporation of the tRNA mimic. As with programmed frameshift and hop sites, the event only occurs when the rate of normal elongation has been reduced to such an extent that the alternative can compete for the A site. Whether other systems as bizarre as the 10Sa system exist remains to be seen.

PROGRAMMED FRAMESHIFTS AS TRANSLATIONAL ERRORS

Programmed frameshifts are by definition translational errors, that is, the polypeptide sequence produced by translation differs from that encoded in the DNA. However, this description could be simply semantic, or the sites could actually have evolved by stimulating the normal very low frequency of errors. The question of the status of programmed frameshifts as amplified translational errors is contentious. The alternative view, reflected in the nomenclature

of "programmed" frameshifting, is that frameshifting results from manipulation of the translational machinery to cause an event that would not occur otherwise. Here I consider the evidence that the concept of amplified error is an appropriate description of these events.

Frameshift errors do occur that resemble programmed +1 frameshifts and translational hops. Amino acid limitation in vivo, or an excess of particular tRNA isoacceptors during in vitro translation can induce frameshifting. Both of these types of events are clearly errors, and most clearly resemble the programmed +1 frameshifts since usually an abnormal effect of the peptidyl-tRNA stimulates frameshifting. Nonprogrammed frameshifts also occur when heterologous genes are overexpressed in *E. coli*. In addition to the case of the hop occurring in the bovine placental lactogen gene described above, an example of +1 frameshifting by apparent peptidyl-tRNA slippage was found when human transferrin was expressed in *E. coli* (21). These events may have been stimulated to high efficiency as a result of the high level of expression achieved, suggesting that frameshift-inducing errors may be more prevalent in these conditions.

What is the evidence, then, that programmed frameshift sites have evolved to amplify the efficiency of these sorts of events? A detailed look at the events underlying some of the +1 frameshifts provides some clues. Frameshifting on the Ty1 site in yeast occurs by slippage of tRNA$^{\text{Leu}}_{\text{UAG}}$ from CUU to UUA during a pause in translation caused by slow recognition of the next codon, AGG (3). Decoding of the CUU, CUC, and CUG codons appears to occur by two-out-of-three pairing mechanism with this tRNA (55), while recognition of CUA is by normal cognate recognition. Recent evidence has shown that this tRNA slips efficiently because it forms only two base pairs with CUU $\left(\begin{smallmatrix}\text{CUC}\\ \text{ | | }\\ \text{GAG}\end{smallmatrix}\right)$ but can form three base pairs in the shifted frame $\left(\begin{smallmatrix}\text{UUA}\\ \cdot\text{ | | }\\ \text{GAU}\end{smallmatrix}\right)$ (101). Frameshifting thus depends on a weak interaction in the normal frame. A second example was discovered in a yeast strain lacking the AGG-decoding tRNA$^{\text{Arg}}_{\text{UCU}}$ (101). In this strain AGG is presumably decoded by the near-cognate tRNA$^{\text{Arg}}_{\text{UCU}}$ that normally decodes AGA. Modification of the wobble U in this tRNA destabilizes base pairing with G, weakening binding to AGG. Peptidyl-tRNA$^{\text{Arg}}_{\text{UCU}}$ frameshifts very efficiently on AGG, whereas peptidyl-tRNA$^{\text{Arg}}_{\text{CCU}}$ is extremely inefficient. The difference once again is the instability of the codon · anticodon interaction in the normal reading frame.

Frameshifting of this type would be expected to occur when a near-cognate tRNA is erroneously selected by the ribosome. When translocated to the P site, a near-cognate peptidyl-tRNA might be able to cause frameshifting by slippage. The efficiency of frameshifting would be limited by the rate at which the next normal frame codon can be selected. Programmed +1 frameshift sites have evolved such that the next codon is poorly recognized. This is a clear

example of an amplified error. Translational hops provide more examples of possible amplified errors. Hops can occur fortuitously when genes are overexpressed. The T4 gene *60* context sequences seem to have evolved to increase the efficiency of such an error.

It is much more difficult to argue for simultaneous slippage −1 frameshifting resembling a translational error. Frameshifting clearly depends strongly on the stimulatory pseudoknot, and recent evidence has suggested that frameshift-stimulatory pseudoknots have a specialized structure that may be required to stimulate the frameshift (12, 86). However, the pseudoknot is not an essential part of the frameshift site, but rather a stimulator of the low level of frameshifting caused by the slippery heptamer alone.

Programmed frameshift sites, then, provide a new tool for dissecting the mechanism of translational accuracy. Some of the stimulatory elements that have evolved at these sites must work by manipulating the ribosome to increase ribosomal inaccuracy. These elements—the pseudoknots of simultaneous slippage frameshift sites, and the upstream and downstream context elements of +1 frameshift, and translational hop sites—may be used to identify the structures in the ribosome controlling ribosomal accuracy.

CONCLUSION

Though rare, programmed translational frameshifting is a ubiquitous mechanism used to express alternative translational products. Given the simplicity of some of the frameshift sites and their similarity to low-efficiency translational errors, many more genes are likely to have evolved similar mechanisms. Since one frameshift system, the *dnaX* gene of *E. coli*, uses frameshifting to create a truncated translational product, it is not possible to identify potential frameshift sites by scanning DNA sequences looking for overlapping reading frames. Further, since many of the frameshift products constitute only a few percent of the primary translational product, they could easily be missed in studies of protein expression. With the advent of genome-level sequencing, it will become increasingly important to identify all possible translational products by inspection of the primary nucleotide sequence, and often without supporting molecular analysis. A clear understanding of the rules of programmed translational frameshifting will be important to enable researchers to fully characterize the translational potential of DNA sequences.

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Literature Cited

1. Atkins J, Gesteland R, Reid B, Anderson C. 1979. Normal tRNAs promote ribosomal frameshifting. *Cell* 18:1119–31
2. Atkins J, Weiss R, Gesteland R. 1990. Ribosome gymnastics—degree of difficulty 9.5, style 10.0. *Cell* 62:413–23
3. Belcourt MF, Farabaugh PJ. 1990. Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. *Cell* 62:339–52
4. Benhar I, Engelberg KH. 1993. Frameshifting in the expression of the E. coli *trpR* gene occurs by the bypassing of a segment of its coding sequence. *Cell* 72:121–30
5. Blinkowa AL, Walker JR. 1990. Programmed ribosomal frameshifting generates the *Escherichia coli* DNA polymerase III γ subunit from within the τ subunit reading frame. *Nucleic Acids Res.* 18:1725–29
6. Brault V, Miller WA. 1992. Translational frameshifting mediated by a viral sequence in plant cells. *Proc. Natl. Acad. Sci. USA* 89:2262–66
7. Brierley I, Boursnell MEG, Binns MM, Bilimoria B, Blok VC, et al. 1987. An efficient ribosomal frame-shifting signal in the polymerase-encoding region of the coronavirus IBV. *EMBO J.* 6:3779-85
8. Brierley I, Jenner AJ, Inglis SC. 1992. Mutational analysis of the "slippery-sequence" component of a coronavirus ribosomal frameshifting signal. *J. Mol. Biol.* 227:463–79
9. Brown JW, Hunt DA, Pace NR. 1990. Nucleotide sequence of the 10Sa RNA gene of the beta-purple eubacterium *Alcaligenes eutrophus*. *Nucleic Acids Res.* 18:2820
10. Bruce AG, Atkins JF, Gesteland RF. 1986. tRNA anticodon replacement experiments show that ribosomal frameshifting can be caused by doublet decoding. *Proc. Natl. Acad. Sci. USA* 83:5062–66
11. Chandler M, Fayet O. 1993. Translational frameshifting in the control of transposition in bacteria. *Mol. Microbiol.* 7:497–503
12. Chen X, Chamorro M, Lee SI, Shen LX, Hines JV, et al. 1995. Structural and functional studies of retroviral RNA pseudoknots involved in ribosomal frameshifting: Nucleotides at the junction of the two stems are important for efficient ribosomal frameshifting. *EMBO J.* 14:842–52
13. Condron BG, Atkins JF, Gesteland RF. 1991. Frameshifting in gene 10 of bacteriophage T7. *J. Bacteriol.* 173:6998–7003
14. Condron BG, Gesteland RF, Atkins JF. 1991. An analysis of sequences stimulating frameshifting in the decoding of gene 10 of bacteriophage T7. *Nucleic Acids Res.* 19:5607–12
15. Craigen WJ, Caskey CT. 1986. Expression of peptide chain release factor 2 requires high-efficiency frameshift. *Nature* 322:273–75
16. Craigen WJ, Cook RG, Tate WP, Caskey CT. 1985. Bacterial peptide chain release factors: conserved primary structure and possible frameshift regulation of release factor 2. *Proc. Natl. Acad. Sci. USA* 82:3616–20
17. Curran JF, Yarus M. 1988. Use of tRNA suppressors to probe regulation of *Escherichia coli* release factor 2. *J. Mol. Biol.* 203:75–83
18. Curran JF. 1993. Analysis of effects of tRNA:message stability on frameshift frequency at the *Escherichia coli* RF2 programmed frameshift site. *Nucleic Acids Res.* 21:1837–43
19. Danilevskaya O, Slot F, Pavlova M, Pardue ML. 1994. Structure of the Drosophila HeT-A transposon: a retrotransposon-like element forming telomeres. *Chromosoma* 103:215–24
20. Dayhuff TJ, Atkins JF, Gesteland RF. 1986. Characterization of ribosomal frameshift events by protein sequence analysis. *J. Biol. Chem.* 261:7491–500
21. de Smit MH, van Duin J, van Knippenberg PH, van Eijk HG. 1994. CCC.UGA: a new site of ribosomal frameshifting in *Escherichia coli*. *Gene* 143:43–47
22. den Boon JA, Snijder EJ, Chirnside ED, de Vries AA, Horzinek MC, Spaan WJ. 1991. Equine arteritis virus is not a togavirus but belongs to the coronavirus-like superfamily. *J. Virol.* 65:2910–20
23. Denison MR, Zoltick PW, Leibowitz JL, Pachuk CJ, Weiss SR. 1991. Identification of polypeptides encoded in open reading frame 1b of the putative polymerase gene

of the murine coronavirus mouse hepatitis virus A59. *J. Virol.* 65:3076–82
24. Dinman JD, Icho T, Wickner RB. 1991. A −1 ribosomal frameshift in a double-stranded RNA virus of yeast forms a gag-pol fusion protein. *Proc. Natl. Acad. Sci. USA* 88:174–78
25. Donly BC, Edgar CD, Adamski FM, Tate WP. 1990. Frameshift autoregulation in the gene for *Escherichia coli* release factor 2: Partly functional mutants result in frameshift enhancement. *Nucleic Acids Res.* 18:6517–22
26. Dunn JJ, Studier FW. 1983. Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J. Mol. Biol.* 166:477–535
27. Eleouet JF, Rasschaert D, Lambert P, Levy L, Vende P, Laude H. 1995. Complete sequence (20 kilobases) of the polyprotein-encoding gene 1 of transmissible gastroenteritis virus. *Virology* 206:817–22
28. Escoubas JM, Prère MF, Fayet O, Salvignol I, Galas D, et al. 1991. Translational control of transposition activity of the bacterial insertion sequence IS*1*. *EMBO J.* 10:705–12
29. Falk H, Mador N, Udi R, Panet A, Honigman A. 1993. Two cis-acting signals control ribosomal frameshift between human T-cell leukemia virus type II *gag* and *pro* genes. *J. Virol.* 67:6273–77
30. Farabaugh PJ. 1995. Programmed translational frameshifting. *Microbiol. Rev.* 60:103–34
31. Farabaugh PJ, Zhao H, Vimaladithan A. 1993. A novel programed frameshift expresses the *POL3* gene of retrotransposon Ty3 of yeast: frameshifting without tRNA slippage. *Cell* 74:93–103
32. Flower AM, McHenry CS. 1990. The γ subunit of DNA polymerase III holoenzyme of *Escherichia coli* is produced by ribosomal frameshifting. *Proc. Natl. Acad. Sci. USA* 87:3713–77
33. Gallant JA, Lindsley D. 1992. Leftward ribosome frameshifting at a hungry codon. *J. Mol. Biol.* 223:31–40
34. Godeny EK, Chen L, Kumar SN, Methven SL, Koonin EV, Brinton MA. 1993. Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV). *Virology* 194:585–96
35. Heller JS, Fong WF, Canellakis ES. 1976. Induction of a protein inhibitor to ornithine decarboxylase by the end products of its reaction. *Proc. Natl. Acad. Sci. USA* 73:1858–62
36. Herbst K, Nichols L, Gesteland R, Weiss R. 1994. A mutation in ribosomal protein L9 affects ribosomal hopping during translation of gene *60* from bacteriophage T4. *Proc. Natl. Acad. Sci. USA* 91:12525–29
37. Herold J, Siddell SG. 1993. An 'elaborated' pseudoknot is required for high frequency frameshifting during translation of HCV 229E polymerase mRNA. *Nucleic Acids Res.* 21:5838–42
38. Hyun NS, Copeland TD, Hatanaka M, Oroszlan S. 1993. Characterization of ribosomal frameshifting for expression of *pol* gene products of human T-cell leukemia virus type I. *J. Virol.* 67:196–203
39. Icho T, Wickner RB. 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.* 264:6716–23
40. Jacks T, Madhani HD, Masiarz FR, Varmus HE. 1988. Signals for ribosomal frameshifting in the Rous Sarcoma virus *gag-pol* region. *Cell* 55:447–58
41. Jacks T, Power MD, Masiarz FR, Luciw PA, Barr PJ, Varmus HE. 1988. Characterization of ribosomal frameshifting in HIV-1 *gag-pol* expression. *Nature* 331:280–83
42. Jacks T, Townsley K, Varmus HE, 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* 84:4298–302
43. Jacks T, Varmus HE. 1985. Expression of the Rous sarcoma virus pol gene by ribosomal frameshifting. *Science* 230:1237–42
44. Jiang B, Monroe SS, Koonin EV, Stine SE, Glass RI. 1993. RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis. *Proc. Natl. Acad. Sci. USA* 90:10539–43
45. Kane JF, Violand BN, Curran DF, Staten NR, Duffin KL, Bogosian G. 1992. Novel in-frame two codon translational hop during synthesis of bovine placental lactogen in a recombinant strain of *Escherichia coli*. *Nucleic Acids Res.* 20:6707–12
46. Keiler KC, Waller PR, Sauer RT. 1996. Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. *Science* 271:990–93
47. Kim KH, Lommel SA. 1994. Identification and analysis of the site of −1 riboso-

mal frameshifting in red clover necrotic mosaic virus. *Virology* 200:574–82
48. Kolor K, Lindsley D, Gallant JA. 1993. On the role of the P-site in leftward ribosome frameshifting at a hungry codon. *J. Mol. Biol.* 230:1–5
49. Komine Y, Kitabatake M, Yokogawa T, Nishikawa K, Inokuchi H. 1994. A tRNA-like structure is present in 10Sa RNA, a small stable RNA from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 91:9223–27
50. Kropinski AM, Farinha MA, Jansons I. 1994. Nucleotide sequence of the *Pseudomonas aeruginosa* insertion sequence IS222: another member of the IS3 family. *Plasmid* 31:222–28
51. Kurland CG, Gallant JA. 1986. The secret life of the ribosome. In *Accuracy in Molecular Processes*, ed. T Kirkwood, R Rosenberger, D Galas, pp. 127–57. London: Chapman & Hall
52. Kurland CG. 1979. Reading-frame errors on ribosomes. In *Nonsense Mutations and tRNA Suppressors*, ed. JE Celis, JD Smith. pp. 97–108. New York: Academic
53. Kurland CG. 1992. Translational accuracy and the fitness of bacteria. *Annu. Rev. Genet.* 26:29–50
54. Kwon DH, Lu CD, Walthall DA, Brown TM, Houghton JE, Abdelal AT. 1994. Structure and regulation of the carAB operon in *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*: No untranslated region exists. *J. Bacteriol.* 176:2532–42
55. Lagerkvist U. 1978. "Two out of three": an alternative method for codon reading. *Proc. Natl. Acad. Sci. USA* 75:1759–62
56. Larsen B, Wills NM, Gesteland RF, Atkins JF. 1994. rRNA-mRNA base pairing stimulates a programmed −1 ribosomal frameshift. *J. Bacteriol.* 176:6842–51
57. Levin ME, Hendrix RW, Casjens SR. 1993. A programmed translational frameshift is required for the synthesis of a bacteriophage lambda tail assembly protein. *J. Mol. Biol.* 234:124–39
58. Lindsley D, Gallant JA. 1993. On the directional specificity of ribosome frameshifting at a hungry codon. *Proc. Natl. Acad. Sci. USA* 90:5469–73
59. Mäkinen K, Ness V, Tamm T, Truve E, Aaspollu A, Saarma M. 1995. The putative replicase of the cocksfoot mottle sobemovirus is translated as a part of the polyprotein by −1 ribosomal frameshift. *Virology* 207:566–71
60. Manch-Citron JN, London JN. 1994. Expression of the *Prevotella loescheii* adhesin gene (*plaA*) is mediated by a programmed frameshifting hop. *J. Bacteriol.* 176:1944–48
61. Marczinke B, Bloys AJ, Brown TD, Willcocks MM, Carter MJ, Brierley I. 1994. The human astrovirus RNA-dependent RNA polymerase coding region is expressed by ribosomal frameshifting. *J. Virol.* 68:5588–95
62. Marlor RL, Parkhurst SM, Corces VG. 1986. The *Drosophila melanogaster* gypsy transposable element encodes putative gene products homologous to retroviral proteins. *Mol. Cell. Biol.* 6:1129–34
63. Matsufuji S, Matsufuji T, Miyazaki Y, Murakami Y, Atkins JF, et al. 1995. Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. *Cell* 80:51–60
64. Matsufuji S, Matsufuji T, Wills N, Gesteland R, Atkins J. 1996. Reading two bases twice: mammalian antizyme frameshifting in yeast. *EMBO J.* 15:1360–70
65. Maurer B, Bannert H, Darai G, Flugel RM. 1988. Analysis of the primary structure of the long terminal repeat and the gag and pol genes of the human spumaretrovirus. *J. Virol.* 62:1590–97
66. McCann PP, Tardif C, Hornsperger JM, Böhlen P. 1979. Two distinct mechanisms for ornithine decarboxylase regulation by polyamines in rat hepatoma cells. *J. Cell. Physiol.* 99:183–90
67. Meulenberg JJ, Hulst MM, de Meijer EJ, Moonen PL, den Besten A, et al. 1993. Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology* 192:62–72
68. Miller WA, Waterhouse PM, Gerlach WL. 1988. Sequence and organization of barley yellow dwarf virus genomic RNA. *Nucleic Acids Res.* 16:6097–111
69. Moazed D, Noller HF. 1989. Intermediate states in the movement of transfer RNA in the ribosome. *Nature* 342:142–48
70. Morikawa S, Bishop DHL. 1992. Identification and analysis of the *gag-pol* ribosomal frameshift site of feline immunodeficiency virus. *Virology* 186:389–97
71. Murakami Y, Matsufuji S, Kameji T, Hayashi S, Igarashi K, et al. 1992. Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. *Nature* 360:597–99
72. Nam SH, Copeland TD, Hatanaka M, Oroszlan S. 1993. Characterization of ribosomal frameshifting for expression of pol gene products of human T-cell leukemia virus type I. *J. Virol.* 67:196– 203
73. Oh BK, Apirion D. 1991. 10Sa RNA, a small stable RNA of *Escherichia coli*, is

functional. *Mol. Gen. Genet.* 229:52–56
74. Pande S, Vimaladithan A, Zhao H, Farabaugh PJ. 1995. Pulling the ribosome out of frame +1 at a programmed frameshift site by cognate binding of aminoacyl-tRNA. *Mol. Cell. Biol.* 15:298-304
75. Parker J. 1989. Errors and alternatives in reading the universal genetic code. *Microbiol. Rev.* 53:273–98
76. Pegg A. 1986. Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem J.* 234:249–62
77. Peter K, Lindsley D, Peng L, Gallant JA. 1992. Context rules of rightward overlapping reading. *New Biol.* 4:520-26
78. Polard P, Prère MF, Chandler M, Fayet O. 1991. Programmed translational frameshifting and initiation at an AUU codon in gene expression of bacterial insertion sequence IS*911*. *J. Mol. Biol.* 222:465–77
79. Priimagi AF, Mizrokhi LJ, Ilyin YV. 1988. The Drosophila mobile element jockey belongs to LINEs and contains coding sequences homologous to some retroviral proteins. *Gene* 70:253–62
80. Prüfer D, Tacke E, Schmitz J, Kull B, Kaufmann A, Rohde W. 1992. Ribosomal frameshifting in plants: A novel signal directs the −1 frameshift in the synthesis of the putative viral replicase of potato leafroll luteovirus. *EMBO J.* 11:1111–17
81. Rezsohazy R, Hallet B, Mahillon J, Delcour J. 1993. IS231V and W from *Bacillus thuringiensis* subsp. *israelensis*, two distant members of the IS231 family of insertion sequences. *Plasmid* 30:141–49
82. Rom E, Kahana C. 1994. Polyamines regulate the expression of ornithine decarboxylase antizyme in vitro by inducing ribosomal frame-shifting. *Proc. Natl. Acad. Sci. USA* 91:3959–63
83. Saigo K, Kugimiya W, Matsuo Y, Inouye S, Yoshioka K, Yuki S. 1984. Identification of the coding sequence for a reverse transcriptase-like enzyme in a transposable genetic element in *Drosophila melanogaster*. *Nature* 312:659–61
84. Sekine U, Ohtsubo E. 1989. Frameshifting is required for production of the transposase encoded by insertion sequence *1*. *Proc. Natl. Acad. Sci. USA* 86:4609–13
85. Sekine Y, Ohtsubo E. 1992. DNA sequences required for translational frameshifting in production of the transposase encoded by IS*1*. *Mol. Gen. Genet.* 235:325–32
86. Shen LX, Tinoco I Jr. 1995. The structure of an RNA pseudoknot that causes efficient frameshifting in mouse mammary tumor virus. *J. Mol. Biol.* 247:963–78
87. Snijder EJ, den Boon JA, Bredenbeek PJ, Horzinek MC, Rijnbrand R, Spaan WJ. 1990. The carboxyl-terminal part of the putative Berne virus polymerase is expressed by ribosomal frameshifting and contains sequence motifs which indicate that toro- and coronaviruses are evolutionarily related. *Nucleic Acids Res.* 18:4535–42
88. Somogyi P, Jenner AJ, Brierley I, Inglis SC. 1993. Ribosomal pausing during translation of an RNA pseudoknot. *Mol. Cell. Biol.* 13:6931–40
89. Steibl HD, Lewecke FM. 1995. IS1222: analysis and distribution of a new insertion sequence in *Enterobacter agglomerans* 339. *Gene* 156:37–42
90. ten Dam EB, Pleij CWA, Bosch L. 1990. RNA pseudoknots: translational frameshifting and readthrough of viral RNAs. *Virus Genes* 4:121–36
91. Tokunaga F, Goto T, Koide T, Murakami Y, Hayashi S, et al. 1994. ATP- and antizyme-dependent endoproteolysis of ornithine decarboxylase to oligopeptides by the 26 S proteasome. *J. Biol. Chem.* 269:17382–85
92. Tsuchihashi Z, Brown PO. 1992. Sequence requirements for efficient translational frameshifting in the *Escherichia coli dnaX* gene and the role of an unstable interaction between $tRNA^{Lys}$ and an AAG lysine codon. *Genes Dev.* 6:511–19
93. Tsuchihashi Z, Kornberg A. 1990. Translational frameshifting generates the γ subunit of DNA polymerase III holoenzyme. *Proc. Natl. Acad. Sci. USA* 87:2516–20
94. Tu C, Tzeng TH, Bruenn JA. 1992. Ribosomal movement impeded at a pseudoknot required for frameshifting. *Proc. Natl. Acad. Sci. USA* 89:8636–40
95. Tu GF, Reid GE, Zhang JG, Moritz RL, Simpson RJ. 1995. C-terminal extension of truncated recombinant proteins in *Escherichia coli* with a 10Sa RNA decapeptide. *J. Biol. Chem.* 270:9322–26
96. Tuohy TMF, Kidd T, Gesteland RF, Atkins JF. 1994. Uninterrupted translation through putative 12-nucleotide coding gap in sequence of *carA*: business as usual. *J. Bacteriol.* 176:265–67
97. Tyagi JS, Kinger AK. 1992. Identification of the 10Sa RNA structural gene of *Mycobacterium tuberculosis*. *Nucleic Acids Res.* 20:138
98. Tzeng T-H, Tu C-L, Bruenn JA. 1992.

Ribosomal frameshifting requires a pseudoknot in the *Saccharomyces cerevisiae* double-stranded RNA virus. *J. Virology* 66:999–1006

99. Ushida C, Himeno H, Watanabe T, Muto A. 1994. tRNA-like structures in 10Sa RNAs of *Mycoplasma capricolum* and *Bacillus subtilis*. *Nucleic Acids Res.* 22:3392–96
100. Ushida C, Muto A. 1993. Small stable RNAs in *Mycoplasma capricolum*. *Nucleic Acids Symp Ser*:157–58
101. Vimaladithan A, Farabaugh PJ. 1994. Special peptidyl-tRNA molecules promote translational frameshifting without slippage. *Mol. Cell. Biol.* 14:8107–16
102. Vögele K, Schwartz E, Welz C, Schiltz E, Rak B. 1991. High-level ribosomal frameshifting directs the synthesis of IS*150* gene products. *Nucleic Acids Res.* 19:4377–85
103. Walker DC, Klaenhammer TR. 1994. Isolation of a novel IS3 group insertion element and construction of an integration vector for *Lactobacillus* spp. *J. Bacteriol.* 176:5330–40
104. Wang AL, Yang HM, Shen KA, Wang CC. 1993. Giardiavirus double-stranded RNA genome encodes a capsid polypeptide and a gag-pol-like fusion protein by a translation frameshift. *Proc. Natl. Acad. Sci. USA* 90:8595–99
105. Weiss RB, Dunn DM, Dahlberg AE, Atkins JF, Gesteland RF. 1988. 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.* 7:1503–7
106. Weiss R, Gallant J. 1983. Mechanism of ribosome frameshifting during translation of the genetic code. *Nature* 302:389–93
107. Weiss RB, Gallant JA. 1986. Frameshift suppression in aminoacyl-tRNA limited cells. *Genetics* 112:727–39
108. Weiss RB, Huang WM, Dunn DM. 1990. A nascent peptide is required for ribosomal bypass of the coding gap in bacteriophage T4 gene *60*. *Cell* 62:117–26
109. Weiss RB, Dunn DM, Shuh M, Atkins JF, Gesteland RF. 1989. *E. coli* ribosomes re-phase on retroviral frameshift signals at rates ranging from 2 to 50 percent. *New Biol.* 1:159–69
110. Weiss RB, Lindsley D, Falahee B, Gallant J. 1988. On the mechanism of ribosomal frameshifting at hungry codons. *J. Mol. Biol.* 203:403–10
111. Willcocks MM, Brown TD, Madeley CR, Carter MJ. 1994. The complete sequence of a human astrovirus. *J. Gen. Virol.* 75:1785–88
112. Wong SC, Abdelal AT. 1990. Unorthodox expression of an enzyme: evidence for an untranslated region within *carA* from *Pseudomonas aeruginosa*. *J. Bacteriol.* 172:630–42
113. Xiong Z, Lommel SA. 1989. The complete nucleotide sequence and genome organization of red clover necrotic mosaic virus RNA–1. *Virology* 171:543–54