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Programmed Translational Frameshifting

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INTRODUCTION

The ribosome is a molecular machine which evolved to translate RNA messages faithfully and efficiently into a protein product. However, the demands of speed and accuracy conflict: to the extent that the translation rate increases, accuracy suffers, and vice versa. The resolution of this conflict produces a ribosome which makes about 5×10^{-4} mistake per amino acid incorporated (152). Although this rate appears to imply an accurate ribosome, this mistake rate would result in no more than 78% of 500 amino acid proteins being accurately decoded (107). Elongation errors are approximately equally divided between missense errors, in which an incorrect tRNA is recruited to read a codon, and processivity errors, leading to premature termination.

Reducing the rate of missense errors depends on a process of kinetic proofreading (80, 146). Kinetic proofreading involves two short timing steps imposed by EF-Tu (196). The EF-Tu · GTP · aminoacyl-tRNA complex enters the ribosomal A site, but GTP is not hydrolyzed for a short period. Cognate and noncognate tRNA complexes bind the ribosome with the same kinetics, but cognate complexes essentially never dissociate from the ribosome whereas noncognate complexes dissociate more quickly than EF-Tu hydrolyzes GTP. Thus, most noncognate complexes are rejected at this stage. After hydrolysis, EF-Tu · GDP does not dissociate from the ribosome for a short period. Again, any noncognate complex still present dissociates from the ribosome much faster than EF-Tu can dissociate from the tRNA. This second stage of selection, the proofreading step, increases the accuracy of selection to the observed level. Achieving this low level of misincorporation errors requires setting the EF-Tu timing steps long enough so that noncognate tRNAs are much less likely to be accepted than are cognate tRNAs.

Processivity errors result either from spontaneous dissociation of peptidyl-tRNA from the ribosome or from translational frameshifting, leading to premature termination at out-of-frame stop codons (107); release factor (RF)-dependent termination at sense codons is very rare (96). Of the two kinds of

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errors, frameshifts occur much less frequently, probably at much less than 5×10^{-5} per codon (107). Apparently, the ribosome eliminates such errors in frame maintenance much more efficiently than it does either missense errors or spontaneous dissociation. In fact, the majority of processivity failures, those involving spontaneous dissociation of peptidyl-tRNA, may not be an error but, rather, may result from an error correction mechanism. It may be a fail-safe mechanism to reduce missense errors which elude kinetic proofreading (133). The process, termed ribosome editing, would discriminate between cognate and noncognate peptidyl-tRNA in the ribosomal P site, releasing noncognate peptidyl-tRNA from the ribosome. Kinetic proofreading should then reduce peptidyl-tRNA dissociation indirectly by reducing the frequency of noncognate tRNA accepted by the ribosome.

The vast majority of elongation errors, then, appear to occur as a result of bypassing the proofreading system; about half result in missense errors, and the other half result in premature termination. The more infrequent residual errors, those occurring by spontaneous frameshifting, may or may not be affected by this system. The fact that some mutations in EF-Tu (84) and its eukaryotic cognate, EF-1α (174), increase the frequency of both missense (in this case, reading nonsense codons as sense) and frameshift errors suggests that kinetic proofreading may also regulate frameshifting, although the mechanism by which these mutations perturb frame maintenance is not known.

Some genes include sites which program altered reading of the code at rates from 1 to 100%. These errors are superficially similar to the random errors, with nonsense codons misread as sense or with the reading frame shifting; however, is the difference between these events and random errors one of extent (the probability of error) or of kind (the difference between random and "programmed" events)? Since the programmed changes in elongation are phenomenologically diverse, there is no one answer to this question. The events run the gamut from truly programmed (for example, incorporation of selenocysteine at special UGA codons depends on a specially encoded analog of EF-Tu [reviewed in reference 16]) to those which are nearly indistinguishable from random error sites (for example, the frameshift which occurs in the Ty1 retrotransposon) (9). However, even the most clearly programmed events probably interact with features of translation responsible for reducing random elongation errors. Therefore, programmed frameshifts provide tools to understand how translational accuracy is maintained, since they help to identify the steps in elongation which are most prone to producing errors and they provide the opportunity to determine how genes evolve to manipulate the mechanism of translational fidelity.

The earliest examples of apparent translational frameshifts derived from studies of leaky frameshift mutants (3). Although analysis of these low-frequency events has proven important in defining the rules of translational frameshifting, their relevance to translational regulation in vivo was obscure. Later, frameshifting in RNA phages was shown in vitro (2) and in vivo (13) to produce a minor protein which includes parts of the coat and lysis products. These events were still infrequent, probably translational errors, although that would not rule out an in vivo role. The case for physiologically relevant frameshifting was bolstered by the apparent dependence of lysis gene expression in bacteriophage MS2 on a frameshift which occurred within the overlapping coat protein gene (99). Apparently, ribosomes translating through the coat cistron would frameshift, encountering an out-of-frame stop codon; lysis translation was believed to depend on translational reinitiation by these ribosomes. Although it was an early canonical, physiologically relevant frameshift event, the reality of this model was later challenged when activation of the lysis gene was shown to depend on normal termination at the end of the coat cistron (14).

Since the errant discovery of frameshifting in MS2, many actual examples of translational frameshifts have been found in systems from bacteria to yeasts to plants and higher animals. These systems have evolved to allow the expression of alternative translational products in a predictable stoichiometry. The purpose of this strategy varies. Its most common purpose is morphogenetic, with the canonical example being the expression of the Gag-Pol fusion peptide from retroviruses, other metazoan viruses, and retrotransposons. Fusing the *pol*-encoded replication enzymatic activities to the *gag*-encoded proteins which are the structural elements of the retroviral capsid causes the enzymes to be packaged into the developing particle. The stoichiometry of expression of Gag and Gag-Pol critically regulates the replication cycle; changing the stoichiometry would result in particles with too many or too few monomers of the replication enzymes. Altering the stoichiometry by less than twofold in either direction can drastically affect propagation of these elements, although the effect seems to be felt at the level of protein processing of the Gag and Gag-Pol polyproteins (reviewed in reference 61). Frameshifting can also be used for autogenous control. Expression of peptide release factor 2 (RF2) occurs by frameshifting which is modulated by the availability of the factor itself. Finally, frameshifting can also allow the expression of alternative enzymatic activities. Frameshifting in the *dnaX* gene of *Escherichia coli*, encoding alternative subunits of DNA polymerase III, results in premature termination when the shifted ribosome encounters a premature termination codon. The truncated product may be a less processive version of the full-length product, with the two products having roles in lagging- and leading-strand synthesis, respectively. The use of this alternative mode of translation is now firmly established among the phenomenology of translational control, and the lessons derived from work on these systems continue to enlighten our understanding of translational accuracy.

ERRORS DURING ELONGATION CAN CAUSE TRANSLATIONAL FRAMESHIFTING

The problem in understanding any system which encodes a discrete product by translational frameshifting is to explain the specificity of the event. How is it that frameshifting occurs at a particular codon and not at others? Two general solutions to this problem have been proposed, when it has been addressed. The specificity could reside in special tRNAs which have the rare capacity to shift reading frame, or it could reside in special mRNA sequences which do the same. In describing the rare translational frameshift in phage f2, which results in fusion of the coat and lysis gene products, Beremand and Blumenthal (13) proposed two possible mechanisms which could cause a shift in reading frame during elongation: quadruplet decoding by an aminoacyl-tRNA (similar to decoding by a frameshift suppressor [168]) or translational bypass of a single nucleotide (i.e., out-of-frame decoding by an aminoacyl-tRNA). The first of these is an example of a special tRNA model; the second could be understood either as a special mRNA model (special mRNA sequences causing the bypass) or as a special tRNA model (incoming aminoacyl-tRNA binding in the wrong frame). It is interesting that until recently, neither of these mechanisms was known to operate in an actual frameshift system. As we shall see, the phenomenology of frameshifting is dominated by "slippery" sequences (special mRNA structures) and by doublet decoding (special tRNAs).

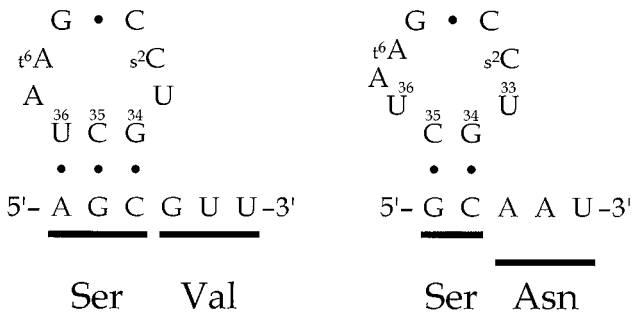

FIG. 1. Doublet decoding by *E. coli* $tRNA^{Ser}_{GCU}$ stimulates −1 frameshifting. The AGY-decoding $tRNA^{Ser}_{GCU}$ can perform either triplet (left) or doublet (right) decoding. In doublet decoding, base pairing occurs between positions 35 and 34, the middle and wobble positions of the normal anticodon, respectively. Doublet decoding is illustrated by using a site in the bacteriophage MS2 coat cistron which provokes frameshifting (2), and triplet decoding is illustrated by using a nonshifty sequence of the same cistron. Adapted from reference 27.

The first indication that some special tRNAs could induce frameshifting came from in vitro work in which particular tRNAs were added in excess to in vitro translation reaction mixtures (2, 48). Excess amounts of two tRNAs, $tRNA^{Ser}_{GCU}$ (which decodes AGU and AGC) and $tRNA^{Thr}_{GGU}$ (decoding ACC), induced frameshifting at GCA and CCG codons, respectively. Frameshifting occurs as a result of doublet decoding, i.e., recognition of a 2-base codon by a 2-base anticodon, as diagrammed in Fig. 1. In doublet decoding, nucleotides (nt) 34 (the wobble base) and 35, but not nt 36, base pair with a 2-base codon (48). The ability of the tRNA to allow doublet decoding depends solely on the sequence of the anticodon loop and, more particularly, on nt 33 to 36, since replacing these nucleotides of a $tRNA^{Phe}$ with those of $tRNA^{Ser}_{GCU}$ allows the chimeric tRNA to promote frameshifting at a GCA codon (27). Detailed mutagenesis of nt 33 to 36 of $tRNA^{Ser}_{GCU}$ showed that frameshift stimulation required either G or C at each of the two base-pairing positions, nt 34 and 35 (the sequences GC, CC, and GG were tested), and U at nt 36 (27). Surprisingly, the universally conserved U at nt 33 is not necessary, since replacing it with A caused no change in frameshifting, although U-33 interacts by a tertiary hydrogen bond with phosphate 36, stabilizing the structure of the anticodon loop (164). Note that these are in vitro experiments; mutating U-33 to any other nucleotide drastically reduces translational efficiency in vivo (5). The conclusion of this work is that certain tRNAs can induce doublet decoding but that this ability depends on strong codon-anticodon interaction by the two base pairs; hence the requirement for two G · C base pairs. The requirement for a U-36 cannot be simply explained, although it is conceivable that it allows an anticodon-loop secondary structure to form which produces doublet decoding (27).

The larger conclusion of this work is that special structural features of some tRNAs allow them to promote disruption of reading frame. Frame disruption depends on providing an excess of these tRNAs. Presumably, unconventional decoding occurs in competition with canonical decoding of the overlapping zero-frame codon, with the excess noncognate tRNA being required to outcompete cognate decoding. No attempt has been made to stimulate frameshifting by overexpressing specific tRNAs in vivo. However, unconventional elongation events can occur as a result of depleting particular aminoacyl-tRNAs by starving cells for particular amino acids. During starvation, noncognate tRNAs may compete much more efficiently than during amino acid sufficiency, producing both missense and frameshift errors (reference 67 and references therein). These events are very strongly influenced by sequence context; a very small number of sites are particularly error prone, accounting for the majority of the errors. The nature of this context effect has illuminated the mechanism underlying the phenomenon of amino acid starvation-induced frameshifting.

Gallant and his coworkers have characterized in a series of papers how amino acid starvation induces either +1 or −1 frameshifting (68, 103, 114, 157, 214, 215, 219). These experiments have demonstrated that limitation for particular amino acids can induce either +1 or −1 frameshifting, whose efficiency depends on the local sequence context. Although initial studies focused on the stimulatory effect of tryptophan limitation (in media supplemented with 3β-indoleacrylic acid, an inhibitor of the tryptophanyl-tRNA synthetase), most of the work concerns the effect of lysine limitation (by using lysine-hydroxamate, an inhibitor of lysyl-tRNA synthetase). The initial assay for frameshifting involved phenotypic suppression of frameshift mutations of the *rIIB* gene of bacteriophage T4. Phenotypic suppression refers to the reversion of the null effect of the frameshift mutation under conditions of amino acid deprivation. Suppression of (+) frameshift mutants (in which 1 nt has been inserted), which shift the translational reading frame 1 nt to the left from the normal, or zero, frame, requires a +1 frameshift event (a shift to the right) to restore the normal reading frame. (−) frameshift mutants (in which 1 nt has been deleted) shift the reading frame 1 nt to the right and are phenotypically suppressed by a −1 frameshift. Eight (+) frameshift mutants and nine (−) frameshift mutants were tested for phenotypic suppression. All of these mutants include an in-frame lysine codon within the suppression window, i.e., the region between nonsense codons in the zero frame and the relevant shifted frame. Of the 12 distinct lysine codons tested, only 1 stimulates +1 frameshifting strongly, and 1 stimulates −1 frameshifting strongly (215). Similarly, of three tryptophan codons tested, deprivation strongly stimulated +1 frameshifting at one codon and −1 frameshifting at another. Clearly, frameshift efficiency varies greatly from site to site, presumably because of sequence context. The codons which stimulate frameshifting in response to amino acid limitation have been termed "hungry" codons by Gallant and his colleagues to accentuate the fact that the cognate aminoacyl-tRNAs for these codons are limiting. Presumably, it is the lack of the cognate that causes frameshifting.

More recent work has identified the sequence elements which stimulate +1 (114, 157, 219) and −1 (68, 103, 114) frameshifting. Using a *lacZ* translational fusion reporter system, Peter et al. (157) demonstrated +1 frameshifting at the sequence GCC-AAG-C (the in-frame lysine codon is underlined). N-terminal sequencing of the frameshifted protein indicated that frameshifting occurs after decoding of the GCC codon as Ala, incorporating Ser at the +1 frame codon overlapping the hungry AAG codon, AGC.

Three distinct elements are essential for +1 frameshifting on this site. First, the hungry codon must be AAG. Replacing it by AAA, a codon recognized by the same isoacceptor, eliminated frameshifting (157). This difference may reflect differences in efficiency of decoding of the two codons. The tRNA which decodes both codons has 5-methylaminomethyl-2-thiouridine at the wobble position (189). This modification causes the tRNA to bind more efficiently to AAA than to AAG in vitro (118, 180, 230). This would suggest that decoding of the AAG codon is slower even in the absence of starvation and that reducing the concentration of cognate ternary complex makes decoding slower still. Although the reduction in the

amount of ternary complex should decrease the rate of decoding of both AAA and AAG by an equivalent factor, assuming that decoding is first order with respect to ternary complex, the relatively slower recognition of AAG, combined with the reduction in the amount of ternary complex, would lengthen the presumptive pause at the AAG codon sufficiently to allow a significant proportion of ribosomes to shift reading frames.

The existence of a translational pause is only inferred; no direct evidence that ribosomes actually pause at the site of the hungry codon has been obtained. We will see that the concept of a translational pause induced in one fashion or another is a common theme in all of the frameshift mechanisms to be discussed below. In only one case has the existence of a translational pause been demonstrated, in the case of programmed −1 frameshifting sites first identified in mammalian viruses. However, the concept of the necessity of a translational pause is a powerful element in our understanding of frameshifting, whether programmed or not. In the case of these +1 frameshifts, limitation for an amino acid reduces the availability of aminoacyl-tRNA for particular codons. Given that the rate of decoding is probably first order with respect to the EF-1α · GTP · aminoacyl-tRNA ternary complex, the reduction in its availability should slow recognition of its cognate codons. This would result in a translational pause but, more importantly, make more likely the aberrant event which causes the shift of translational frame in competition with normal in-frame decoding.

The second required element is the last zero-frame codon, GCC. This codon is the only Ala codon exclusively recognized by the rare $tRNA^{Ala}_{GGC}$; GCU can be decoded by either this tRNA or the major isoacceptor, $tRNA^{Ala}_{U^*GC}$ (the wobble base denoted by U* is uridine-5-oxyacetic acid); GCA and GCG are exclusively decoded by $tRNA^{Ala}_{U^*GC}$ (79). Changing GCC to GCA or GCG eliminated frameshifting, and changing it to GCU reduced frameshifting severely (157); changing it to either ACC or GAC also eliminated frameshifting (114). These data suggest that frameshifting occurs only when a peptidyl-$tRNA^{Ala}_{GGC}$ occupies the P site during a pause at the adjacent AAG codon (157). The data do not rule out an alternative conclusion that the primary sequence GCC in some way stimulates frameshifting. What is surprising about this result is that frameshifting occurs while peptidyl-$tRNA^{Ala}_{GGC}$ is bound in the P site. Frameshifting has been thought to involve slippage of one or two tRNAs between cognate or near-cognate codons (see below for a discussion of this point). If $tRNA^{Ala}_{GGC}$ were to slip from GCC to CCA, it could lose 2 of the 3 bp. Therefore, it is unlikely that frameshifting occurs with slippage of the +1 frame codon (157), although the actual mechanism has not been determined.

The third element in the frameshift site is the first +1 frame codon, AGC. Replacing the first +1 frame AGC codon with AGU (Ser), AGA (Arg), or AGG (Arg) eliminated frameshifting (114, 219). It is perhaps significant that this codon is decoded by the rare $tRNA^{Ser}_{GCU}$, which when present in excess, stimulates in vitro −1 frameshifting, as described above. Of course, $tRNA^{Ser}_{GCU}$ uses doublet decoding to cause −1 frameshifting, and presumably its ability to promote triplet decoding in the +1 frame stimulates +1 frameshifting. It is tempting to suggest that this tRNA is particularly shifty, stimulating both types of frameshifting by distinct mechanisms, as suggested recently (1). The alternative explanation would be that frameshifting is sensitive to the availability of the first +1 frame codon. All four of the codons tested—AGU, AGC, AGA, and AGG—are decoded by low-abundance isoacceptors, AGU and AGC by $tRNA^{Ser}_{GCU}$ and AGA and AGG each by a distinct $tRNA^{Arg}$. Perhaps the low availability of these tRNAs limits the frequency of frameshifting. Surprisingly, although AGU and AGC are both decoded by the same isoacceptor, they behave differently. That difference could again reflect a difference in the rate of recognition of the two codons by $tRNA^{Ser}_{GCU}$, with the codon recognized by a G · C Watson-Crick base pair being recognized more rapidly than the codon requiring G · U wobble pairing. Codon pairs like this do show such a bias in vitro (195). In addition, a difference in decoding rate in vivo was inferred from the differing effect of the same two codons on frameshifting on the Ty3 frameshift site (151), as discussed below.

The context requirements for leftward −1 frameshifting are, by contrast, more conventional. Again, lysine deprivation induces frameshifting at AAG codons. −1 frameshifting occurs by leftward slippage of the peptidyl-tRNA, allowing decoding of the first −1 codon (the codon which overlaps the AAG hungry codon) (68, 103). The minimal −1 frameshift site consists of the hungry codon and the 4 nt 5′ to it (114). Whether the hungry codon must be AAG rather than AAA has not been demonstrated, although given the requirement in +1 frameshifting, this seems likely. The quadruplet upstream of the hungry codon allows slippage of peptidyl-tRNA during the pause caused by slow decoding of the hungry codon (114). Changing the first U in the sequence U-UUC-AAG, which promotes −1 frameshifting, to A or C reduces frameshifting, and changing the UUC codon to AUC or AUG eliminates it (103). All of these changes reduce the ability of the peptidyl-tRNA to slip −1. The model, then, for −1 frameshifting at such sites is that during a pause caused by the lack of aminoacyl-tRNA for the hungry codon, the peptidyl-tRNA slips −1 and elongation continues in the −1 frame by first decoding the −1 codon overlapping the hungry codon.

This mechanism is distinct from the mechanism of −1 frameshifting stimulated in vitro by excess $tRNA^{Ser}_{GCU}$, which involves doublet decoding by the aminoacyl-tRNA entering the ribosome. In fact, analysis of these +1 and −1 frameshifting systems identifies three paradigms: doublet decoding of aminoacyl-tRNA, out-of-frame binding of aminoacyl-tRNA, and slippage of peptidyl-tRNA. All of these events occur very infrequently during normal elongation, as reflected by the extremely low level of spontaneous frameshifts in vivo. Each can occur at unusually high levels at specific sites. High-level frameshifting depends on either limiting the efficiency of normal elongation, which reduces the availability of an aminoacyl-tRNA, or stimulating noncanonical decoding, with excess amounts of tRNA driving noncognate recognition in preference to normal decoding. Each of these translational errors can be seen as paradigms for programmed frameshift events discussed below.

PROGRAMMED +1 FRAMESHIFTING

The *prfB* gene of *E. coli*

One of the earliest identified examples of translational frameshifting occurs in the *prfB* gene of *E. coli*, encoding the peptide release factor 2 (RF2). Expression of the gene involves an autogenous regulatory loop in which expression of the gene by translational frameshifting is negatively regulated by the gene product. This system is a paradigm for all other programmed translational frameshifts.

In bacteria, two factors function with the ribosome to identify termination codons. The factors, peptide release factor 1 (RF1) and RF2, are codon specific, with RF1 recognizing UAG and UAA and RF2 recognizing UAA and UGA (203). How they function has not been fully determined. The genes

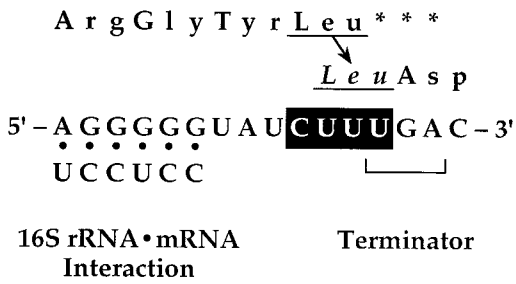

FIG. 2. Sequence of the programmed frameshift site of the *prfB* of *E. coli*. +1 slippage of peptidyl-tRNA^{Leu} at a CUU-U sequence of the *prfB* gene is stimulated by an in-frame UGA stop codon and an upstream 16S rRNA interaction site. The Leu is shown in italics in the +1 frame to indicate that the tRNA reads the noncognate Phe codon (UUU) after slippage.

for RF1 (*prfA*) and RF2 (*prfB*) are very similar, showing 31% amino acid identity overall (41). Surprisingly, the *prfB* gene includes an in-frame UGA terminator at codon 26. Translation past this position, through the remaining 340 codons of the gene, requires a +1 shift of reading frames. N-terminal peptide sequencing demonstrated that translation of RF2 actually does initiate 25 codons upstream of the UGA codon and that translation continues past the terminator in the +1 reading frame. This could be accomplished by a programmed +1 translational frameshift (Fig. 2). The sequence at the UGA codon is CUU-UGA-C (shown as codons in the initiation reading frame), which is decoded as Leu-Asp (reading the underlined codons) as shown by peptide sequencing. Craigen et al. (41) hypothesized that frameshifting at this site could be regulated autogenously. An elongating ribosome having translated the CUU codon as leucine would then face two alternative fates. If sufficient RF2 were present, it could bind to the ribosome and promote recognition of the UGA terminator, prematurely terminating translation of *prfB*. Since termination cannot occur in the absence of RF, if insufficient RF2 were present, the ribosome would pause, allowing the peptidyl-tRNA^{Leu} to shift reading frames +1 from CUU to UUU, a near-cognate codon. Slippage of the CUU-specific tRNA$^{Leu}_{GAG}$ to UUU would require a first-position U · G wobble. This hypothesis was validated by experiments showing that excess RF2 (40, 56) but not RF1 (40) suppresses frameshifting on this site in vitro and that expression of partially functional mutants of RF2 in vivo induced up to 100% frameshifting (56), an increase from the normal 30 to 50%. These experiments confirm that the efficiency of frameshifting varies inversely with the efficiency of termination at the UGA codon. If frameshifting normally competes with recognition of the nonsense codon by RF2, it should also compete with decoding by a nonsense suppressor tRNA. This form of competition was demonstrated by using a version of the *prfB* frameshift site in which the UGA codon was replaced with UAG (44). Competition with various forms of the Su7 amber-suppressor form of tRNA^{Trp} in vivo showed that frameshift efficiency varied inversely with the translational efficiency of the suppressor tRNA; that is, the more efficiently the tRNA recognized the UAG codon, the less frameshifting occurred, again confirming that frameshifting competes with an alternative fate. The concept of competition between alternative fates during a translational pause is a universal feature of programmed recoding sites.

Detailed mutagenesis of the region surrounding the *prfB* frameshift site indicated that frameshifting depends on a combination of three elements. First, the in-frame UGA codon can be replaced by either UAA or UAG terminators, causing no more than a twofold reduction in frameshifting (217). Replacing it with three other sense codons causes at least an 8-fold (UGG) and as much as a 12-fold (UUA and UUG) reduction. Second, the identity of the peptidyl-tRNA present on the ribosome during frameshifting is very critical. Changing the last decoded zero-frame codon, CUU, to GUU reduced frameshifting only 3.5-fold, but changing it to five other codons (UUA, CUA, GUA, GUG, or AUA) severely reduced frameshifting, from 110- to 440-fold (217). The severity of the mutations is roughly correlated with the ability of the tRNAs to slip +1 on the mRNA and still remain bound. The GUU-decoding tRNA (anticodon GAC) could still make two base pairs (G · U and A · U) after shifting, while those which have much less capacity to induce frameshifting can form one Watson-Crick base pair (UUA), one U · G wobble pair (CUA), or no base pairs. These data suggest that frameshifting is related to the ability of the peptidyl-tRNA to slip +1, an idea which was explored more fully by Curran (45) and will be discussed below.

The third feature is a sequence immediately upstream of the frameshift site. Mutations changing sequences in this region reduced frameshift efficiency with different severities. The sequence implicated by these mutations, AGGGGG, resembles the Shine-Dalgarno sequence of ribosome-binding sites (182); base pairing between the 3′ end of 16S rRNA and this site positions the ribosome for initiation. Weiss et al. (217) suggested that an interaction of this sort might enhance frameshifting. The spacing between the Shine-Dalgarno site and the frameshift site is critical; increasing it by only 1 nt reduces frameshifting 17-fold (217). This implies that formation of the Shine-Dalgarno interaction may strain the ribosome, pushing the peptidyl-tRNA into the +1 frame (44, 213). Consistent with this hypothesis, inserting a second Shine-Dalgarno site immediately upstream, to compete for binding to the 16S rRNA, reduced frameshifting up to 47-fold; the ability to form a less strained interaction appears to preclude the effect of the normal site.

The involvement of an rRNA-mRNA duplex was directly demonstrated by an elegant experiment with a mutant form of 16S rRNA with an altered Shine-Dalgarno complementary sequence (213). Mutations of this sort were used to demonstrate that complementarity between the 16S rRNA and mRNA directs ribosome recognition of initiators in *E. coli*. Changing the sequence of the Shine-Dalgarno complementary sequence reduces the ability of mutant ribosomes to bind to wild-type initiation regions, and complementary changes to the Shine-Dalgarno region limit initiation on mutated mRNAs; combining complementary changes to the rRNA and mRNA restores normal translation of the mRNA by the dedicated mutant ribosomes (85, 94). To determine if complementarity between the 16S rRNA and the Shine-Dalgarno analog upstream of the *prfB* frameshift site stimulates frameshifting, the 16S rRNA was changed to recognize AGCGGG instead of AGGGGG (recognized by the wild-type form). AGCGGG-specific ribosomes stimulated frameshifting when that site was present about fourfold more efficiently than when AGGGGG was, and the AGGGGG-specific ribosomes induced frameshifting on that site almost fivefold more efficiently. This confirms that the interaction is responsible for stimulation of frameshifting.

Using the *prfB* system to study general frameshifting in *E. coli*. Frameshifting in *prfB* sensitively responds to the kinetics of decoding in the ribosomal A site. The efficiency of the event also depends on the ability of the peptidyl-tRNA to slip +1 during a translational pause introduced by slow decoding in the A site. The system is therefore a sensitive tool to measure the

relative rates of both decoding and slippage on *E. coli* ribosomes in vivo.

Curran and his coworkers have used the *prfB* system to measure the rate of decoding in the ribosomal A site (46, 155), the relationship of +1 frameshifting to slipperiness of the peptidyl-tRNA (44, 45), and the context effect of 3′ neighbor nucleotides on RF recognition of terminators (155). As discussed above, Curran and Yarus (44) measured the effect of UAG suppressor tRNAs on the efficiency of frameshifting at a variant of the *prfB* frameshift site in which the UGA pause codon was replaced by UAG. The fact that efficiency was indirectly related to the efficiency of suppression suggested that the efficiency of frameshifting could be used as an indirect measure of translational efficiency of other codons. Consequently, Curran and Yarus (46) generated mutations of the *prfB* frameshift signal in which the nonsense pause codon was replaced by each of 29 sense codons. They found that the apparent rate of selection of these aminoacyl-tRNAs varied 25-fold. The data showed a correlation between apparent rate of selection of a codon by a tRNA and usage of the cognate codon in highly expressed genes—codons used in these genes tend to be those which are more rapidly decoded, and those which tend not to be used also tend to be less rapidly decoded. By contrast, in weakly expressed genes, between pairs of codon read by the same tRNA, the slower codon is actually slightly preferred.

These results confirm the prediction of a correlation between rate of decoding and codon usage (154, 207). The rate of decoding is composed of two variables, the intrinsic rate of binding of the aminoacyl-tRNA and the concentration of that isoacceptor. Surprisingly, codon usage in highly expressed genes has not evolved to use codons with high intrinsic decoding rates (determined by dividing the apparent rate of decoding by the concentration of the tRNA, the rate constant of this first-order reaction). Codon usage appears to have evolved to avoid tRNAs with rate constants at either extreme, high or low, and to use tRNAs with intermediate rate constants. This implies that codon usage has evolved to use tRNAs with features other than sheer speed; Curran and Yarus suggest that such a feature might be accuracy of decoding (46).

Curran (45) has also attempted to correlate intrinsic slipperiness of peptidyl-tRNAs with their ability to stimulate +1 frameshifting at the *prfB* frameshift site. Versions of the frameshift site were constructed, as a *prfB*::*lacZ* reporter fusion, in which the CUU codon immediately upstream of a UAG pause codon was replaced by each of 32 codons. Frameshifting on these sites varied over 1,000-fold, with the most efficient being the normal CUU (45). The five most efficient codons, CUU, CCC, UUU, GUU, and CCU, are similar in that they include at least two repeated nucleotides and all except GUU are runs of pyrimidines. Since this arrangement is most likely to allow the aminoacyl-tRNA to be able to repair with the overlapping +1 frame codon, it appears that these codons function well as a result of their ability to slip. Curran attempted to quantitate the propensity to slip of all of the tRNAs tested. The metric he developed involves estimating the stability of each tRNA after +1 slippage. The stabilities were based on data of Grosjean et al. (74), who estimated tRNA · mRNA binding strength by using the strength of interaction of anticodon-anticodon complexes between tRNAs. Although this data may be somewhat flawed—how accurately does the strength of formation of these paired tRNA dimers predict the affinity between tRNA and mRNA?—they may be used to estimate the affinity. Curran showed a clear exponential relationship between frameshift efficiency and estimated stability after +1 slippage (45). This result suggests strongly that the efficiency of frameshifting depends on the ability of the tRNA to slip, as often predicted.

It has long been recognized that various nonsense mutations are suppressed at highly variable rates. This context effect depends on the identity of the base immediately 3′ to the nonsense codon (18, 135). The efficiency of suppression depends on two variables: the rate of decoding of the nonsense codon by a suppressor tRNA and the rate of recognition of the codon by RF (228). The context effect could consist of the effect of the 3′ neighbor on each of these rates or only on one. The rate of recognition can, in principle be measured indirectly by using the *prfB* frameshift system, since the efficiency of frameshifting depends on the rate of recognition of a terminator by RF. Pedersen and Curran (155) compared the effect of the 3′ neighbor nucleotide on the efficiency of frameshifting at versions of the *prfB* frameshift site in which the pause codon is UAG. From these data, they derived the efficiency of recognition of each tetranucleotide (UAGX) by RF, finding a variation of 2.6-fold in apparent rate of selection (UAGU > UAGG > UAGC > UAGA). Comparison of the sequences of terminators of genes in various organisms has suggested that RF may recognize a tetranucleotide signal (26), although no in vivo experiment which confirms this conclusion has been reported. Significantly, the frequency of UAGX codons used as terminators and the apparent efficiency of their recognition as measured by Pedersen and Curran are highly correlated. This suggests that terminators have evolved to maximize efficiency of RF recognition, presumably to maximize gene output. Differences in rate of recognition by RF do not explain the suppression context effect. Instead, there is an apparent additional large variation in the rate of decoding by suppressors with respect to the 3′ neighbor nucleotide, which may reflect stacking effects between the codon-anticodon complex and the 3′ neighbor nucleotide (155).

Ty Retrotransposons in *S. cerevisiae*

Ty elements are a family of retrotransposons in the yeast *Saccharomyces cerevisiae* (reviewed in reference 17). Like metazoan retroviruses, Ty elements replicate via an RNA intermediate by a process of reverse transcription. They are not true viruses since they do not move horizontally within a population by producing virus particles. Rather, replication results in reintegration of the element into the genome of the same cell at a new chromosomal location. The enzymatic activities and structural proteins required for Ty replication are encoded in two genes, the analogs of the retroviral *gag* and *pol* genes. The *pol* product is expressed as a translational fusion to the upstream and partially overlapping *gag* product (35). Expression of this translational fusion in two types of Ty elements is now known to require +1 translational frameshifting (9, 62). Although the two elements both use frameshifting, the phenomenology is very different and in one case unexpected.

Frameshifting in retrotransposon Ty1 occurs by tRNA slippage. Frameshifting was first demonstrated for the Ty1 class of elements. The *gag* analog of Ty1, termed *TYA*, overlaps the first 38 bp of the *pol* analog, *TYB*, with *TYB* shifted into the +1 reading frame (34). *TYB* expression, as a TYA-TYB fusion peptide (34), occurs by +1 translational frameshifting within a short sequence of the overlap, identified first as a 31-nt sequence (222) and later reduced to a 14-nt sequence (35). Later, detailed analysis identified a sequence of only 7 nt that is both necessary and sufficient to promote frameshifting (9). Frameshifting occurs on the sequence CUU-AGG-C, shown as codons of the upstream *TYA* gene, which is decoded as Leu-Gly reading the underlined codons. All single base changes at

any position of this heptamer either eliminate or greatly reduce frameshifting. The partially functional mutations fall in the wobble position of the CUU codon (CUG and CUC) and in the AGG codon (CGG, UGG, and AGU).

The heptameric minimal site has two notable features: overlapping Leu codons (CUU and UUA) and an Arg codon (AGG) decoded by a low-abundance tRNA encoded by a single-copy gene (66). In *S. cerevisiae*, one tRNA recognizes all four of the CUX family of Leu codons, $tRNA^{Leu}_{UAG}$. An unmodified U in the wobble position of the anticodon (165) confers the relaxed specificity on the tRNA. In fact, in vitro the tRNA will recognize all six Leu codons. These data suggested a mechanism for Ty1 frameshifting: during a pause caused by the low availability of the cognate $tRNA^{Arg}_{CCU}$, specific for AGG, $tRNA^{Leu}_{UAG}$ slips +1 from CUU to UUA. If this model were correct, one would expect that overexpression of $tRNA^{Arg}_{CCU}$ would interfere with frameshifting. In fact, 5-fold overexpression of this tRNA did interfere, reducing frameshifting 43-fold (9). Second, the model predicts that changes in $tRNA^{Leu}$ which reduce its ability to slip +1 would reduce frameshifting. The UUG-specific $tRNA^{Leu}_{CAA}$ was mutated to change its anticodon to AAG, an anticodon cognate for CUU but not for UUA. Overproduction of this tRNA was predicted to reduce frameshifting by interfering with slippage of the tRNA; in fact, overproducing this tRNA also reduced frameshifting 43-fold whereas overproducing a control mutant in which the anticodon was changed to the putative slippery UAG had no effect (9). These data were interpreted as demonstrating that frameshifting occurs with the ribosomal A site poised over the AGG codon but empty of an aminoacyl-tRNA. During the pause induced by the lack of $tRNA^{Arg}_{CCU}$, $tRNA^{Leu}_{UAG}$ slips +1 from CUU to UUA.

This frameshifting mechanism strongly resembles +1 frameshifting at *prfB* (217): during a translational pause, a tRNA slips from a cognate to a near-cognate codon. In both cases, the ribosomal A site is empty during the translational pause. Of course, according to the three-site model of the ribosome (139), after translocation of the peptidyl-tRNA to the P site, the deacylated tRNA, which had been bound to the P site before peptide transfer, moves to the E (exit) site. The function of the E site is controversial. One hypothesis states that occupation of the E site by the deacyl-tRNA allosterically regulates selection of aminoacyl-tRNA in the A site, creating a low-affinity A site and thus reducing the probability of acceptance of noncognate aminoacyl-tRNA (167). In this model the deacyl-tRNA is bound by a codon-anticodon interaction to the mRNA in the E site. An alternative hypothesis states that binding of the deacyl-tRNA to the E site facilitates its release from the P site; binding of deacyl-tRNA is seen as a transient intermediate, not bound by a codon-anticodon interaction (223). Whether the deacyl-tRNA does base pair in the E site is not clear. Some data suggest that cognate tRNA associates in the E site more efficiently than noncognate tRNA does (70). However, the measured free-energy contribution of codon-anticodon pairing in the E site suggests that the increase in affinity for a cognate codon is up to 3 orders of magnitude less than for binding in the P site, deemphasizing the importance of pairing of deacyl-tRNA (113).

The ability or inability of the tRNA which occupies the E site to slip has no effect on +1 frameshifting in either the *prfB* system (217) or the Ty1 system (9). These results support the hypothesis that the deacyl-tRNA does not base pair with the mRNA in the E site during the shift. This is in marked contrast to the effect of changes in the codon bound by the peptidyl-tRNA, which can drastically reduce frameshifting. The lack of base pairing in the E site, however, does not invalidate the allosteric three-site model of Nierhaus and his colleagues (reviewed in reference 167), since the necessity for base pairing in that model is not clear.

The AGG codon in the Ty1 site clearly stimulates frameshifting because of the low availability of its cognate $tRNA^{Arg}_{CCU}$. This simple role for the codon mimics the role of the AAG codon in stimulating frameshifting in response to lysine deprivation in *E. coli* (219). In its simplest form, the model for the Ty1 event would predict that any sufficiently hungry codon could induce the slippage of a susceptible peptidyl-tRNA. This proved not to be the case (9). Replacing the AGG codon with a codon recognized by the equally rare $tRNA^{Arg}_{CCG}$ (CGG) or $tRNA^{Ser}_{GCU}$ (AGU) reduced frameshifting about 20-fold, and replacing AGG with UGG, recognized by the apparently abundant $tRNA^{Trp}_{CCA}$, allowed the same 20-fold-lower frameshifting. Most significantly, the AGG codon was replaced by UAU, decoded by $tRNA^{Tyr}_{GUA}$, and tested in a set of strains with progressive deletions of the eight genes encoding the tRNA. No frameshifting was observed in any of these strains, although with only two gene copies remaining, the lack of the $tRNA^{Tyr}_{GUA}$ restricted the growth rate of the strain (28). Thus, the fact that a codon is decoded by a limiting tRNA is not sufficient to cause a translational pause capable of inducing detectable frameshifting (9).

Overexpression of $tRNA^{Arg}_{CCU}$ suppresses frameshifting at CUU-AGG-C, suggesting that the AGG codon stimulates frameshifting as a hungry codon, i.e., a codon whose cognate $tRNA^{Arg}_{CCU}$ is present at a limiting concentration in vivo (9). Since overexpression of the tRNA would reduce the expression of the Ty1 *pol* analog, *TYB*, relative to the *gag* analog, *TYA*, it is not surprising that it also reduces Ty transposition (227). Correspondingly, underexpression of $tRNA^{Arg}_{CCU}$ leads to increased frameshifting. Although only one gene encodes $tRNA^{Arg}_{CCU}$, the gene is not essential for growth; a cell lacking $tRNA^{Arg}_{CCU}$ survives, probably because of near-cognate decoding of AGG by $tRNA^{Arg}_{UCU}$, which normally decodes AGA exclusively (100). However, the effect of loss of $tRNA^{Arg}_{CCU}$ is to greatly stimulate frameshifting, to virtually 100% efficiency, and in this strain, transposition of Ty1 elements is also greatly decreased (100). Although frameshifting can be regulated in response to the concentration of this tRNA, there is no evidence for any in vivo modulation of frameshifting by altering the level of its expression or aminoacylation.

Frameshifting in retrotransposon Ty3 occurs by out-of-frame binding of tRNA. Almost all of the examples discussed present a common paradigm for +1 translational frameshifting. During a translational pause induced in any of several ways, a peptidyl-tRNA slips onto a near-cognate codon in the +1 reading frame. After this slippage, translation continues in the new reading frame to produce the frameshifted product. The only exceptions to this common mechanism come from the work of Gallant and his collaborators (114, 157), who showed that +1 frameshifting at certain hungry lysine codons occurs despite the apparent inability of the peptidyl-tRNA to slip; the frameshift site GCC-AAG stimulates +1 frameshifting although the GCC decoding $tRNA^{Ala}_{GGC}$ retains only one $\frac{G \cdot C}{CGG}$ base pair when it slips onto the +1 CCA codon ($\frac{CCA}{CGG}$). This event occurs extremely infrequently in the absence of a strong starvation signal, and the relevance of the event to frameshifting errors in vivo has been open to debate.

However, an efficient programmed frameshift system which induces frameshifting without peptidyl-tRNA slippage has recently been described (62). The retrotransposon Ty3 of *S.*

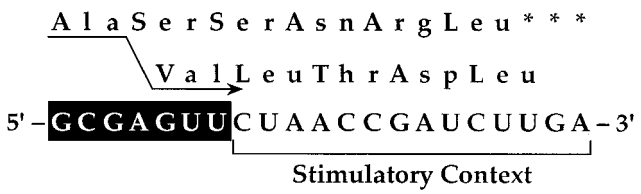

FIG. 3. Sequence of the programmed frameshift site of the Ty3 retrotransposon of *S. cerevisiae*. +1 frameshifting on the Ty3 retrotransposon occurs without tRNA slippage by decoding of a +1 frame Val codon (GUU) while peptidyl-tRNA$^{Ala}_{CGC}$ is in the P site. A downstream context stimulates frameshifting.

cerevisiae is distantly related to the Ty1 retrotransposon. It encodes a *gag* analog, *GAG3*, and a *pol* analog, *POL3*. Expression of *POL3* is as a translational fusion to the upstream *GAG3* product. Frameshifting on the sequence GCG-AGU-U (shown as codons of *GAG3*) encodes Ala-Val (reading the underlined codons [see Fig. 3]). The tRNA which decodes the GCG codon has not been identified; however, by the rules of decoding in *S. cerevisiae*, it should have the anticodon CGC or, less likely, CGU. Surprisingly, given these putative anticodons, frameshifting must occur even though it cannot form the required two base pairs with the +1 frame overlapping codon ($\begin{matrix} CGC \\ CGA \end{matrix}$ or $\begin{matrix} CGA \\ CGU \end{matrix}$). We assume that frameshifting on the Ty3 site must occur by out-of-frame binding of aminoacyl-tRNA in the A site. Two features stimulate this unusual event. First, a hungry codon, AGU, decoded by the limiting tRNA$^{Ser}_{GCU}$ causes a translational pause with peptidyl-tRNA$^{Ala}_{CGC}$ bound at the GCG codon. Second, a 14-nt "context" sequence immediately distal to the GCG-AGU-U frameshift site by an unknown mechanism stimulates frameshifting about 7.5-fold (Fig. 3).

Two alternative mechanisms for the unusual Ty3 frameshift event are possible. First, the structure of the mRNA may be unusual, leading to bypassing of the A between the decoded GCG and GUU codons. Alternatively, frameshifting may depend on a special characteristic of one of the tRNAs which decode the site, either the GCG-decoding tRNA$^{Ala}_{CGC}$ or the GUU-decoding tRNA$^{Val}_{IAC}$. Genetic analysis has shown that frameshifting on the Ty3 site does depend on a special peptidyl-tRNA$^{Ala}_{CGC}$. A frameshift-incompetent tRNA$^{Ala}_{UGC}$ was mutated to change its anticodon from UGC to CGC such that it would now recognize the GCG codon. Overexpression of this mutant tRNA, competing out binding by the low-abundance tRNA$^{Ala}_{CGC}$, drastically reduced frameshifting (208). This effect demonstrates two things. First, it is the structure of peptidyl-tRNA$^{Ala}_{CGC}$ which promotes frameshifting; changing the tRNA which recognizes GCG to another isoacceptor reduces frameshifting drastically. Second, the result eliminates any alternative model in which frameshifting results from an unusual mRNA structure causing the A in the GCG-AGU-U sequence not to be available to base pair with incoming aminoacyl-tRNA$^{Ser}_{GCU}$.

tRNA$^{Ala}_{CGC}$ is not the only peptidyl-tRNA which induces frameshifting. When the GCG codon of the Ty3 site was replaced by all 63 other codons and frameshifting was induced by the AGG pause codon in the absence of its cognate tRNA$^{Arg}_{CCU}$, eight tRNAs were found to induce +1 frameshifting to different degrees (208). Four tRNAs induce very high levels of frameshifting, including tRNA$^{Leu}_{UAG}$, responsible for Ty1 frameshifting; tRNA$^{Ala}_{CGC}$, which induces Ty3 frameshifting; tRNA$^{Pro}_{CGG}$; and tRNA$^{Gly}_{CCC}$. The others induce 5 to 45% frameshifting. Whereas Curran (45) found a tight correlation between slipperiness of peptidyl-tRNAs and induction of frameshifting in *E. coli*, these data do not support that conclusion for *S. cerevisiae*. Among the tRNAs which induce frameshifting, only half appear capable of slipping +1. More importantly, several tRNAs predicted to slip do not induce frameshifting (e.g., tRNA$^{Phe}_{GAA}$, decoding UUU; tRNA$^{Lys}_{UUU}$, decoding AAA; tRNA$^{Glu}_{UUC}$, decoding GAA; and tRNA$^{Gln}_{UUG}$, decoding CAA). There appears to be no correlation between peptidyl-tRNA slippage and +1 frameshifting in *S. cerevisiae*.

The Rat Ornithine Decarboxylase Antizyme Gene

Ornithine decarboxylase (ODCase) catalyzes the first, rate-limiting step in polyamine biosynthesis, converting ornithine to putrescine, which is then converted to spermidine and then spermine by the enzymes spermidine synthase and spermine synthase, respectively (156). The level of ODCase is modulated both transcriptionally and posttranscriptionally. Recently, it has become clear that ODCase is regulated largely by degradation which is controlled by an inhibitory protein, ODCase antizyme. Production of the antizyme has recently been shown to occur by a programmed +1 frameshift which is sensitively regulated in response to polyamines (69, 127, 170). Other than the autogenous control loop in RF2 synthesis, this is the only known example of physiological control of frameshifting. It appears that polyamines are general regulators of +1 frameshifting (6, 7), although the mechanism of that control is unclear.

The ODCase antizyme inhibits the activity both of ODCase and of the polyamine transport protein (137, 191). The antizyme was initially identified as an activity induced by putrescine which inhibited ODCase stoichiometrically (76, 131), although in fact the in vivo role of antizyme is to target ODCase for ubiquitin-independent degradation by the 26S proteasome (142, 198). Synthesis of the antizyme was assumed to be regulated by polyamines at the translational level, since its induction was sensitive to cycloheximide but not actinomycin D (65, 126).

The sequence of the antizyme cDNA (138) revealed that the region of the gene included two open reading frames (ORFs), a short ORF, which could encode a 7.4-kDa protein, overlapping a longer ORF, which could encode a 24-kDa protein, approximately the size of the antizyme but lacking an ATG initiation codon (127, 170). The longer ORF is in the +1 frame with respect to the shorter. Initial analysis showed that the longer ORF could be expressed to produce active antizyme if it were provided with an in-frame initiator (128, 143), although its expression was independent of polyamines (143). Subsequent work showed that expression of antizyme normally occurs by initiation within the short upstream ORF followed by frameshifting into the second ORF and that the efficiency of the frameshift was regulated by polyamines (127, 170). The first published data supporting a frameshift mechanism of expression, from Rom and Kahana (170), showed that expression of a full-length product required the UGA termination codon at the end of the upstream ORF. This result is consistent with a frameshift mechanism, rather than RNA editing, although it did not rule it out, since the nonsense codon could provide a required translational pause.

A more extensive analysis by Matsufuji et al. (127) provided clearer evidence of +1 translational frameshifting. Rom and Kahana had demonstrated that the expression of a protein of the correct molecular weight depended on the sequence surrounding the in-frame termination codon. Matsufuji et al. were

able to produce a partial protein sequence of the segment of the protein encoded across this site. The sequence showed that the frameshift occurs at the stop codon, since the Ser codon (UCC) immediately preceding it was decoded and the Cys codon following it was not. In addition, the sequence past the Ser residue was consistent with the sequence of the +1-shifted longer ORF. These data are consistent with a +1 frameshift occurring at the UCC codon, although again an editing event which removes one nucleotide cannot be formally eliminated. Since polyamines are known to modulate translation, the fact that polyamine concentrations modulate expression argues for a translational mechanism.

Five nucleotides downstream of the UCC codon is a potential pseudoknot. Mutations which eliminated the pseudoknot reduced frameshifting up to fourfold. Importantly, compensatory double mutations which altered the primary structure of the pseudoknot but restored the secondary structure restored frameshifting to wild-type levels. Thus, the pseudoknot appears to stimulate the frameshift event, although it is of secondary importance with respect to the termination codon, since in a mutant lacking the terminator, deleting the pseudoknot region had no effect. There is no precedent for the involvement of such a secondary structure in +1 frameshifting. The only correlate is the downstream context in the Ty3 frameshift, although in that case it is the primary sequence of the region, not its secondary structure, which stimulate frameshifting.

The pseudoknot is almost certainly not a specific sensor of polyamines. In an unrelated series of experiments, Balasundaram et al. (6, 7) have shown that the efficiency of +1 frameshifting on the Ty1 site in *S. cerevisiae* is regulated by polyamines. In apparent contrast to the ODCase antizyme system, frameshifting is stimulated by spermidine deprivation rather than by an excess of polyamine (7). The deprivation was created in vivo by inactivating the *SPE2* gene, which specifies *S*-adenosylmethionine decarboxylase, required for synthesis of both spermidine and spermine. In cells that were grown with exogenous spermidine and then switched to medium lacking spermidine, the intracellular spermidine level gradually dropped and frameshifting increased about 10-fold (7). However, as the spermidine concentration decreased, the intracellular concentration of putrescine also drastically increased from an initially undetectable amount. It was the presence of this excess putrescine which induced frameshifting, since blocking putrescine biosynthesis, by deleting the yeast ODCase gene, *SPE1*, eliminated the induction. Simply overexpressing putrescine in the presence of normal amounts of spermidine increased frameshifting only slightly, indicating that the increase resulted from the imbalance of putrescine and spermidine.

Thus, +1 frameshifting in both the yeast Ty1 and rat ODCase antizyme systems is adjusted when the balance of polyamines is disturbed. The response to polyamines appears different in the two systems, since the antizyme is induced by either putrescine, spermidine, or spermine and the yeast system appears specific to putrescine. Since experiments were not done to test the effect of excess spermidine or spermine, it may be that the yeast and ODCase antizyme systems are more similar than was expected. If that proves correct, the effect of polyamines on +1 frameshifting would be unrelated to the specific system used. The fact that two such distant +1 frameshift sites respond to modulation of polyamines already suggests that this may be correct.

Addition of any of several polyamines to a cell-free protein synthesis system increases the yield of products, especially high-molecular-weight proteins (4). In addition, in the presence of physiological levels of Mg^{2+}, polyamines are essential for aminoacylation of tRNAs (117). Polyamines also regulate the fidelity of translation. They regulate the fidelity of aminoacylation; increasing concentrations of polyamines reduce the frequency of misacylation, especially in the absence of Mg^{2+} (116). This effect might explain why increasing the concentration of polyamines increases the fidelity of protein synthesis in vitro (88) and in vivo (132). However, the effect of polyamines on translation appears to be more complex, affecting the conformation of aminoacyl-tRNAs and thus the efficiency and accuracy of codon-anticodon recognition (reference 145 and references therein). These experiments show that increasing the concentration of polyamine causes an increase in the accuracy of decoding by reducing the likelihood of noncognate tRNA being accepted. This seems an effect opposite to the stimulation of frameshifting by polyamines. However, consistent with that observation, polyamines also stimulate readthrough in vitro of termination codons, both UGA (82) and UAG (140). In addition to binding tRNAs, polyamines are known to bind ribosomes (97) and polynucleotides (124); it is unclear how polyamines might stimulate mistranslation by readthrough or frameshifting.

PROGRAMMED −1 FRAMESHIFTING

By far the most numerous and ubiquitous of frameshift sites are a class of −1 frameshift sites first described in metazoan retroviruses. These sites have been found in retroviruses (60, 86, 90–93, 121, 130, 141, 144), coronaviruses (21, 49, 50, 58, 78), toroviruses (186), arteriviruses (71, 134), astroviruses (95, 123, 220), giardiaviruses (211), plant viruses (20, 101, 136, 163, 226), *Drosophila* retrotransposons (47, 125, 162, 173), a virus-like element in *S. cerevisiae* (52, 87, 205), a bacterial gene (15, 64, 201), bacteriophage genes (38, 39, 57, 112), and bacterial insertion sequences (59, 105, 160, 166, 176, 188, 209, 210). Sequence comparison and molecular genetic analysis of many of these sites have identified a canonical structure for these frameshift sites. Their prevalence across such an evolutionarily diverse distribution suggests that such sites may have evolved multiple times, converging on a single simple solution.

Programmed −1 Frameshifting in Eukaryotes

In retroviruses, expression of the primary translational product of the *pol* gene occurs as a translational fusion to the upstream *gag* gene (93). The understanding that expression of the Gag-Pol fusion protein occurred by this mechanism was slow in coming and faced formidable ideological obstacles. The favorite mechanism for expression of the Gag-Pol fusion, by analogy to the expression of the *env* product, was through an inefficient splice, uniting *gag* and *pol* into one reading frame in the spliced mRNA. In a review published in 1984, Coffin states that "the only reasonable hypothesis to explain the expression of *pol* is that there is a distinct mRNA that can be translated into the *gag-pol* precursor and that this mRNA is created by splicing around the UAG codon [at the end of *gag*] to shift the reading frame somewhere near the *gag-pol* boundary" (36). However, by the next year the same author was describing the idea of splicing to allow expression of the *gag-pol* product as "losing ground" (37). Coffin was, of course, reflecting the common state of belief in the field, which was finally radically altered with the publication of the paper which demonstrated unequivocally a translational mechanism of expression (93). This episode could be offered as a small example of "normal science," as defined by Kuhn (106). In a small way, the paper by Jacks and Varmus revolutionized our thinking, forcing us to

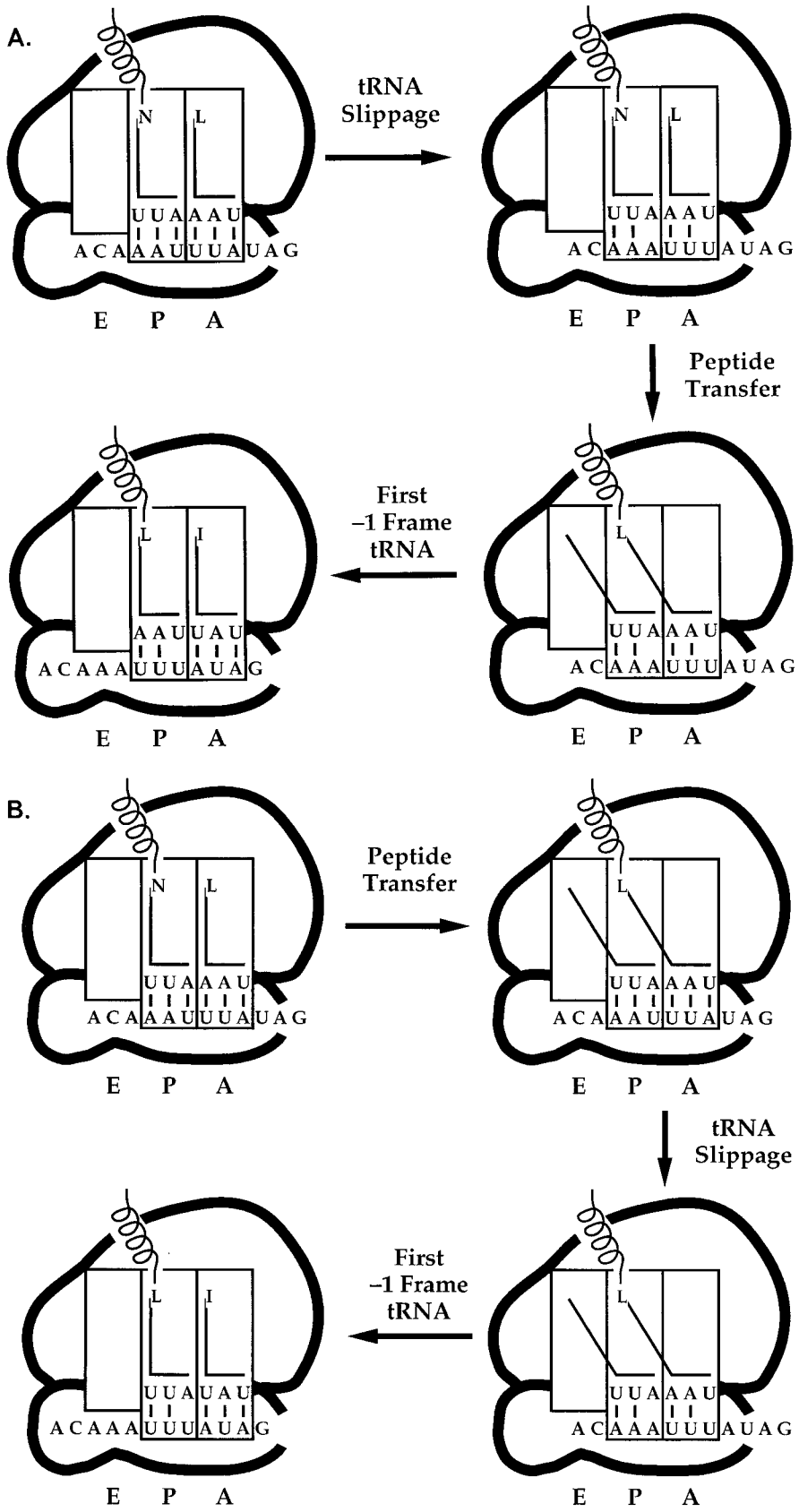

FIG. 4. Models of simultaneous-slippage −1 frameshifting. Two competing models are illustrated by using the hybrid-site model for tRNA translocation (139). (A) In the Jacks et al. model (90), slippage of the two tRNAs occurs after aminoacyl-tRNA enters the A site but before peptide transfer. (B) The Weiss et al. model (218) proposes that the slip occurs after peptide transfer, when the tRNAs occupy the hybrid E-P and P-A sites. In both models, slippage allows the tRNAs to form less than 3 bp with the mRNA after slippage. The site illustrated is from RSV. N, L, and I are the one-letter codes for the amino acids asparagine, leucine, and isoleucine, respectively.

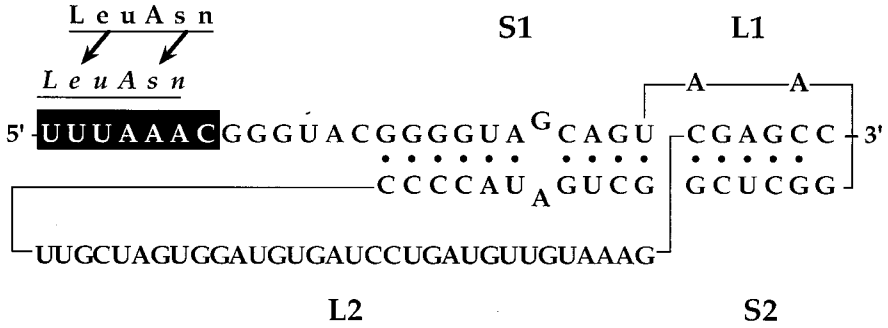

FIG. 5. Sequence of the programmed frameshift site of the coronavirus IBV. −1 slippage occurs on the sequence U-UUA-AAC by slippage of Leu and Asn tRNAs stimulated by a downstream pseudoknot. The four components of the pseudoknot, stem 1 (S1), loop 1 (L1), stem 2 (S2), and loop 2 (L2), are labeled.

consider the idea that the ribosome might at some sequences be programmed to make what appears to be a translational error. Much of the work in this field during the last 10 years has focused on understanding how the ribosome can do this.

−1 Frameshifting occurs on a "slippery heptamer." The initial discovery of −1 frameshifting in Rous sarcoma virus (RSV) simply indicated that expression of the Gag-Pol fusion protein occurred translationally but did not specify how this event occurred. Later, sequence comparison and site-specific mutagenesis identified a motif as responsible for inducing the shift. The motif consists of two elements, a heptanucleotide sequence of the form X-XXY-YYZ, shown as codons of the upstream *gag* gene (for example, in RSV A-AAU-UUA [90] or in the coronavirus infectious bronchitis virus [IBV] U-UUA-AAC [22]), and an RNA secondary structure, usually a pseudoknot, beginning about 6 nt downstream (193). Mutational analysis suggested that changes to the "slippery-heptamer" sequence which interrupt its repetitive structure can drastically reduce frameshifting (90). Changes made upstream or downstream of the heptamer had no effect on expression, but almost all changes to the heptamer itself reduced expression of the Gag-Pol fusion protein. Changing any of the first 3 nt in the heptamer, for example, changing A-AAU-UUA to C-AAU-UUA, A-CAU-UUA, or A-ACU-UUA, reduced expression about fivefold. The effect of all single base changes to the next 3 nt were much more severe, essentially abolishing expression. These data suggest that the repetitive nature of the site, i.e., the existence of tandemly repeated nucleotides, is essential to frameshifting.

Sequence comparison of the many available sites confirms the importance of the motif. ten Dam et al. (193) identify 11 different heptamer sequences from among 38 −1 frameshift sites: 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 which would conform do not appear in the list. In an attempt to determine how many potential heptamer sequences there are, Brierley et al. (23) constructed a nearly exhaustive collection of heptamers. They found that almost all of the sequences tested stimulated measurable frameshifting, although the efficiency varied widely. All of the sites listed above which were tested stimulated frameshifting efficiently (from 16.1% for G-GGU-UUA to 41.7% for U-UUA-AAC). As had been noted by Dinman et al. (52), introducing GGG or CCC into the YYY position of the motif severely reduced frameshift efficiency; however, efficient sites could be constructed with any homopolymeric sequence at the XXX position.

A definitive analysis of frameshifting required determination of the protein sequence encoded across the frameshift site. The mutational analysis argued strongly that the heptamer was an essential element but did not prove that frameshifting happened there. However, peptide sequencing has demonstrated that the heptamer is the site of shifting. For example, the frameshift on the RSV site, A-AAU-UUA (90), occurs after decoding of the zero frame codon UUA as Leu and without decoding the −1 frame codon UUU as Phe. This means that the frameshift occurs after the entire heptamer has been decoded.

The simultaneous-slippage model. On the basis of the repetitive structure of the heptamer and the nature of the protein product produced, Jacks et al. suggested a model for −1 frameshifting in RSV termed the simultaneous-slippage model (90) (Fig. 4A). The model proposes that each of two ribosome-bound tRNAs slip in the 5′ direction simultaneously from their initial position in the zero frame (XXY-YYZ) to the −1 frame (XXX-YYY). The model explains why the YYZ codon is decoded in the final product and the necessity for the homopolymeric triplets XXX and YYY. The model proposes that frameshifting can occur only when tRNAs can continue to form at least two base pairs in the shifted frame; therefore, an XXY-decoding tRNA can still base pair at least to the first 2 nt of XXX. The fact that the first 2 nt of the anticodon must be able to pair in overlapping frames creates the need for the 3-nt repeat.

The original model imagined this happening before peptide transfer by slippage of the peptidyl- and aminoacyl-tRNAs bound to the ribosomal P and A sites, respectively. At this point in the ribosome cycle, there are two tRNAs, each base paired to the mRNA. However, as suggested by Weiss et al. (218), the shift may occur at a second step in the elongation cycle during which two tRNAs engage the mRNA, after peptide transfer but before translocation of the tRNAs (Fig. 4B). These two mechanisms cannot be distinguished by their protein products or by site-directed mutagenesis of the slippery heptamer.

Surprisingly, on the human immunodeficiency virus type 1 (HIV-1) site, U-UUU-UUA (91), there appeared to be two products synthesized across the heptamer. About 70% of the product, as on the RSV site, is synthesized by decoding the zero-frame UUA codon as Leu; however, about 30% of the time, the ribosome appears to shift before decoding UUA. In this product, the Leu is replaced by Phe, presumably encoded by the overlapping −1 frame codon UUU. The more prevalent product, but not the alternative product, could have been produced by simultaneous slippage. Jacks et al. suggested that the less abundant product could have been produced by the nor-

mal mechanism of simultaneous slippage, followed by spontaneous release of the UUA-decoding $tRNA^{Leu}$ from the UUU codon and subsequent decoding of UUU by phenylalanyl-$tRNA^{Phe}$ (91). A simpler alternative would be that a single $tRNA^{Phe}$ bound to the zero frame UUU codon slips onto the overlapping −1 frame UUU codon followed by decoding of the −1 frame UUU codon (note that the sequence immediately upstream of the heptamer, U-AAU, should not allow slippage of peptidyl-$tRNA^{Asn}$, necessitating a single tRNA slippage model).

Support for this alternative mechanism comes from an analysis of frameshifting on the HIV-1 site in *E. coli*. Yelverton et al. (229) show that starvation of *E. coli* cells for leucine induced frameshifting on the HIV-1 site, although starvation for phenylalanine or arginine had no effect. As in eukaryotic cells, frameshifting on the HIV-1 site in *E. coli* resulted in two products but starvation of the cells for leucine specifically stimulated the product in which the −1 frame UUU codon, overlapping the UUA Leu codon, was decoded. Apparently, the inability to decode the Leu codon efficiently stimulated the alternative product. This result is consistent with the one-tRNA slippage model; with the ribosome paused with the UUA codon in the A site by the low availability of the cognate $tRNA^{Leu}$, peptidyl-$tRNA^{Phe}$ slips −1 between identical UUU codons. Decoding then resumes in the +1 frame by reading the next codon, UUU, as Phe. Earlier, Weiss et al. (218) found that *E. coli* ribosomes can shift on the mouse mammary tumor virus (MMTV) slippery heptamer, A-AAA-AAC, by an apparent two- or one-tRNA slippage model, either decoding the in-frame Asn codon, AAC, before slippage or slipping and reading the overlapping Lys codon, AAA. Interestingly, the presence of a downstream hairpin biased frameshifting toward the two-tRNA slippage whereas removing the hairpin favored one-tRNA slippage (the construct used lacked the downstream region necessary to form the MMTV pseudoknot) (218). This result makes it unlikely that one-tRNA slippage contributes greatly to −1 frameshifting on such sites in eukaryotic cells, although a minority of products may be made by that mechanism.

More recently, Horsfield et al. (81) introduced termination codons (UGA, UAA, and UAG) immediately downstream of the HIV-1 slip site (e.g., creating the sequence U-UUU-UUA-UGA) and found that frameshift efficiency in *E. coli* was influenced by the efficiency of recognition of the terminators by peptide RFs. Most convincingly, they showed that expression of a defective RF2 protein, capable of nonsense codon-directed binding to ribosomes but deficient in peptide release, stimulated frameshifting from the UGA and UAA constructs but not the UAG or sense codon control constructs. Since RF2 recognizes UGA and UAA but not UAG, this suggests that inefficient termination at the inserted terminator stimulated frameshifting. Since this stimulation could occur only after the codon has entered the A site, they propose that simultaneous slippage occurs in this case after translocation of deacyl-$tRNA^{Phe}$ and peptidyl-$tRNA^{Leu}$ into the E and P sites, which would place the termination codon in the A site. A less radical model would propose that frameshifting in this case again occurs by slippage of a single tRNA, in this case peptidyl-$tRNA^{Leu}$, during a pause caused by the slow recognition of the termination codon. This mechanism resembles the alternative mechanisms of Yelverton et al. (229) and Weiss et al. (218). This model is consistent with the data from several sources that base pairing by the tRNA in the E site does not influence frameshifting (see, e.g., references 9, 114, and 217). Distinguishing between these models would require introducing mutations into the putative E-site codon (e.g., U-UUU → G-UGU) to determine if they interfere with frameshifting.

The existence of an alternative mode of frameshifting on the HIV-1 site does not decrease the generality of the simultaneous-slippage model. Clearly, the simultaneous-slippage model better explains frameshifting on the vast majority of sites, including all of the eukaryotic sites. In bacteria, as discussed below, the situation is less monolithic; several of the putative simultaneous-slippage sites probably actually employ a mechanism more similar to that proposed by Yelverton et al. (229). Before dealing with those cases, though, we will discuss other aspects of simultaneous slippage.

Stimulation of −1 frameshifting by a downstream pseudoknot. The vast majority of simultaneous-slippage sites include a pseudoknot downstream of the slippery heptamer, although some sites substitute a simple hairpin or, in rare cases, no structure at all (193). Early papers suggested that a hairpin might stimulate frameshifting (21, 90, 92), yet mutagenesis showed that the region distal to the hairpin in the RSV site was also required, suggesting the possibility of a pseudoknot (90). The first clear demonstration of a pseudoknot came from analysis of the coronavirus IBV by Brierley et al. (22). −1 frameshifting occurs between the 1a and 1b genes of the virus, producing a 1a-1b fusion protein (21). The site of frameshifting was identified as the slippery heptamer, U-UUA-AAC, by flanking it with stop codons in the zero and −1 frames (89). Initially, a hairpin loop located 6 nt downstream of this heptamer was thought to stimulate frameshifting (21). Later, however, Brierley et al. (22) recognized that the region distal to the heptamer could form a pseudoknot (Fig. 5) in which an 8-nt region of the hairpin loop (5′-GAGG CUCG-3′) could base pair with an 8-nt segment (5′-CGAGC CUU-3′) 32 nt downstream. Site-directed mutagenesis demonstrated the requirement for the two base-pair interactions (the stem of the hairpin loop is termed S1, and the pairing between the loop and the downstream region is termed S2). Changes to each of the putative paired regions were made by replacing a stretch of 6 nt of S1 with their pairing partners (i.e., changing apparent G · C pairs to C · C and G · G), and the 8 nt of S2 with their pairing partners. Each of these four mutations eliminated the ability to form the pseudoknot and eliminated frameshifting. Re-creating the pseudoknot by making both compensatory changes in each stem (i.e., changing apparent G · C pairs to C · G) restored frameshifting. Since there is no chemical similarity between the alphabetic palindromes created by these mutations (5′-GAGGCUCG-3′ has no chemically relevant similarity to 5′-GCUCGGAG-3′), apparently the ability to form a pseudoknot, not its primary sequence, stimulates frameshifting.

Similar experiments were done with other putative pseudoknots, including those of the L-A virus of *S. cerevisiae* (52), MMTV (30), feline immunodeficiency virus (141), and simian retrovirus 1 (SRV-1) (192, 194), as well as a more extensive analysis of the IBV site performed by Brierley et al. (24). The results in each case were qualitatively similar: mutations which interfere with formation of the pseudoknot reduce frameshifting, and compensatory double mutations restore frameshifting, although not always to wild-type levels. Although it is not possible to assign a free energy value for a pseudoknot, since the effects of the loops on stability are unknown, the effect of mutations on the stability of each of the stems in isolation can be estimated. In the case of both SRV-1 (192, 194), and IBV (24), it was difficult to directly correlate stability with frameshift efficiency. In several cases, mutant pseudoknots with similar estimated stabilities promoted different levels of frameshifting. ten Dam et al. (194) pointed out

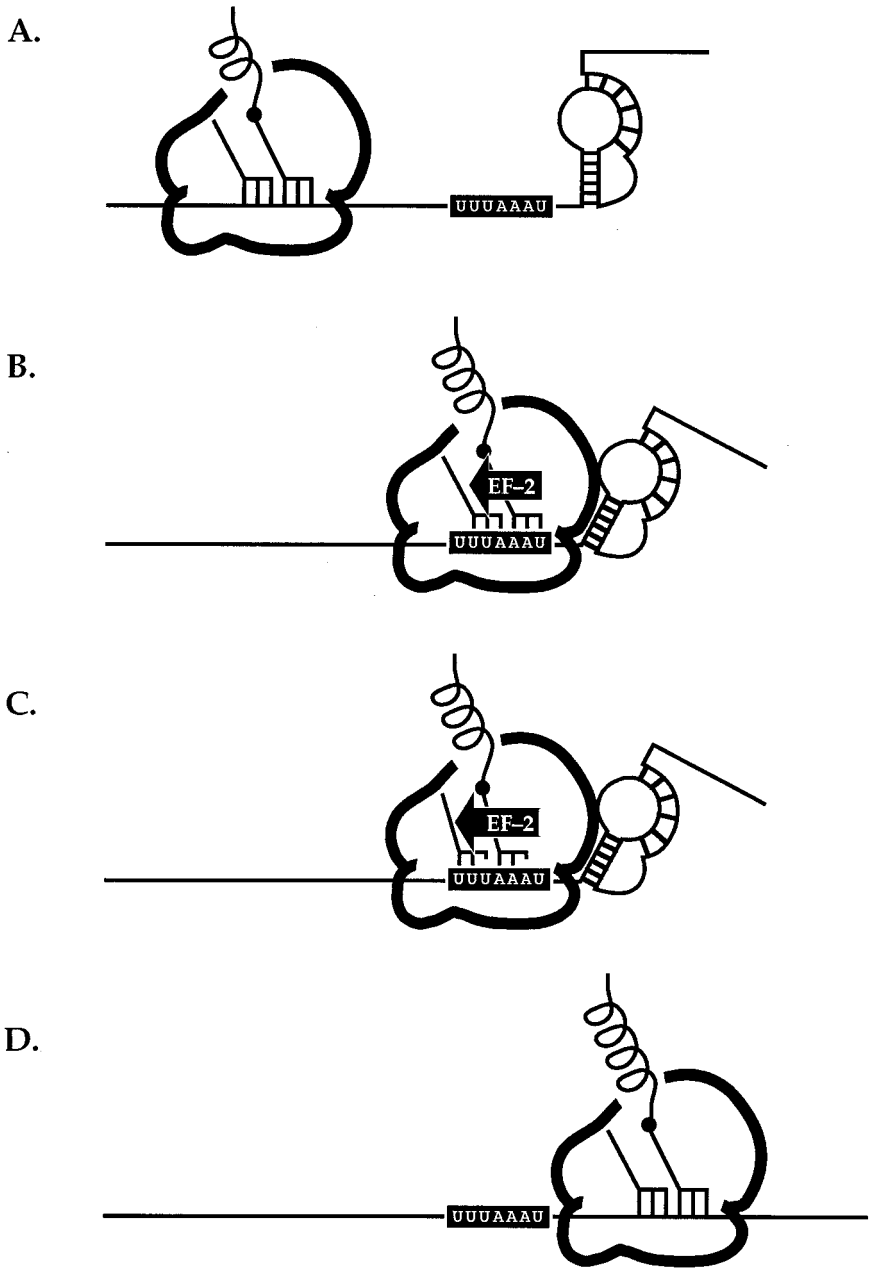

FIG. 6. A model in which EF-2 stimulates simultaneous-slippage frameshifting. A translating ribosome (A) approaches the frameshift site, and a downstream pseudoknot (B) pauses the ribosome. During the pause, the ribosome transfers the nascent peptide to the aminoacyl-tRNA, which then occupies the P-A site. EF-2 then enters the ribosome and attempts to cause translocation. Because the pseudoknot precludes movement of the mRNA, EF-2 (C) pushes the two tRNAs 1 base to the left. After unwinding of the pseudoknot, the ribosome (D) resumes translation of the mRNA in the −1 frame.

that the correlation was better in S2 than in S1, and Brierley et al. (24) found highly variable effects of mutations at the junction between S1 and S2, where the two stems are proposed to stack coaxially. The lack of correlation between a simple stability model and the mutational data suggested that perhaps there was a more complicated effect of pseudoknot structure.

Is the only role of the pseudoknot to block passage of the ribosome? The spacing between the heptamer and secondary structure, which averages about 6 nt (193), is also critical. Changing the spacing by deleting or inserting as little as 2 bp can reduce frameshifting, although not as much as creating mutations which destabilize the structure (23). An early hypothesis proposed that the structure would stall the ribosome with the slippery heptamer in the decoding sites, allowing time for the tRNAs to slip −1 (92). In this case, spacing would be critical, since the slippery heptamer would have to occupy the decoding sites during the induced pause. In fact, direct evidence now shows that ribosomes do pause when traversing the region including a pseudoknot (187, 202).

Is the only role of the pseudoknot to pause the ribosome with the heptamer in the decoding sites? Both of the competing models of simultaneous slippage envision such a pause, either before (90) or after (218) peptide transfer. Peptide transfer is known to occur extremely quickly once an amino-

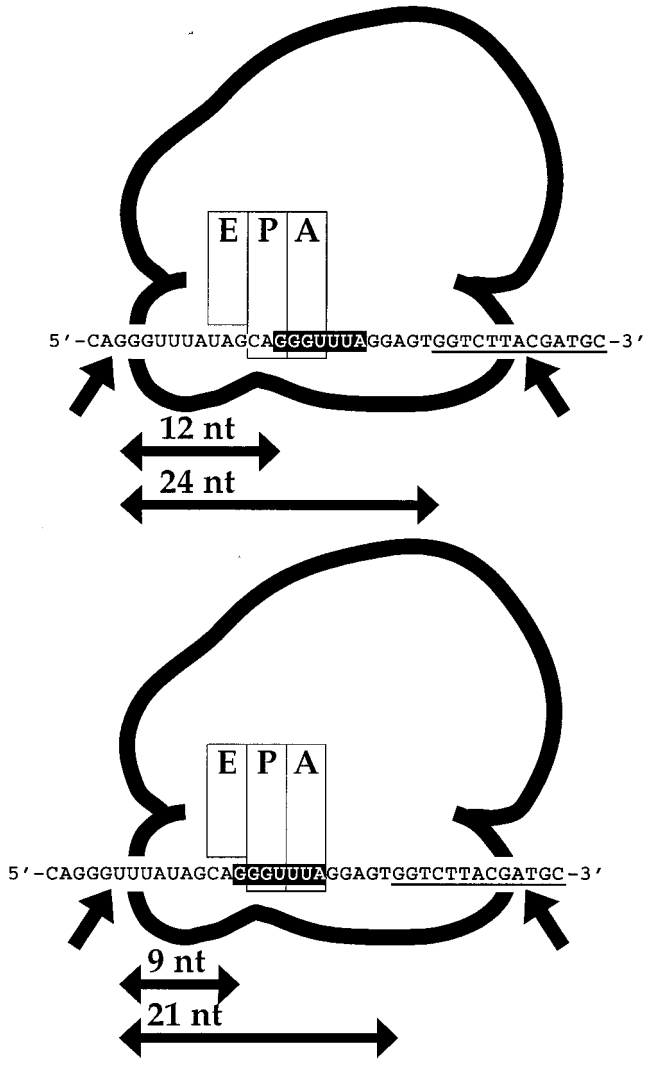

FIG. 7. Position of the decoding sites during pseudoknot-induced pausing on the L-A virus site. The positions of the putative first (top) and second (bottom) pauses on the L-A virus site are illustrated, with the ends of the ribosome-protected region indicated by arrows. The positions of the slippery heptamer (white letters in black box) and pseudoknot (underlined) are shown relative to the positions of the decoding sites predicted from the data of Wolin and Walter (224). The slippery heptamer occupies the P and A sites only during the second pause.

acyl-tRNA has been accepted into the A site of the ribosome. In fact, a rate constant for this step cannot be measured, and for experimental purposes it is considered instantaneous (197). Therefore, the Jacks et al. model (90) suggests that slippage occurs during a state which normally is extremely transient. Of course, the Weiss et al. model (218) imagines slippage before elongation factor-stimulated translocation (stimulated by EF-G in bacteria and its homolog EF-2 in eukaryotes), which also occurs rapidly although not as rapidly as peptide transfer. If the ribosome must be paused at a specific step in the elongation cycle, does the pseudoknot act as a roadblock or does it directly interfere with a biochemical activity of the ribosome, thereby causing the ribosome to pause in a way which facilitates frameshifting? If its effect is more specific, which activity does it interfere with? This problem is central to understanding the mechanism of −1 simultaneous-slippage frameshifting, although resolving it appears to be for the present beyond our technical capacity.

Frameshifting in the Weiss et al. model (218) could result from a mechanical effect of the pseudoknot on the ribosome. The presence of the pseudoknot may physically interfere with movement of the mRNA on the ribosome. The translocation step occurs by slippage of the tRNA-mRNA complex across the face of the ribosome. Specifically, the mRNA must slip 3 nt along the ribosome during translocation, with the codon which occupied the A site moving into the adjacent P site and the P-site codon moving into the E site (Fig. 6). The pseudoknot may physically block movement of the mRNA, allowing time for the two mRNA-bound tRNAs to slip −1. In fact, movement of the tRNA-mRNA complex on the ribosome occurs by an EF-G/EF-2-stabilized change in conformation of the peptidyl-tRNA, moving it into the P site. This movement of the tRNA could, in the absence of mRNA slippage, cause the −1 slippage that occurs in frameshifting, as diagrammed in Fig. 6. This is in essence a mechanical model for frameshift induction by the ribosome, caused by physical restriction of movement.

The Jacks et al. model (90) requires a more sophisticated effect of the pseudoknot. Since frameshifting occurs in preference to peptide transfer, the pseudoknot could function in either of two ways. First, it might interfere with the activity of the peptidyl transferase center itself, precluding peptide transfer. Since the peptidyl transferase activity appears to reside in the large-subunit rRNA, one might imagine that the pseudoknot would interact with the rRNA, perhaps disrupting its structure or acting as a competitive inhibitor by mimicking one of the tRNAs. Alternatively, since peptide transfer cannot occur until EF-Tu (or its eukaryotic analog, EF-1α) leaves the ribosomal A site, the pseudoknot could interfere with dissociation of the elongation factor.

The fact that a pseudoknot causes a translational pause has been demonstrated in two laboratories (187, 202). The experimental approaches were different. Tu et al. (202) used a "heelprinting" technique (224) to map the position of paused ribosomes near the −1 frameshift site of the yeast double-stranded RNA L1, also known as L-A (52, 205). This approach allows mapping of the position of ribosomes to the nucleotide level but cannot distinguish a transiently paused ribosome from a permanently trapped ribosome. Somogyi et al. (187) assayed the production of discrete nascent peptides during a synchronized pulse of translation, allowing the indirect assignment of the position of the elongating ribosomes. This approach does not allow precise mapping but does allow identification of transiently paused ribosomes. The two papers come to largely complementary conclusions, demonstrating that ribosomes pause at the position of pseudoknots, although the ability to induce a pause does not appear to be sufficient to induce efficient frameshifting.

The heelprinting assay identifies a ribosome positioned immediately upstream of the pseudoknot. The assay involves isolating short segments of mRNA protected from micrococcal nuclease digestion. A population of mRNAs including a proportion of paused ribosomes generates an excess of a specific protected fragment. The 5′ end of that fragment is assigned by using it to block primer extension when it is annealed to the single-stranded minus-strand DNA. Tu et al. map a pair of apparently paused ribosomes whose 5′, or trailing, edges map to 12 and 9 nt upstream of the slippery heptamer (202). They argue that the two paused complexes correspond to unshifted and −1-shifted ribosomes, although one might expect that the shift would cause a 1-nt difference in position. They did not demonstrate that formation of two complexes depends on the presence of the slippery heptamer as this model would predict.

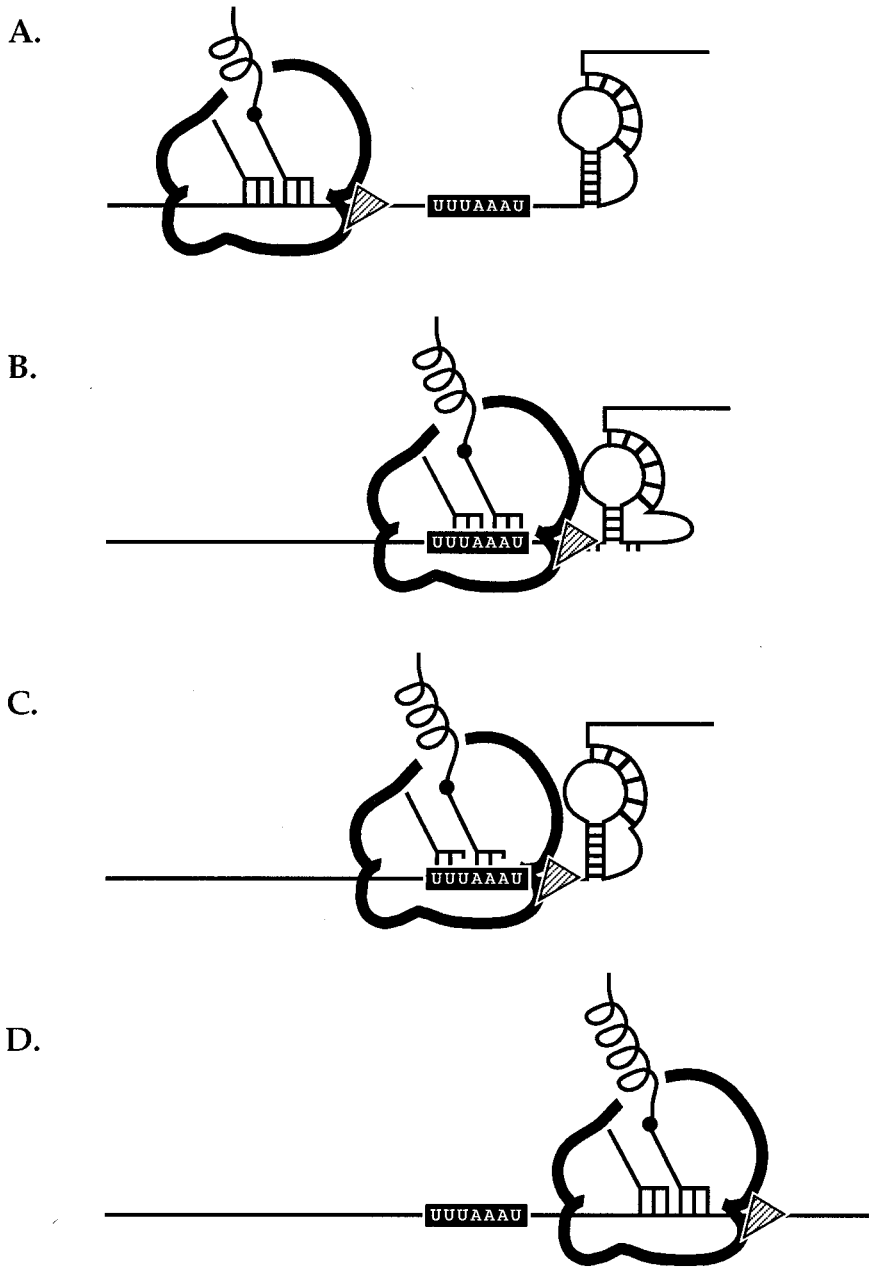

FIG. 8. A pseudoknot-rewinding model for simultaneous-slippage frameshifting. (A and B) A translating ribosome approaches the frameshift site (A) and pauses after partially unwinding S1 of the downstream pseudoknot (B) (the hatched triangle represents a putative ribosome-associated helicase). (C) Slippage occurs as a result of re-formation of 1 bp or more of S1, causing the mRNA to move 1 nt to the right with respect to the helicase. (D) After unwinding the pseudoknot, the ribosome resumes translation of the mRNA in the −1 frame.

On the other hand, it may be significant that the two complexes differ in position by precisely one codon, with the ribosome pausing at two successive codons. Wolin and Walter (224) precisely mapped the distance along the mRNA from the trailing border to the ribosomal A site in a ribosome paused at the terminator of the bovine prolactin mRNA. They found 12 nt between the edge and the A site. If elongating yeast ribosomes behave similarly, that would put the slippery heptamer, G-GGU-UUA, in the decoding sites during each of the two pauses. As illustrated in Fig. 7, in the first of these paused complexes the GGU codon would occupy the A site and in the second GGU would be in the P site and UUA would be in the A site. This suggests that simultaneous-slippage frameshifting occurs at the second of the two pauses.

The 5′ borders of the two protected regions are 24 and 21 nt upstream of the pseudoknot. Given the 30- to 31-nt size of the protected region (202), this suggests that the ribosome progresses until the pseudoknot is either 7 to 8 nt or 10 to 11 nt into the protected region. Why are there two pauses? One possibility is that the intact pseudoknot interferes with two successive translocation steps by the ribosome, although it is unclear why the ribosome would not pause uniquely. Alternatively, the two pauses may be caused by partial unwinding of the pseudoknot. The ribosome may pause 23 nt upstream of

the pseudoknot and then partially unwind the pseudoknot (perhaps by 3 bp) in translocating 3 nt. It may then pause a second time, after which it completely unwinds the structure and resumes normal elongation.

The proposal that partial unwinding of the pseudoknot occurs as the ribosome approaches the slippery heptamer explains why eliminating the first base pair in S1 had little effect on IBV frameshifting (24). Brierley et al. explained this as demonstrating the effect of overall stability of the structure, since such changes should have a minimal effect on stability (204). It is possible, alternatively, that the effect is minimal because the base pair is normally disrupted before slippage occurs on the IBV site; in that case, eliminating it would be expected to have no effect. Unwinding may explain another odd result. Morikawa and Bishop (141) showed that increasing or decreasing the spacing by 3 nt between the slippery heptamer and the pseudoknot in the feline immunodeficiency virus frameshift site greatly reduced −1 frameshifting. They also mutated the sequence of loop 2 to allow an additional 3 bp to form at the bottom of S1, effectively moving the pseudoknot 3 nt nearer the heptamer while increasing its stability slightly. Surprisingly, this mutation had little or no effect on frameshift efficiency. Although they did not explain this result, it may reflect the fact that the longer pseudoknot would be unwound as the ribosome approached the heptamer. Increasing the length of S1 might induce an additional translational pause but would not eliminate the frameshift-producing pause caused by the wild-type pseudoknot.

Tu et al. (202) correlate the ability of mutational variants of the pseudoknot to induce pausing with induction of frameshifting. The correlation is nearly perfect; all pseudoknots which stimulate frameshifting induce a pause, and five of six defective mutants appear not to do so. The simple conclusion from these data is that frameshift-inducing pseudoknots position ribosomes, probably transiently, over the slippery heptamer and that this pausing may be necessary for frameshifting. Since frameshifting may occur after the ribosome has partially unwound the pseudoknot, it is possible that frameshifting requires partial unwinding. This requirement for partial unwinding suggests a model for simultaneous-slippage frameshifting in which re-formation of 1 bp or more induces the slippage of the mRNA with respect to the ribosome-bound tRNAs, as shown in Fig. 8. In this model, the free energy associated with re-forming 1 bp or more drives frameshifting. Like the EF-2 model described above (Fig. 6), this model provides a mechanical explanation for the slippage. If a ribosome-associated helicase unwinds secondary structures in advance of the ribosome to facilitate elongation, and the pseudoknot pauses the ribosome with, in this model, the pseudoknot bound to the helicase, re-forming base pairs would require the mRNA to move slightly downstream with respect to the helicase and ribosome. This would slip the mRNA in the 5′ direction with respect to the bound tRNAs, causing a leftward shift of frame.

Not all pseudoknots which cause ribosomes to pause can stimulate −1 frameshifting. The limitation of the experiments of Tu et al. (202) is that the transient nature of the pause cannot be proven. The approach of Tsuchihashi (199) and Somogyi et al. (187), on the other hand, directly visualizes this transient pause. Tsuchihashi (199) used an in vitro transcription-translation system to demonstrate the formation of a product produced by −1 simultaneous-slippage frameshifting in the *dnaX* gene of *E. coli*. As discussed below, frameshifting at the *dnaX* gene causes premature termination at an out-of-frame stop codon (15, 64, 201). In a control experiment, in which shifting at the slippery heptamer had been eliminated by a site-directed mutation, a polypeptide the size of this prematurely terminated product still appeared, although transiently, since it disappeared with continued incubation. Tsuchihashi interpreted this fragment as being a nascent peptide which accumulated on ribosomes paused at the *dnaX* shift site. This directly demonstrated pausing at the frameshift site and demonstrated its transient nature. Somogyi et al. (187) extended these experiments by using the well-characterized IBV frameshift site. They inserted the site into a reporter RNA construct and translated it in vitro. Translation was allowed to continue for increasing lengths of time at 26°C rather than 37°C, which would slow elongation sufficiently to emphasize any transiently paused complexes. To facilitate visualizing transient species, a quasisynchronous pulse of translation was created by adding the initiation inhibitor edeine 5 min after the start of the reaction, which eliminated any further initiation. Somogyi et al. observed transient nascent peptides, one apparently present on ribosomes paused immediately upstream of the pseudoknot. Because of limitations of the method, the precise location of the apparent pause could not be determined. The pause, however, did depend on the presence of the pseudoknot. Replacing the pseudoknot with mutationally destabilized versions drastically reduced pausing, as did replacing it with a stem-loop structure. In either case, though, pausing was not eliminated but only reduced less than 10-fold. Again, there was a good

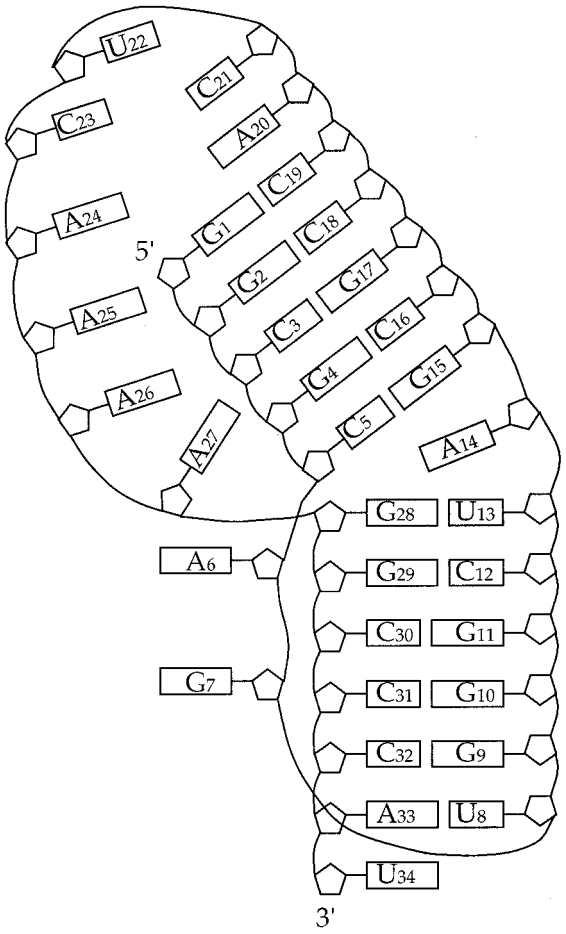

FIG. 9. Structure of a frameshift-inducing pseudoknot. The structure is shown schematically, with ribose (pentagons), bases (rectangles), and phosphate bridges (lines). Bases shown in parallel orientation are involved in stacking interactions. The intercalated adenosine 14 causes a bend in the overall structure. Adapted from reference 181 with permission of the publisher.

correlation between pausing and frameshifting, since all of the constructs which induced pausing poorly also induced frameshifting at barely detectable or undetectable levels.

Significantly, the correlation between pausing and frameshifting was not perfect, since some pseudoknots incapable of inducing measurable frameshifting can still induce measurable pausing. This brings into question the causal relationship between pausing and frameshifting. Both groups concluded that frameshifting may require some additional function provided by the pseudoknot—one that cannot be provided by the pause-inducing structures that cannot stimulate frameshifting.

If translational pausing and frameshifting are not necessarily correlated, it is possible that the pseudoknot has a second function in inducing frameshifting (32, 187). If the structure does play a second critical role, one might expect that fulfilling that role would require a particular primary or secondary structure. Chen et al. (32) maintain that there are two general mechanistic roles for a pseudoknot in frameshifting, either to act simply as an impediment to ribosome passage because of the difficulty of unwinding the structure, which seems unlikely, or to bind some component of the translational apparatus to directly induce −1 frameshifting. Fulfilling this alternative role would require a specific primary or secondary structure to allow specific recognition. They therefore attempted to analyze the structure of a particular pseudoknot to determine the nature of the structural constraints on its action. The pseudoknot they chose was a variant of the *gag-pro* pseudoknot of MMTV (30), termed VPK, which efficiently stimulates frameshifting. Structural mapping with enzymatic and chemical probes demonstrated that VPK forms a pseudoknot which resembles previously characterized model pseudoknots (109, 159, 225). The main difference between VPK and the model pseudoknots is the presence of an unpaired nucleotide at the interface between the two helices. In the models, the two helices, S1 and S2, stack coaxially, with the stacking presumably contributing to the stability of the molecule. The presence of the extra nucleotide should interfere with stacking and therefore might be expected to destabilize the structure. If stability were the only determinant of pseudoknot activity, the extra nucleotide might be expected to decrease the ability to stimulate frameshifting. Quite the opposite seems to be the case, since a mutation that removes the nucleotide but still allows formation of a pseudoknot actually interferes with frameshift stimulation. A related variant of the MMTV pseudoknot termed APK is not able to stimulate frameshifting. One of the differences between APK and VPK was an A · U base pair replacing a G · U base pair at the helix interface (Fig. 9). The G → A change from VPK to APK appears to be the main reason that APK does not induce frameshifting. Structural mapping demonstrates that the presence of the A · U base pair makes the S1-S2 interface more accessible, which suggested to Chen et al. (32) that the A · U base pair does not form, leaving two unpaired nucleotides at the APK S1-S2 interface. Replacing the A · U base pair of APK with a G · U both increases frameshift stimulation and makes the S1-S2 interface less accessible in APK. These data suggest strongly that the presence of a single nucleotide, probably intercalated into the pseudohelix formed by S1 and S2, is essential to frameshift stimulation.

Shen and Tinoco (181) have used a nuclear magnetic resonance spectroscopy approach to determine the structure of a 34-nt VPK oligonucleotide. From the nuclear magnetic resonance spectroscopy data, molecular modeling produced four very similar structures, which generally confirm the predictions of Chen et al. (32). The pseudoknot consists of two A-form helices with an intercalated A residue at the interface. The presence of that nucleotide and the length of the two loops introduce a bend of about 112° at the interface. The loops lie along the grooves of the structure, L1 on the wide minor groove of S2 and L2 on the narrow major groove of S1. The structure is highly ordered with all but one of the loop nucleotides even participating in base stacking. The nuclear magnetic resonance spectroscopy data do indicate that the structure is dynamic, with rapid movement indicated for four non-base-paired residues and fraying at the ends of S2. Shen and Tinoco (181) speculate that frameshift stimulation depends both on the bent structure of the pseudoknot, which is very different from the suspected straight structure of pseudoknots lacking the intercalating base, and on its dynamic structure, which they propose may allow the structure to react more favorably with the translational machinery. They note that the mutational data suggest that the primary sequence of frameshift-stimulating pseudoknots is not constrained (22, 24, 30, 32). Therefore, they favor a role for the pseudoknot in which its overall structure is recognized by the ribosome. They note a similarity between the VPK structure and that of a base-paired complex between a codon and the anticodon stem of a tRNA, suggesting that the ability of the pseudoknot to stimulate frameshifting may depend on its being recognized by one or more factors which are responsible for monitoring codon-anticodon pairing. How this might stimulate frameshifting is not described.

Is there a pseudoknot-recognizing factor? How does the pseudoknot induce frameshifting? The two possible mechanisms mentioned by Chen et al. (32), with the pseudoknot either blocking progress by the ribosome or interacting with a component of the translational machinery, may not account for all the possibilities. In particular, I have already mentioned one possibility, that the re-formation of base pairs by a partially unwound pseudoknot might drive slippage. At a more basic level, there are two extreme models for the function of the pseudoknot. At one extreme, an RNA catalysis model would predict that the structure of the pseudoknot alone would be sufficient, not requiring the intervention of any other protein or RNA. At the other extreme, a factor recruitment model would predict that the pseudoknot has no direct role in stimulation but, rather, that it simply binds some factor on the surface of the ribosome. A more moderate version of the ribonucleoprotein complex model would predict that the complex of the pseudoknot and such a factor would induce frameshifting, acting in concert.

The structural data of Chen et al. (32) and Shen and Tinoco (181) seem to argue strongly for the factor recruitment model. If pseudoknots must adopt a particular conformation to promote frameshifting, it may be that this conformation is the target recognized by the factor. It is true that the ribosome must specifically recognize polyribonucleotides, tRNAs, which bear little resemblance to each other. The concept that the pseudoknot is a molecular mimic of the codon-anticodon pair structure is attractive, although how this mimicry would promote frameshifting is ill defined (the pseudoknot is unlikely to mimic tRNA-binding in either decoding site, since those sites are occupied when the frameshift occurs). Chen et al. (32) demonstrate that the pseudoknots of both SRV-1 (192, 194) and feline immunodeficiency virus (141) share the unusual structure at the S1-S2 interface and that the structure is necessary for their stimulating frameshifting. However, it is not clear how general this structure is. In the IBV pseudoknot, for example, the two stems are predicted to stack coaxially and mutant forms of the pseudoknot which disrupt base pairing at this position tend to interfere with frameshifting (see Fig. 5 in reference 24). In addition, some frameshift sites replace the pseudoknot with a conventional hairpin or no structure what-

ever, as we will see below. For these reasons, the idea of a pseudoknot recognition factor must be treated as extremely hypothetical.

Various laboratories have attempted to test if pseudoknot-binding factors are required for frameshift stimulation. ten Dam et al. indirectly tested if such a factor exists (192). They reasoned that if frameshifting in an in vitro system depended on a pseudoknot-binding factor, an excess of the pseudoknot should titrate the factor out of the extract, thus reducing frameshifting. Using the SRV-1 site, which functions in an in vitro system, they found that addition of an excess of the SRV-1 pseudoknot in *trans* did not inhibit frameshifting. Even a 2,000-fold molar excess of competitor pseudoknot caused no decrease in frameshifting from the reporter RNA. This result is consistent with the hypothesis that there is no pseudoknot-binding factor, although it is also possible that the factor is tightly ribosome associated. If this is the case, the factor might not be extracted from translating ribosomes and the pseudoknot competitor would not interfere with frameshift induction. Tinoco's laboratory reports having done a similar experiment with the same result (32).

Two other groups have taken a genetic approach to the identification of factors which stimulates −1 simultaneous-slippage frameshifting, which might include pseudoknot-binding factors. Dinman and Wickner (54) used a genetic screen to identify mutations which significantly reduced −1 frameshifting. Mutations were identified in eight complementation groups, termed *mof1* through *mof8* (maintenance of frame). They were identified as mutations which increased frameshift-dependent expression of a *lacZ* reporter gene 2.7- to 8.9-fold. These were not bypass suppressors, increasing *lacZ* expression by a mechanism unrelated to the normal −1 frameshift mechanism, since their ability to stimulate *lacZ* expression depended on the presence of a functional frameshift site. In addition to this direct phenotype, the *mof* mutations had other relevant phenotypes. Previously, Dinman and Wickner (53) had demonstrated that the efficiency of −1 frameshifting critically regulates virus propagation, as measured by the ability of a cDNA clone of the yeast virus-like double-stranded RNA to support propagation of the M_1 satellite double-stranded RNA. Increasing or decreasing efficiency by as little as twofold interfered with propagation. Accordingly, five of the *mof* mutations confer the same phenotype. It is not clear why not all the mutations affect M_1 propagation. Those which fail to affect propagation are among those with a small effect on frameshifting, but mutants with indistinguishable effects on frameshifting can differ in their ability to support M_1. In addition, mutations in three of these five genes, *MOF2*, *MOF5*, and *MOF6*, cause temperature-sensitive growth with unique cell cycle arrest at the restrictive temperature, and both *MOF2* and *MOF5* are essential for growth on nonfermentable carbon sources. These pleiotropic phenotypes suggest that these *MOF* genes perhaps encode elements of the translational apparatus (54).

None of the *MOF* genes is a clear candidate for a pseudoknot-binding factor. Mutations in four of six tested genes significantly increase +1 frameshifting on the Ty1 frameshift site. Since the pseudoknot is not expected to stimulate +1 frameshifting, these genes are unlikely to encode pseudoknot-binding factors. In fact, the *MOF* products could affect frameshift-dependent expression of the L-A *pol* gene indirectly. Mutations in the *UPF1* gene provide a precedent for an indirect effect. The *upf1* mutants were selected as increasing the expression of a frameshift mutant form of the *HIS4* gene, ostensibly by increasing the efficiency of decoding by a frameshift suppressor tRNA. However, the mutation actually increases the stability of the *HIS4* mRNA, made unstable by the lack of translation distal to the frameshift mutation. Although the *mof* effect may not be related to mRNA stability (the amount of L-A mRNA is unchanged in the mutant [54]), the mutations could affect a step other than frameshifting itself and still have the observed effect on expression. Determining the mechanism of action of these mutations will, of course, require further analysis.

Surprisingly, another *mof* mutation, *mof9*, has an unexpected target, the gene encoding the 5S rRNA (55). A multicopy plasmid which suppresses the mutation carries a segment of the rDNA including the 5S rRNA gene. Subcloning demonstrated that the 5S rRNA gene itself suppressed the phenotype, and classical genetic analysis showed that the *mof9* mutation is linked genetically to the rDNA array. In fact, Dinman and Wickner (55) found that an rDNA cistron which had been marked by integration of a $URA3^+$ gene fortuitously conferred a Mof^- phenotype. Presumably, this genetic background carries a mutant form of the 5S rRNA gene, although this has not been directly shown. However, two characterized 5S rRNA gene mutations (206) confer the Mof^- phenotype when overexpressed in a wild-type genetic background. This is somewhat surprising, since the mutations were constructed to test the ability to detect mutant forms of 5S rRNA in vivo and confer no phenotype. It is odd that the Mof^- phenotype occurs in three allelic forms of 5S rRNA, suggesting that it may be relatively easy to generate such a phenotype. The *mof9* mutations increase frameshifting at both −1 and +1 frameshift sites, suggesting that the 5S rRNA gene may have a role in ensuring maintenance of translational frame and that the mutants are somewhat defective in that function, allowing increased programmed frameshifting. Alternatively, *mof9* could be a gain-of-function mutation, altering 5S rRNA to interfere with the frame maintenance function of another ribosomal component without 5S rRNA normally having a role in fidelity.

A second screen for suppressors that increase the efficiency of −1 frameshifting has been performed in *S. cerevisiae* by using the MMTV *gag-pro* frameshift site (111). Using a combination of a selection for increased copper resistance, with a gene fusion to *CUP1*, the yeast copper metallothionein, and a screen for increased expression of β-galactosidase from a *lacZ* fusion, they identified mutations in two genes, *IFS1* and *IFS2*, which increase frameshifting up to threefold at −1 frameshift sites. These mutations also show increased suppression of nonsense mutations in the presence of the drug paromomycin, which causes decreased fidelity of translation (150, 184). The wild-type *IFS1* gene has been cloned and shown to be identical to the previously characterized gene *SUA1*/*UPF2*/*NMD2* (42, 75, 158), a gene involved in degradation of mRNAs containing premature termination codons. The *IFS2* gene is allelic to a second gene involved in nonsense codon-mediated mRNA degradation, *UPF1* (51). Although Lee et al. (111) state that the *ifs* mutants do not alter the stability of mRNAs, including programmed frameshift sites (reference 111 and data not shown), the fact that the genes also affect the stability of mRNAs which do not include frameshift sites suggests that the effect of increased expression from programmed frameshift sites may be caused by increased availability of mRNAs. Alternatively, the proteins encoded by these genes may have a dual function in modulating translational frameshifting and in signaling the lack of translation of nonsense mutation-containing mRNAs. It is not clear how the protein would accomplish the two tasks, since the first would seem to be a ribosomally associated function and the second would not.

Some simultaneous-slippage sites do not include a stimulatory pseudoknot. The requirement for a pseudoknot to stimulate −1 frameshifting is nearly universal. However, there are

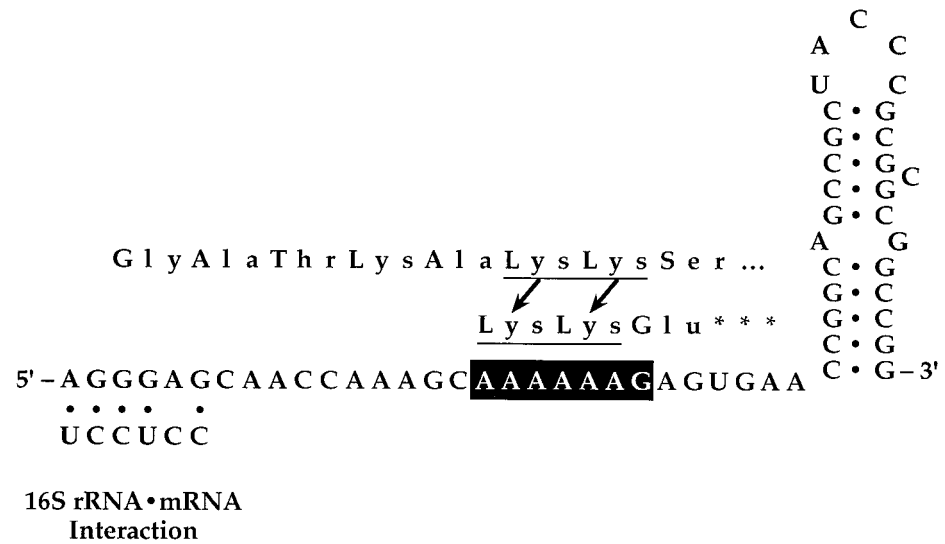

FIG. 10. Sequence of the programmed frameshift site of the *dnaX* gene of *E. coli*. In the *dnaX* gene, base pairing between the 16S rRNA and a Shine-Dalgarno site and a downstream stem-loop stimulates slippage on the A-AAA-AAG heptamer.

genes which either have no apparent structure or utilize a stem-loop structure instead of a pseudoknot. For example, no structure was found distal to −1 frameshift sites from either retrotransposon 17.6 of *Drosophila melanogaster* or an isolate of simian immunodeficiency virus isolated from African green monkeys (SIV_{AGM}) (193). In addition, stem-loops rather than pseudoknots were predicted distal to the *gag-pro* frameshift sites of human T-cell leukemia viruses 1 and 2 (HTLV-1 and HTLV-2) and simian T-cell leukemia virus (STLV-1); the *pro-pol* sites of SRV-1 and SRV-2 and Mason-Pfizer monkey virus; and the *gag-pol* sites of retrotransposon *gypsy* of *D. melanogaster*, HIV-1, and HIV-2 (193). Most of these structures are merely predicted by computer analysis, and no data exist to prove that the RNA adopts the predicted conformation. The fact that, as we have seen, frameshift-stimulating pseudoknots cannot be replaced by similarly or more stable stem-loop structures (24) raises the question of why a pseudoknot is not required in some cases.

One explanation could be that the heptanucleotide at these sites is unusually slippery, obviating the need for stimulation by the secondary structure. This may often be the case, as exemplified by the case of the HIV-1 frameshift site. Wilson et al. (221) analyzed the signals which specify −1 frameshifting in HIV-1 and found that only the six uracils of the homopolymeric sequence U-UUU-UUA appeared to be necessary for efficient frameshifting and that this signal functioned in vitro in a rabbit reticulocyte extract translation system and in vivo in a heterologous yeast system. Later, in a careful quantitative analysis, Parkin et al. (153) demonstrated that in fact the distal stem-loop was necessary for maximal frameshifting. Using a construct in which the entire HIV-1 *gag-pol* region was expressed under control of a foreign promoter, Parkin et al. showed that mutations which destabilized or eliminated the stem-loop reduced frameshifting four- to sevenfold in vivo, and two- to threefold in vitro.

Clearly, the HIV-1 *gag-pol* stem-loop stimulates frameshifting at the slippery heptamer, although in other contexts, stem-loops do not. It is not yet clear if the reason why the stem-loop functions is the presence of an especially slippery heptamer or of other features of the sequence surrounding the shift site. An experiment which involved swapping downstream structures showed that the HIV-1 *gag-pol* stem-loop does stimulate much less efficiently than the pseudoknot derived from the MMTV *gag-pro* shift site (30). However, the converse experiment, introducing other slippery heptamers into the HIV-1 context, has not been done. It is possible that stimulation by the HIV-1 heptamer requires other sequences either upstream or downstream of the shift site. Wilson et al. (221) used a 26-nt minimal site and could not demonstrate a role for the stem-loop. It could be, as suggested by Madhani et al. (120), that the region encompassing the stem-loop plays a role in stimulating frameshifting. The possibility of a long-range interaction, in the form of a pseudoknot, cannot be rigorously excluded.

Programmed −1 Frameshift Sites in Bacteria

By analogy to the characterized eukaryotic sites, putative simultaneous-slippage −1 shift sites have been identified in several insertion sequences (IS) and in the chromosomal gene for two subunits of DNA polymerase III, *dnaX*, in *E. coli*. However, the analysis of these sites has revealed significant phenomenological differences, implying the existence of mechanistic differences between the bacterial and eukaryotic sites. Many of the bacterial sites do not include a stimulatory pseudoknot, with many substituting a stem-loop structure and some showing no evidence for any secondary structure. Some of the sites do not show evidence of a heptameric sequence being required at the shift site, which would certainly imply a different mechanism. Finally, in vitro mutagenesis indicates that several of the sites include a Shine-Dalgarno interaction site upstream of the recoding site, which stimulates −1 frameshifting in a fashion analogous to its stimulation of +1 frameshifting at the *prfB* gene (44, 213, 217).

The *dnaX* gene: −1 frameshifting stimulated by both upstream and downstream elements. Three groups independently discovered the single known instance of a simultaneous-slippage −1 frameshifting in a bacterial chromosomal gene, *dnaX* (15, 64, 201). The site is unusual in two ways. First, frameshifting in *dnaX* is extremely efficient, with about 80% of ribosomes shifting at the site. Second, unlike most programmed frameshifts, in which shifting leads to expression of a C-terminally extended product, frameshifting in *dnaX* leads to

premature termination at a −1 frame UGA stop codon. However, the consequence, as with other programmed sites, is to express two alternative primary translation products, the two subunits of *E. coli* DNA polymerase III, τ (tau, 71 kDa) and γ (gamma, 47 kDa). The *dnaX* ORF corresponds to a 71-kDa protein (643 residues) approximately the size of τ, which was initially identified as the *dnaX* product. A second gene, *dnaZ*, was defined genetically and demonstrated biochemically to encode the γ subunit. These genetic results contrasted with peptide sequencing results and immunological tests which suggested that the shorter γ subunit corresponded to an N-terminal fragment of τ. This conflict was resolved when the *dnaX* and *dnaZ* mutations were found to be complemented by the same 2.2-kb DNA fragment, which included only the *dnaX* reading frame. Apparently, by an unknown mechanism, the *dnaZ* mutations interfered with expression of γ from the gene which encodes τ. An early model suggested that γ was a proteolytic fragment of τ, on the basis of in vitro data showing that limited tryptic digestion of τ released a fragment the size of the γ subunit (110). The cleavage occurs between Lys-472 and Lys-473. Replacement of the codons specifying these residues to eliminate the putative process had no effect on production of γ (15, 201), and no evidence was found in vivo for the C-terminal fragment of τ, which should be generated by proteolysis (64). These results were inconsistent with the proteolysis model.

The surprising result of the three papers published in 1990 was that the production of γ required −1 translational frameshifting. A site resembling a eukaryotic simultaneous-slippage −1 frameshift site occurs in *dnaX* (Fig. 10) with a putative slippery heptamer, A-AAA-AAG (codons 429 and 430), followed 6 nt downstream by predicted stem-loop (64, 201). A ribosome shifting −1 at this site would encounter a UGA stop codon two codons downstream, resulting in the expression of a truncated protein of 47 kDa, the predicted size of γ. Production of γ was eliminated when this terminator was changed to a sense codon (CGA); the mutation allowed continued translation into a *lacZ* reporter gene placed downstream in the −1 frame (64). As expected, site-directed mutations which altered the slippery heptamer eliminated production of γ, showing that the heptamer was essential to production of γ (15, 201). Sequences downstream of the slippery heptamer also stimulated frameshifting. Deleting into this region reduced frameshifting 2.5-fold (64) to 7.9-fold (201). The effect was limited to the region of the stem-loop; mutations altering nucleotides involved in forming the stem reduced frameshifting, whereas those targeting other sequences had little or no effect. Mutations targeting the bottom of the stem-loop had a larger effect (up to fourfold) than those targeting the top (about twofold). Finally, amino acid sequencing of a small C-terminal tetrapeptide generated by mild proteolysis indicated that its sequence was NH_2-Ala-Lys-Lys-Glu-COOH. The Ala-Lys-Lys sequence is encoded in the normal reading frame; the Glu occurs in the −1 frame immediately after the putative slippery heptamer. This sequence demonstrates that frameshifting occurs after decoding of the AAA-AAG codons, with only one amino acid being incorporated before termination.

These results generally support the simultaneous-slippage model but do not eliminate the idea that slippage occurs by a single $tRNA^{Lys}$ bound to either of the Lys codons (which would also produce the Lys-Lys depicted). However, detailed mutagenesis of the *dnaX* heptamer indicated that both Lys codons were essential to frameshifting. Single base changes across the entire heptamer uniformly reduce frameshifting 2- to 16-fold, and two changes, A-AAA-GAG and A-AAA-AUG, eliminate frameshifting (200). Previous in vitro work with rabbit reticulocyte extract (23) and in vivo work with *S. cerevisiae* (52) indicated that the A-site codon of a slippery heptamer (e.g., A-AAA-AAG) was more restricted than the P-site codon (A-AAA-AAG). The P-site codon could be any redundant run of nucleotides (AAA, UUU, CCC, or GGG), whereas if the A-site codon were GGG or CCC, frameshifting was much reduced. In *dnaX*, changing the heptamer to A-AAG-GGA also greatly reduced frameshifting (200). This preference for AAA or UUU in the A site may reflect the fact that frameshifting consists of two steps—codon-anticodon dissociation and reassociation—and that in the A site, G · C base pairs interfere with dissociation, as hypothesized by Dinman et al. (52). The fact that G · C base pairs do not bar slippage in the P site may indicate that the strength of the codon-anticodon pair is weaker in the P site. This difference is consistent with the differing roles for the two sites; selecting cognate codons in the A site may require tighter base pairing.

From all these data, the *dnaX* frameshift appears to be a bacterial counterpart of a eukaryotic simultaneous-slippage frameshift site. But why is the *dnaX* site so unusually efficient? The fact that the site includes a downstream stimulatory stem-loop, rather than a pseudoknot, suggests that it actually should be inefficient. At least part of the reason for its efficiency is that the heptamer is unusually slippery. The sequence A-AAA-AAG is uniformly at least twofold more efficient than any of the substitutes tried. Tsuchihashi and Brown (200) noted that the last base of the heptamer is essential for efficient frameshifting at *dnaX*. Changing the final G to any other base severely reduced frameshifting. Strangely, in eukaryotes, a G in this position inhibits frameshifting (23, 52). Tsuchihashi and Brown noted that in *E. coli*, only one $tRNA^{Lys}$, with the anticodon U*UU (where U* is 5-methylaminomethyl-2-thiouridine), recognizes both AAA and AAG codons whereas in eukaryotes, each codon has a dedicated tRNA, with AAG recognized by a tRNA with C in the wobble position of the anticodon. For example, in rabbits, AAG is recognized by a tRNA with anticodon CUU and AAA is recognized by a tRNA with 5-methoxy-carbonylmethyl-2-thiouridine as the anti-wobble base. The uridine modifications in both cases act to restrict base pairing with A and not G (230). Recognition of AAG by the U*UU tRNA in *E. coli* should therefore be much weaker than its recognition of AAA. The difference in recognition may also be because the anticodon loop of this tRNA has an unusual structure. Circular dichroism and enzymatic digestion analyses suggest that the wobble base may be involved in hydrogen bonding (212), which might alter its base pairing properties, weakening pairing with the AAG codon. Presumably, the reason that G-ending heptamers induce little frameshifting in eukaryotes is because the G · C base pair, perhaps combined with a normal anticodon loop structure, strengthens the codon-anticodon interaction, interfering with slippage. Tsuchihashi and Brown (200) tested whether the abnormal codon-anticodon interaction causes increased frameshifting by constructing a mutant of $tRNA^{Lys}$ in which the U*UU anticodon was replaced with CUU. Expressing $tRNA^{Lys}_{CUU}$ reduced frameshifting on A-AAA-AAG and virtually eliminated frameshifting on A-AAG-AAG but had no effect on the sequences containing noncognate A-site codons A-AAA-AAA and A-AAA-AAC.

This result demonstrated that frameshifting efficiency depends on the stability of the codon-anticodon pair in the normal frame in addition to the stability after shifting. The importance of stability after shifting has been demonstrated clearly (most clearly by Curran [45]). However, the effect of instability in the normal frame can be seen in other systems, as noted by Tsuchihashi and Brown (200). In the MMTV *gag-pro*

shift site, the A-site codon AAC promotes more efficient shifting than does AAU (30), possibly because $tRNA^{Asn}$ with the hypermodified base queosine at the antiwobble position binds more strongly to AAU than to AAC. Both Phe codons, UUC and UUU, are recognized by a tRNA with 2′-*O*-methylguanosine at the antiwobble position, which should recognize UUC more strongly than it recognizes UUU. This difference might explain why UUU induces more frameshifting than UUC in the A-site codon (90, 218). The same effect has been seen in a +1 frameshift in *S. cerevisiae*, in which recognition of the codon AGG by a tRNA with 5-methoxy-carbonylmethyluridine at the antiwobble base, which normally restricts decoding to AGA, causes extremely high levels of frameshifting (208).

A second reason for the efficiency of the *dnaX* site is the presence of an upstream stimulatory element. As in the *prfB* gene, interaction between a Shine-Dalgarno site and the 3′ end of 16S rRNA stimulates frameshifting on the *dnaX* site, although in the −1 rather +1 direction. The potential Shine-Dalgarno interaction site is present 10 nt upstream of the A-AAA-AAG heptamer of *dnaX*. Larsen et al. (108) tested if this site stimulated frameshifting in *dnaX*. They found that changing the spacing to 7 or 16 nt eliminated the stimulatory effect of the Shine-Dalgarno site. In fact, changing the spacing to the optimum for +1 frameshifting, 3 nt, virtually eliminated −1 frameshifting, to a level less than was observed when the Shine-Dalgarno site was absent. They concluded that the Shine-Dalgarno site could be either stimulatory or inhibitory toward frameshifting depending on the type of frameshift and the distance to the shift site. Maximal frameshifting at the *prfB* +1 site required a spacing of 3 nt, while maximal −1 frameshifting required a spacing of about 10 to 14 nt. The optimum spacing between the Shine-Dalgarno site and the initiator AUG is intermediate between these values. If the Shine-Dalgarno interaction serves to push the ribosome into the shifted reading frame, one would expect this difference in spacing. The spacing of the *prfB* gene would tend to stress the ribosome, causing the mRNA to slip to the left to attempt to achieve a more normal distance between decoding sites and the Shine-Dalgarno interaction (Fig. 2). The *dnaX* spacing would have the opposite effect, since to achieve the smaller normal spacing, the mRNA would have to slip to the right, promoting a −1 shift (Fig. 10).

This mode of stimulation of frameshifting would appear to be a prokaryotic adaptation. Eukaryotic ribosomes do not use a mRNA-rRNA pairing to identify translation initiation sites. Rather, they scan in a 5′-to-3′ direction starting at the 5′-capped end of the mRNA and identify initiators by using the complementarity of the initiator $tRNA^{Met}$ (104). Therefore, they are not expected to use a Shine-Dalgarno interaction to stimulate −1 frameshifting.

Programmed −1 frameshifts in insertion sequences are mechanistically diverse. Insertion sequences (IS) are a family of simple transposable elements in bacteria. Like other transposable elements, they encode an enzymatic activity, termed transposase, which catalyzes insertion of copies of the IS into novel chromosomal locations. The DNA sequences of several IS elements, including IS*10* (102), IS*50* (171), and IS*903* (73), include a long ORF which was assumed to encode transposase. By contrast, the DNA sequences of a larger number of other elements show no sign of such a gene product. In these cases, the IS transposase is encoded by translational frameshifting.

IS*1* includes an unusual −1 frameshift site. The DNA sequence of IS*1* presented a conundrum, since it did not include any ORF long enough to encode transposase. IS*1* includes six ORFs. Comparison of several iso-IS*1* sequences showed only silent substitutions (those which do not alter the predicted protein product) in two of these frames, termed *insA* and *insB*. These two genes are encoded on the same strand of IS*1* from partially overlapping reading frames (119). Site-specific mutagenesis of these genes demonstrated that they are both required for the related processes of transposition and cointegration (119). Original models suggested that both proteins might be necessary or that mutations of a putative inessential *insA* gene might be polar on the downstream *insB* gene. Subsequently, detailed mutagenesis (59, 176) and analysis of proteins produced from IS*1* (59) demonstrated that *insA* and *insB* were expressed as an InsA-InsB fusion protein by continuous translation from *insA* into *insB* via a 5′ extension of *insB* termed B′ (176). Since *insB* is in the −1 frame with respect to *insA*, this would require either a translational shift −1 within the 103-nt overlap between the ORFs or the synthesis of an alternative form of the mRNA in which *insA* and *insB* are in the same reading frame. Expression of the fusion product is inefficient, as judged by direct comparison of the amounts of InsA and InsA-InsB expressed and from expression of a *insB*::*lacZ* reporter construct (59). Quantitative *lacZ* assays suggested that InsA-InsB is expressed at less than 1% the rate of InsA. Although a pretranslational mode (transcriptional stuttering or RNA editing) has not been explicitly excluded, several lines of evidence point toward a translational mechanism of fusion peptide expression.

The InsA-InsB fusion protein was proposed to be expressed by −1 frameshifting at a sequence which resembles the eukaryotic simultaneous-slippage frameshift sites. A heptamer sequence, A-AAA-AAC (shown as codons of *insA*) occurs 15 nt upstream of the putative *insA* terminator. An *insA* nonsense mutation immediately upstream of this element eliminates cointegration (an indirect assay of transposase), while one immediately downstream has no effect. Putative *insB* mutations upstream had no effect, while those downstream interfered (176). This is consistent with the idea that ribosomes shift from *insA* into *insB* at this point. Finally, a mutation of the putative slippery heptamer, A-AAA-AAC to A-CAA-AAC, which had previously been shown to interfere with frameshifting in MMTV (218), also interfered with expression of the downstream gene (59). Escoubas et al. proposed that, like eukaryotic simultaneous-slippage sites, IS*1* frameshifting requires a region distal to the putative slippery heptamer (59). The region distal to the IS*1* heptamer can be folded into several secondary structures, both stem-loops and pseudoknots. Deletion of this region reduced frameshifting almost twofold. On the basis of these similarities, the IS*1* shift site has been proposed as a prokaryotic analog of the eukaryotic simultaneous-slippage frameshift sites (31).

More recent work has brought this conclusion into question. Near-saturation mutagenesis of the putative IS*1* slippery heptamer indicates that frameshifting depends only on the first 4 nt of the motif, A-AAA (179). Mutations were introduced at each of the positions of the heptamer, as well as at the 3 nt preceding it, and their effect on cointegration was tested. This assay is indirect, and some of the mutations clearly interfered with cointegration by altering essential amino acids in the *insA-insB* fusion product. However, by using careful controls for this effect and by combining tests of some of these mutations using a *lacZ* reporter construct, Sekine and Ohtsubo (179) showed that several mutations of the A-AAA motif interfered with expression whereas several of the last 3 nt, AAC, had little effect. Since a second potential slippery heptamer, U-UUA-AAA, overlaps the A-AAA-AAC motif, the requirement for the three bases upstream of the A-AAA was tested; none of the mutations of the U-UU sequence had an effect on

expression. These data suggest that frameshifting occurs by −1 slippage of a single $tRNA^{Lys}$ bound to AAA onto the overlapping AAA codon and not by simultaneous slippage of two tRNAs (179). Direct sequencing of the peptide encoded across this sequence showed that frameshifting does indeed occur at this motif and that the shift occurs before decoding of the AAC codon, resulting in the expression of two successive Lys codons, one in each of the two reading frames (178).

Thus, though IS*1* uses −1 frameshifting to express its transposase, it does not use the canonical simultaneous-slippage mechanism but, rather, the alternative mechanism which occurs inefficiently on the HIV-1 site, as shown by Jacks et al. (91). In this case, I have argued above that frameshifting occurs on U-UUU-UUA by −1 slippage of peptidyl-$tRNA^{Phe}$ on U-UUU before the UUA codon can be recognized by aminoacyl-$tRNA^{Leu}$. Sekine and Ohtsubo (179) proposed this slippage model, although they preferred two others which require abnormal codon-anticodon interaction by the tRNA present during the shift. One of the alternative models proposes that $tRNA^{Lys}$ recognizes the AAA codon of the IS*1* site normally but subsequently disengages from the wobble base, allowing another $tRNA^{Lys}$ to bind to the overlapping −1 codon; a similar mechanism was proposed to account for −1 frameshifting induced at a GCU codon by $tRNA^{Ser}_{GCU}$ ($tRNA^{Ser}_{3}$) described above (27). Interestingly, as mentioned above, Watanabe et al. (212) have found evidence that the anticodon of $tRNA^{Lys}$ adopts an unusual structure in which the wobble base is involved in an intramolecular hydrogen bond. They proposed that this interaction promotes the two-of-three decoding suggested by Sekine and Ohtsubo. A second model, actually a variation on the first, suggests that $tRNA^{Lys}$ recognizes the zero-frame AAA codon but uses a shifted anticodon which includes the U at position 33 adjacent to the anticodon (U-33); subsequently, this nucleotide would disengage from the codon, again allowing access by $tRNA^{Lys}$ to the −1 frame AAA codon. This second model is unlikely because U-33, a nearly universal base in tRNAs, is involved in a tertiary interaction which stabilizes the structure of the anticodon loop (164) and so cannot stack underneath nt 34 as part of a shifted anticodon (5, 19, 43). Sekine and Ohtsubo (179) found that changing the first A of A-AAA had less effect on expression than changes to the other positions; since they believed that this change would interfere with slippage, they suggested that the slippage model was unlikely. However, two-of-three pairing after slippage is common among both −1 (see reference 193 for a review) and +1 (45, 208) frameshift sites. Thus, the single tRNA slippage model cannot be discounted.

Does frameshifting on the IS*1* shift site, like eukaryotic simultaneous-slippage frameshifting, depend on the formation of a stimulatory downstream secondary structure? Escoubas et al. (59) suggested that the region immediately distal to the shift site stimulated frameshifting, but they failed to provide direct proof in the form of comparison of constructs with and without the region. Sekine et al. (178) later showed that the 67 nt downstream of the A-AAA shift site stimulated frameshifting about fourfold and that a shorter, 42-nt region was nearly as effective. The region distal to the shift site can be folded into several possible secondary structures. Two alternative stem-loop structures would begin 9 nt downstream of the A-AAA shift site (176), a spacing similar to the structures following eukaryotic sites (193). In addition, several structures located more distally are also possible (59, 179), including one possible pseudoknot. Surprisingly, mutants created by site-specific mutagenesis, predicted to eliminate all of these secondary structures, failed to interfere with expression of the *insA-insB* fusion product, as measured indirectly in the cointegration assay (179). This indicates that the secondary structures probably play no role in frameshift stimulation. Sekine and Ohtsubo (179) suggested that the *insA* UAA termination codon, located 18 nt downstream of the shift site, may stimulate frameshifting. Changing this codon to a sense codon (CAA) eliminated the fourfold stimulation by the downstream region, and changing it to either UGA or UAG increased stimulation a further 2.5- and 3.3-fold, respectively. How a distant stop codon could stimulate the IS*1* shift is unclear. Sekine and Ohtsubo suggest that the effect may resemble the ability of a nonsense codon in an artificial construct to induce −1 frameshifting (217). The analogy is incorrect, though, since in that case the stop codon is the next codon past the shift site, so that during the shift the stop codon occupies the ribosomal A site. The stimulatory effect of slow recognition of termination codons in the ribosomal A site on frameshifting has been amply demonstrated. The *insA* terminator could not have such an effect. One possibility is that the effect is indirect, with a ribosome paused on the terminator causing a second ribosome to queue up 5′ to it over the *insA* shift site. Arguing against this model is the lack of effect of introduction of a premature terminator two codons past the shift site on either the cointegration assay (178) or frameshift-dependent *lacZ* expression (179). Determination of the mechanism of IS*1* frameshifting must await further work.

The IS*1* frameshift is extremely inefficient, allowing only about 1% of ribosomes to shift frames. This low efficiency may explain the very unusual mechanism used. Had IS*1* used a more canonical frameshift mechanism, much more efficient frameshifting would certainly have resulted. Other IS elements do use much more efficient frameshifts, up to at least 30% in the case of IS*150* (209). Although not as efficient as the *dnaX* event, these higher-efficiency events use a mechanism which much more closely resembles the metazoan paradigm, although unusual elements specific to prokaryotes have been added on.

IS*150*: a prokaryotic analog of a metazoan simultaneous-slippage site. Like IS*1*, IS*150* includes two ORFs, *ins150A* and *ins150B*. The *ins150B* gene overlaps the last 55 nt of *ins150A* in the −1 reading frame. Within the overlap region is a motif very similar to a metazoan simultaneous-slippage site: a putative slippery heptamer, A-AAA-AAG, followed 7 nt later by a stem-loop structure (209). By overexpressing the entire IS*150* element with the highly efficient, inducible *tac* promoter, Vögele et al. (209) demonstrated that the element encodes three products, the *ins150A* and *ins150B* products and an apparent fusion product of the two frames. The *ins150A* and *ins150AB* products are expressed in about equimolar amounts, while the *insB* product is about fivefold less abundant. Expression of the *insAB* product involves an alternative translational event, rather than resulting from RNA editing or transcriptional stuttering, as demonstrated by cDNA sequencing of the transcript. Sequencing of the protein product expressed across the putative frameshift site showed that the shift occurs at the expected location, with decoding of the two Lys codons (AAA-AAG) followed by decoding of the first −1 frame codon (GCU, Ala), consistent with the simultaneous-slippage model. The result does not rule out a shift of the IS*1* type, with slippage by a single $tRNA^{Lys}$, followed by decoding in the +1 frame. Vögele et al. (209) did not mutagenize the slippery heptamer, so either model is possible. However, the data do prove that expression of *ins150AB* occurs by translational frameshifting.

The efficiency of the frameshift is unaffected by any IS*150* gene product and does not require sequences outside an 83-nt cassette extending from 9 nt upstream of the slippery heptamer to 66 nt downstream. Whether this is the true minimal cassette is unknown, since shorter regions were not tested. The down-

stream stem-loop consists of a 10-bp stem and a 28-nt loop. The presence of the stem-loop stimulates frameshifting fivefold. The possibility that the stimulatory structure is a pseudoknot, as is the case in virtually all such sites in eukaryotes, has not been tested. In fact, the 8 nt immediately downstream of the stem-loop could base pair with a portion of the loop region. If the structure were in reality a pseudoknot, IS*150* would be a perfect prokaryotic analog of eukaryotic simultaneous-slippage frameshift sites.

In eukaryotes, when a frameshift mechanism is used to fuse the translation of a gene to an upstream gene, expression of a downstream gene occurs only by this mechanism. In particular, initiation at an AUG in frame with a downstream gene does not occur in any case described. Internal initiation does occur on eukaryotic mRNAs, for example, in the expression of picornavirus genes and of other cellular genes including the *antennapedia* homeotic gene of *D. melanogaster* (reviewed in reference 147). In none of these cases, however, does initiation occur downstream of a second normally expressed gene, although such model chimeric constructs have been made. In addition, no eukaryotic gene has been found which is expressed by translational coupling, a process whereby terminating ribosomes efficiently reinitiate translation at overlapping or nearly overlapping AUG or GUG initiation codons (8, 149, 175).

In prokaryotes, the existence of the phenomenon of translational coupling means that translation of a gene could be coupled to a second upstream gene both by translational frameshifting and by translational coupling. IS*150* is an example of such a system, since the element expresses an internally initiated *ins150B* product in addition to the *ins150A* and *ins150AB* products. The precise site of initiation has not been demonstrated. There are two potential initiation sites which would produce a protein of the size observed: an AUG in the *ins150A* frame 3 codons upstream of the IS*150* slippery heptamer, and a GUG codon which overlaps the *ins150A* termination codon in the *ins150B* reading frame. Vögele et al. (209) tested the ability of the 83-nt cassette region including these two potential initiators to direct initiation of translation and found very inefficient expression of *ins150B*, much less efficient than in the intact element. They concluded that translation must occur from the AUG but depended on a initiation region at least partly located upstream of the 83-bp minimal frameshift cassette. They did not, however, exclude the alternative hypothesis that initiation of translation of *ins150B* occurs at the GUG codon by translational coupling. As predicted by this hypothesis, translation initiation in the 83-nt cassette was very low since translation in the upstream *insA* frame had been eliminated. Of 33 IS elements predicted to express a product by simultaneous slippage, 10, including IS*150*, show evidence of translational coupling. In five other cases, expression of a downstream gene may occur exclusively by coupling. The involvement of translational coupling has been demonstrated in the case of IS*3*.

IS*3*: a single site modulates both −1 frameshifting and translational coupling. IS*3* also includes a putative −1 frameshift site within the overlap between the upstream *orfA* and downstream *orfB* genes. Near the end of *orfA* is a sequence, A-AAG, which could allow −1 slippage of a $tRNA^{Lys}$ into the *orfB* frame, as in IS*1*. This site is followed 5 nt downstream by a pseudoknot. The existence of this region within the overlap suggested that expression of an *orfA-orfB* fusion product could occur by −1 frameshifting similar to that in IS*1*. A second sequence at the end of *orfA* suggests that an additional regulatory mechanism may also allow independent expression of the *orfB* product. The terminator of *orfA* overlaps the initiator of *orfB* (AUGA), again suggesting the presence of translational coupling between the two genes. This would predict that IS*3* encodes three protein products, the *orfA* product, the *orfA-orfB* fusion protein, and the *orfB* product, which has been confirmed by in vivo by overexpressing the IS*3* transcript in vivo with a T7 polymerase-based expression system (177).

IS*3 orfB* is expressed about equally by coupled initiation and by translational frameshifting. Targeted mutagenesis changing the A-AAG motif to A-CAG eliminates −1 frameshifting, whereas changing the ATGA motif to CTGA eliminated internal initiation of *orfB* (177). Each mutation reduced expression of a *orfB*::*lacZ* reporter fusion by about half. In addition, internal initiation of *orfB* depended on termination at the overlapping *orfA* terminator; introducing an upstream premature termination codon in *orfA* virtually eliminated expression of *orfB*::*lacZ* dependent on internal initiation. Amino-terminal peptide sequencing by micro-Edman degradation of the purified *lacZ* products demonstrated that internal initiation occurred at the coupling site and that frameshifting occurred at the A-AAG motif.

The pseudoknot following the IS*3* −1 slip site affects both frameshifting and coupling (177). Mutations destabilizing and restoring each of the two pseudoknot stems were tested for their effect on both processes. Mutations destabilizing S1 of the pseudoknot reduced frameshifting about sevenfold. Destabilizing S2 had a more drastic effect, reducing frameshifting about 40- to 50-fold; the greater effect of these mutants probably reflects the fact that the mutation of S2 reduced the stability of the pseudoknot more. Thus, as in other −1 frameshift site, the secondary structure stimulates the shift, probably by inducing a translational pause at the shifty A-AAG sequence.

The pseudoknot also affects translational initiation of *orfB* by ensuring that initiation requires coupling to *orfA*. Destabilizing either of the stems of the pseudoknot has the same effect, reducing internal initiation of *orfB* 10-fold while only slightly decreasing translational coupling of *orfA* and *orfB* (177). The pseudoknot structure apparently precludes direct binding by the ribosome at the *orfB* initiation codon. However, since ribosomes translating into the region from *orfA* unwind the structure, reinitiation by these ribosomes at the same site should be relatively unaffected. The two translational mechanisms operating on the IS*3* transcript allows expression of the three primary translational products (OrfA, OrfB, and OrfA-OrfB) in a constant ratio. Presumably, these ratios are important for efficient transposition of the element. Genetic evidence implicate the OrfA-OrfB fusion peptide as the transposase (177). Sekine et al. suggest that the *orfA* product may negatively regulate transposition in competition with the *orfA-orfB* transposase; it is not clear what function the *orfB* product may perform (177).

IS*911*: a Shine-Dalgarno interaction stimulates both −1 frameshifting and translational initiation. IS*911* is an insertion sequence from *Shigella dysenteriae* that is closely related to the *E. coli* element IS*3*. Like IS*1*, IS*911* includes two ORFs, a shorter 5′ *orfA* with a longer 3′ *orfB* overlapping it in the −1 frame. The overlap between the two genes includes a distinctive A-AAA-AAG motif (shown as codons of *orfA*), which is also found in five other members of the IS*3* family although not in IS*3* itself (161). In addition, 6 nt downstream of the motif is a "rabbit ear" stem-loop structure, a Y-shaped structure consisting of a single stem off the top of which two other hairpin loops branch (31).

The similarity in structure between this site and a eukaryotic simultaneous-slippage site again suggested that this site was

probably the site of −1 frameshifting in the expression of IS*911* transposase. Polard et al. (160) demonstrated that overexpression of an IS*911* transcript allowed expression of two products which appeared to be the products of the *orfA* and *orfB* genes and a third product consistent with frameshifting at the A-AAA-AAG motif. In fact, the N-terminal sequences of the *orfA* product and putative *orfAB* fusion product are identical, as expected by this model. The sequence of the *orfB* product showed that it initiates at an AUU codon immediately upstream of the A-AAA-AAG, dependent on a Shine-Dalgarno site located 8 bp upstream of the AUU. The only other gene known to initiate with an AUU codon is the gene for initiation factor 3 of *E. coli* (172), to which the initiator region of *orfB* bears significant homology (160). Internal initiation of *orfB* complicated measurement of the presumed −1 frameshift. Using a *lacZ* reporter fusion system, Polard et al. (160) compared expression of *orfA* and *orfB* fusions, finding that *orfB* was expressed at about 16% the efficiency of *orfA*. Only 1/10 of this expression occurred as a result of internal initiation at the *orfB* AUU codon. The residual expression appeared to occur by frameshifting at the A-AAA-AAG motif, since it could be eliminated by mutating the motif to A-CAA-AAG, which would interfere with tRNA slippage. Although neither detailed mutagenesis of the motif nor sequencing of the peptide product in this region has been performed, the conclusion that −1 frameshifting expresses the *orfAB* product is clear. Given the unexpected result with IS*1*, though, one cannot assume that the mechanism involves simultaneous slippage.

The conjunction of the *orfB* initiator and the *orfAB* frameshift site creates a region in which ribosomes recognize two signals, specifying two alternative forms of the *orfB* product. Polard et al. (160) suggested that this conjunction might indicate that initiation at *orfB* was "coupled" to *orfAB* frameshifting. It is unclear, however, how initiation, which occurs by binding of the 30S subunit, might be coupled to frameshifting, which occurs on elongating 70S ribosomes. The processes are "coupled" in the sense that they utilize the same sequence signals in the mRNA. Both initiation of *orfB* and *orfA-orfB* frameshifting depend on a Shine-Dalgarno 16S rRNA interaction site located 8 nt upstream of the AUU and 11 nt upstream of the slippery heptamer. These spacings are each optimal for the processes of translational initiation (182) and −1 frameshifting (108). Fayet et al. (63) first showed that the Shine-Dalgarno site in IS*911* stimulates frameshifting by site-directed mutagenesis of the Shine-Dalgarno site looking for changes which would affect frameshift efficiency. Changing this site from GGAG to GGAA, predicted to reduce the stability of mRNA-rRNA pairing, reduced frameshifting twofold, while changing it to CCCG, which should completely block pairing, reduced frameshifting eightfold, indicating that the Shine-Dalgarno interaction stimulates frameshifting. Second, they tested whether the spacing between the Shine-Dalgarno site and the heptamer was critical. In the *prfB* gene, frameshift efficiency was maximal when the spacing was 3 nt (108). Apparently, the optimal spacing to stimulate −1 frameshifting is somewhat greater. Frameshifting was only slightly reduced by changing the wild-type spacing of 11 nt to 7 nt but was drastically reduced by changing it to 4 nt, the optimal distance for +1 frameshifting (63). Changes which increased spacing to as much as 22 nt had a slight effect on frameshifting. This differs from the observations of Larsen et al. (108), who found that spacings of 16 nt or more eliminated the effect of the Shine-Dalgarno site. The reason for this difference is unclear.

tRNA HOPPING

Up to this point, the discussion has focused on events in which the reading frame shifts between overlapping codons. However, shifts can occur in which the frame shifts by larger increments, to as much as 50 nt. Such an event is termed a translational hop. Translational hopping was first observed in synthetic constructs made to test the sequence requirements of frameshifts in *E. coli*. Hopping occurred by re-pairing of tRNAs on cognate or near-cognate codons shifted +2 to +6 (217). All of these events were very inefficient, occurring with apparent efficiencies of only 0.4 to 1%. More recently, extremely efficient translational hops have been identified in phage T4 gene *60*, encoding topoisomerase (83, 216), and in the *trpR* gene, encoding the *E. coli trp* repressor (10). Clearly, the sequences surrounding these sites must stimulate translational hopping in the same way that sequence contexts stimulate frameshifting. It is not immediately clear whether the distinction between translational frameshifts and translational hops has a functional significance or if it is just semantic, i.e., whether hopping is mechanistically distinct from frameshifting or if it is just frameshifting over longer distances. Analysis of the efficient hop sites suggests that they involve mechanisms not previously found in frameshifting, suggesting that the distinction is not merely semantic.

Nonprogrammed Translational Hopping

One of the earliest studies which identified suppressors of frameshift mutations was performed with the *trpE91* allele of *Salmonella typhimurium*, a −1 frameshift mutation (169). The mutation is leaky, allowing very low level expression of the *trpE* product by spontaneous shifting of frame near the site of the frameshift mutation. Weiss et al. characterized the low-level frameshifting which occurs at this site by transferring the region surrounding the mutation into a *lacZ* expression vector (217). Unexpectedly, the peptide expressed across the site indicated that the frameshift occurred by a translational hop of +2 rather than by −1 frameshifting. The original mutation changed the sequence GGA-GUG-GUG-AGG to GGA-GUG-UGA-GG, introducing an in-frame stop codon. Spontaneous suppression occurs inefficiently (0.5%) by a translational hop, with a $tRNA^{Val}$ apparently hopping +2; the sequence is read as Gly-Val-Arg, decoding the GGA, GUG, and AGG codons (217). Hopping on this sequence appears to resemble +1 frameshifting. Ribosomes probably pause at the in-frame UGA stop codon after decoding the Val codon, GUG. At a very low frequency during that pause, peptidyl-$tRNA^{Val}$ can dissociate from the in-frame GUG codon and repair with the +2-shifted GUG codon. After the hop, the ribosome resumes normal decoding, reading the next codon, AGG, as Arg.

Weiss et al. identified two other sequences on which the ribosome hops, either +5 on the sequence AAC-UCA-AU (in which peptidyl-$tRNA^{Asn}$ hops between cognate codons) or +6 on the sequence CUU-UAG-CUA (in which peptidyl-$tRNA^{Leu}$ hops over an in-frame stop codon between near-cognate codons). Each of these events was confirmed by N-terminal sequencing of the protein product, and each occurs inefficiently (the +5 hop at 0.4%, and the +6 hop at 1.0%). Two of these hops occur at in-frame stop codons, which one presumes provide the pause necessary for the unconventional event to occur. However, the third occurs at an in-frame sense codon (UCA); this codon is not rare and is not predicted to cause a translational pause, as do the AGG and AGU codons in Ty frameshifting in *S. cerevisiae*. It is not clear that translational hopping depends on a translational pause, although that would be very unexpected.

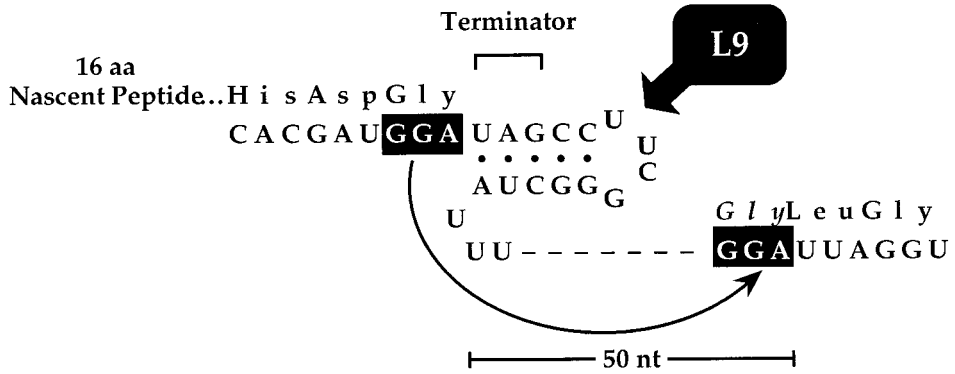

FIG. 11. The translational hop site of the bacteriophage T4 gene *60*. The hop occurs by repositioning of a $tRNA^{Gly}$ from a takeoff to a landing GGA codon (white letters in black rectangles). This is stimulated by an in-frame UAG terminator, a hairpin loop, an upstream 16-amino-acid nascent peptide, and an approximately 50-nt spacer. Ribosomal protein L9 modulates the ability of the hairpin to stimulate the hop (illustrated by the arrow).

A similar 6-nt hop was discovered serendipitously when the bovine placental lactogen gene was overexpressed in *E. coli* (98). Expression of the gene in *E. coli* resulted in the production of two forms of the protein, the full-length product and a second product in which a 2-codon hop appears to have occurred. The hop occurs at the sequence CTA-TTG-AGG-TTG-GTT, which encodes Leu-Leu-Val, presumably by reading the CTA codon, decoding TTG and hopping over the AGG codon, and decoding GTT. The authors suggest that the low abundance of the AGG-decoding tRNA in *E. coli*, possibly exacerbated by the high demand for that tRNA generated by overexpression of a gene with a high proportion of AGG codons (as suggested in reference 25), causes a translational pause and that the peptidyl-$tRNA^{Leu}$ then hops between cognate codons. The event is efficient, occurring with an efficiency of about 20%. This model for the expression of the protein has not been confirmed, for example, by modifying the AGG codon to an abundant Arg codon.

The high-level occurrence of translational hopping in this case may mean that alternative translational events may become a problem for the biotechnology industry. It has long been recognized that differences in codon usage and, by implication, tRNA abundance might profoundly affect the yield of proteins expressed in heterologous contexts; however, it is now evident that this problem may be compounded by the heterogeneity of products brought about by translational errors (for a recent review of this problem, see reference 148). This difficulty adds to the importance of our understanding the mechanisms underlying translational errors and alternative translational events.

Programmed Translational Hopping

Just as programmed translational frameshifts resemble frameshift errors, some genes are encoded by using programmed translational hops which resemble these spontaneous nonprogrammed hops. Apparently, programmed translational hops are much less prevalent than frameshifts, since there are now only three cases of proposed translational hops, all in bacterial systems. The mechanisms of two of these hops, those of the T4 gene *60* and the *trpR* gene of *E. coli*, have been studied in some detail. One of these resembles the spontaneous hops described above, while the other does not. In the case of the third putative hop, in the gene encoding *Bacteroides ruminicola* 1,4-β-D-endoglucanase, the mechanism is not known.

Hopping in the topoisomerase gene of phage T4. The type II topoisomerase of bacteriophage T4 is a hexameric protein made up of dimers of three proteins, the smallest of which is encoded by gene *60* (115, 190). This gene is one of four genes encoded by the bacteriophage which are interrupted; that is, they include a region which is not expressed in the protein product. The other three genes, *td*, *nrdB*, and *sunY*, all include group I self-splicing introns (33, 72, 183, 185); after removal of these introns, the genes are translated normally. This in itself is unusual enough for prokaryotic genes. However, in gene *60*, a 50-nt interruption is not removed by RNA splicing but remains present in the mature mRNA. A programmed translational hop allows the ribosome to bypass this region to encode the correct protein product. This hop is quite specific and is very efficient (83). The mechanisms underlying this unusual mechanism of gene expression have been very well characterized. This mechanism can be considered the canonical form of programmed translational hopping against which other subsequently discovered examples must be compared.

The existence of a 50-bp interruption was apparent from the sequence of gene *60*. The seven N-terminal residues of that subunit (115, 190) match the beginning of a 45-bp ORF. However, this frame could not encode the 18-kDa gene *60* product. Marker rescue experiments showed that gene *60* extended far beyond the end of this frame, into a region including a second, nonoverlapping reading frame (83). The combination of these two regions could encode an 18-kDa protein. Direct protein sequencing of the gene *60* product showed that the gene was translated up to a GGA Gly codon in the first frame, the last codon before an in-frame UAG nonsense codon, and then continued to be decoded 50 nt downstream, starting immediately distal to another GGA Gly codon at the beginning of the second frame (83). This 50-nt interruption in the continuity of the gene was present in the mRNA, and no evidence of splicing of this region could be obtained (an in vitro-synthesized mRNA, unlike the mRNAs of the other interrupted T4 genes, would not splice in an in vitro reaction). However, the gene could direct the synthesis of the 18-kDa product in a cell-free protein synthesis system (83). The simplest explanation for the expression of the protein was that the translational machinery simply bypassed the interruption by a translational hop. Although the authors of the original paper thought it unlikely, it was still formally possible that the 18-kDa gene *60* product were produced by a protein-splicing event, joining the products of the first reading frame with a putative second primary translation product, perhaps initiating translation of that frame at an in-frame AUU codon (as occurs in IS*1* [160] and the initiation factor 3 gene [172]).

In an elegant paper, Weiss et al. (216) characterized the mechanism of translational hopping in gene *60*. Hopping occurs in gene *60* at an extremely high rate, estimated as 94% (216). There are five distinct components of the hop site, which together promote the extremely efficient alternative decoding event (Fig. 11): a pair of matched "takeoff" and "landing" codons, an in-frame stop codon, an upstream nascent polypeptide sequence, and a hairpin structure which includes the takeoff codon (216). A region of 143 nt, including the 50-nt coding gap and the 93 nt upstream of it, constitutes the minimal sequence of the hop site. The requirement for the upstream sequence reflects the fact that the nascent peptide encoded in that region stimulates the hop. The codons specifying amino acids 17 to 30 of the gene *60* product are essential to stimulate the hop, which occurs after reading codon 47. Combinations of frameshift mutations of the region preceding the translational gap were tested for their effect on hopping. Any combination of mutations which retained normal decoding of codons 17 to 30 had little effect on hopping efficiency, yet mutations including some of the same changes which altered the reading of this region reduced hopping to background levels. This clearly suggests that it is the primary amino acid sequence encoded by codons 17 to 30 which stimulates hopping. This conclusion was further strengthened by introducing synonymous codon changes at each of these codons; changing the codons in this region to synonymous codons had no effect on the efficiency of hopping, demonstrating that the amino acids encoded by the region, not the RNA sequence, stimulate the event.

The nascent peptide stimulates hopping after decoding of the last sense codon in the upstream frame, GGA. Translation then resumes 50 nt downstream at a UUA codon immediately 3′ to a second GGA codon. To demonstrate that a peptidyl-tRNA reading the first GGA must dissociate from it and reassociate with the second GGA to promote the hop, Weiss et al. (216) mutated either one or both of these codons. Changing either of the codons to GCA eliminated hopping, while changing both restored it. The requirement for a match between the two codons implies that both must be read by the ribosome, consistent with the hypothesized reengagement model. Further, the fact that the double mutant allows hopping at near-normal levels suggests that the tRNA does not play an important role in the hop. Some sequence features of the site must explain the high efficiency of hopping. One feature is the nonsense codon immediately after the first GGA codon. Changing it to a sense codon reduced hopping by up to 59-fold. Presumably, slow recognition of the in-frame nonsense codon causes a translational pause; as predicted, the tetranucleotide sequence at this site, UAG-C, is underrepresented in termination sites in *E. coli* (26).

The second feature is a hairpin immediately after the takeoff codon (Fig. 11). The stability of this structure is essential. Mutations which increase its stability by increasing the length of the stem tend to decrease hopping efficiency. Strangely, mutations which decrease its stability, by disrupting base pairs near the loop, interfere equally well with hopping. Herbst et al. (77) have proposed a dynamic model to explain this behavior. They proposed that the ribosome destabilizes the hairpin as it approaches the takeoff codon. When the ribosome pauses at that site, the upper portion of the hairpin, past the GGA codon, can re-form within the ribosomal A site, and it is the re-formation of the structure which then stimulates the hop. Apparently, the hairpin interacts with the ribosome, specifically with ribosomal protein L9, since *hop1* mutations which target the *rplI* gene encoding that subunit increase the efficiency of hopping on mutant sites with extended hairpins, although they have no effect on shorter hairpins. Herbst et al. suggest that hopping is inhibited by a shortened hairpin because it cannot re-form in the A site. They also suggest that a elongated hairpin cannot re-form for a different reason; i.e., it cannot disengage from the putative ribosome-associated helicase. The *hop1* mutation would reduce the ability of the helicase to inhibit re-formation of the longer helices; in this model, it would be predicted to affect only elongated hairpins.

Although the details of this model are still very hypothetical, the stimulatory role of the hairpin is clear. An early model (83) invoking an alternative stacked-hairpin structure which would juxtapose the takeoff and landing codons to allow direct transfer of the peptidyl-tRNA now seems unlikely. Weiss et al. showed that mutations which specifically destabilize this alternative structure had little or no effect on hopping efficiency (216). Interestingly, hopping efficiency decreased significantly when the length of the coding gap was either substantially decreased or increased. It may be that the hairpin occupying the A site mimics tRNA occupation or simply interferes with recognition by release factor to prolong the translational pause at the site. Alternatively, the hairpin may directly promote the hop, although how it would do this is not clear.

Hopping in the *trpR* gene of *E. coli*. In general terms, the T4 gene *60* translational hop resembles programmed frameshifts. During a translational pause, the ribosome-bound peptidyl-tRNA shifts between cognate codons and continues translating in the new reading frame. The shift depends on structural features of the site, although one of those features is unexpectedly expressed in the structure of the nascent protein rather than in the RNA of the site. The event, although extreme, exemplifies the attributes of the other programmed translational elongation events which I have discussed.

A translational hop which occurs in the *trpR* gene does not conform well to the paradigm. It occurs without the need for repositioning of the peptidyl-tRNA, it is apparently stimulated by a nonspecific sequence of amino acids in the nascent peptide, and it does not appear to depend on a well-defined translational pause site. Understanding the mechanism of this event may fundamentally challenge our thinking about how programmed alternative coding events occur.

Inspection of its sequence suggested to Benhar et al. (12) that a programmed +1 frameshift might occur in the *trpR* gene, the repressor of several operons regulated by Trp in *E. coli*. They found a sequence, A-AAA-AAT, which resembled a slippery sequence associated with frameshifts. Although this sequence resembles most closely a −1 simultaneous-slippage site, they found no evidence of −1 frameshifting but, rather, a low level of apparent +1 frameshifting (as measured with a *lacZ* reporter fused downstream in either frame) (12). If frameshifting were to occur at or before this site, it would result in the expression of a product identical to the *trpR* product over the first 73 amino acids but would then shift into the +1 frame and terminate translation at a +1 TGA terminator 15 codons downstream, to encode a 10-kDa protein slightly shorter than the 12-kDa normal translation product. As expected, translation of the *trpR* gene in vitro produced a 10-kDa protein which reacted to antibody directed against the 15 C-terminal residues of the putative frameshift product. Further confirming the assignment, this protein required the normal *trpR* initiation codon to be expressed, reacted against antibodies which recognized an N-terminal peptide of the *trpR* product, and had an N-terminal amino acid sequence identical to that of the full-length *trpR* product (12). All these data argue that a +1 frameshift occurs near the A-AAA-AAT motif to produce an alternative form of the *trpR* product.

More detailed analysis of the phenomenon showed that translational hopping rather than frameshifting might be hap-

pening in *trpR*. Since a *lacZ* fusion to *trpR* seemed a faithful reporter of *trpR* frameshifting, Benhar and Engelberg (10) used this fusion to attempt to characterize the frameshift further. Using the fusion, they identified a region from codons 54 to 70 of *trpR* which could direct the apparent frameshift. A 5′ deletion which removed codons 2 to 65 eliminated *lacZ* expression. Strangely, expression could be restored by introducing an unrelated 13-codon sequence upstream of codon 65 but not by introducing a 9-codon sequence. Benhar and Engelberg (10) concluded that the region distal to codon 65 was essential but that a random sequence of at least 10 amino acids was necessary as well, although the role of a random amino acid sequence remains obscure. By introducing a cleavage site for the protease factor Xa immediately before codon 65 (11), they were able to determine the amino acid sequence encoded by the *trpR-lacZ* fusion gene. Quite unexpectedly, the sequence indicated that a translational hop into the +1 frame occurs immediately after codon 65, a Met codon, although the hop occurs not into *trpR* sequences but into the adjacent *lacZ* sequence. The hop of 55 nt was confirmed by genetic analysis which showed that a +1 frame termination codon introduced immediately upstream of the UUA Leu codon at which elongation resumes had no effect on *lacZ* expression whereas a terminator inserted 3 codons downstream in the +1 frame blocked *lacZ* expression, as did a terminator inserted immediately upstream of codon 65. This is consistent with the idea that the ribosome hops from codon 65 to the UUA codon and then continues elongation. However, unlike the gene *60* hop, there are not matching takeoff and landing codons at the ends of the coding gap. The last codon read before the hop is an AUG, and the codon immediately before the next codon decoded is a GUU. Clearly, peptidyl-tRNA^{Met} is not able to base pair with GUU, so it cannot act as a landing codon. Additionally, mutating the GUU codon to UAA, the nonsense codon mutation referred to above, had no effect on hopping. Therefore, with matching takeoff and landing codons lacking, it is unlikely that hopping at this site could involve repositioning of the peptidyl-tRNA.

A final puzzling result is the fact that the *trpR* hop requires translation through the coding gap in the normal reading frame. Nonsense codons were inserted at four positions downstream of codon 65 in the zero frame. Each of these mutations would block expression of *lacZ* by translational hopping. This is odd because this region would be skipped over by the ribosomes which hop. Therefore, it is unclear how ribosomes which do not decode this region would detect the presence of these terminators. The only possible explanation is that one of the majority of ribosomes which do not hop must be required to decode these codons to allow hopping by other ribosomes. Benhar and Engelberg-Kulka suggested that a ribosome which normally translates through the region may in some fashion stabilize a ribosome which bypasses the region, although the nature of that stabilization is unclear. Alternatively, they imagine that the bypassing ribosome may have to "ratchet" through the region without incorporating amino acids, a mechanism which they admit is unprecedented and one which is again ill defined. Since translocation of the ribosome occurs only after successful peptide transfer to an incoming aminoacyl-tRNA and is concomitant with the movement of a tRNA from the A to the P site, it is unclear how a ribosome might ratchet through a 55-nt region without incorporating amino acids.

The only conclusion which can be drawn about the *trpR* story is that it is a conundrum. The mechanisms proposed for the hop are, to say the least, unconventional as well as undefined. It is not clear whether this event can be considered a model for hopping which may occur at presently undiscovered sites or whether we should attempt to use these results to speculate about the mechanism of translational frame maintenance. The result obtained for the *trpR-lacZ* fusion may not even be relevant for the intact *trpR* gene. Since the described hop occurs into *lacZ* sequences, lacking in *trpR*, the same mechanism cannot explain the expression of the truncated form of *trpR*. In fact, the putative +1 frame coding region which, as indicated by immunoblotting, is encoded in the 10-kDa form of *trpR* is specifically bypassed in the *trpR-lacZ* hop. One is forced to conclude that the region of *trpR* around codon 65 may be a hot spot for unconventional decoding events, although the identity of that event depends on what sequences are adjacent.

Does translational hopping occur in other cellular genes? Only the T4 gene *60* and *trpR* genes are proposed to use a programmed translational hop. Are there other genes which could use the same mechanism? Since the two known examples differ in so many ways, it is not possible to identify other potential sites by sequence inspection. There are some genes which are candidates for a translational hop. The carboxymethyl cellulase gene of *Bacteroides ruminicola* expresses two primary translation products, one of which may be expressed from two overlapping reading frames (129). The carboxymethyl cellulase gene includes two ORFs, the second of which overlaps the first by 13 nt in the −1 reading frame. The N terminus of an 88-kDa product is encoded within the upstream ORF, while the N terminus of an 82-kDa product is encoded in the second ORF. The first ORF could encode a protein of only 18 kDa, so it must be encoded by some mechanism which fuses the translational product of the two reading frames. However, the predicted size of such a product is 106 kDa. This suggests that in fusing the two frames, a large region is excluded potentially by a translational hop. A second carboxymethyl cellulase gene from *Fibrobacter succinogenes* has a similar structure (29) and could conceivably be expressed by either −1 frameshifting or translational hopping. A third gene, the *plaA* gene of *Prevotella loescheii*, may also be expressed by translational hopping (122). In none of these cases is the simpler hypothesis of RNA splicing ruled out, making speculation about possible hopping mechanisms premature. It remains to be seen if the gene *60* and *trpR* hops are archetypes of a new class of programmed frameshift events or idiosyncrasies.

CONCLUDING REMARKS

Programmed translational frameshifts are not a unitary phenomenon but, rather, a varied collection of unconventional events, all of which cause the ribosome to alter its reading of the genetic code to produce an alternative product not encoded directly by the mRNA. Although this review does not attempt to be exhaustive—I have focused on a few systems in which the mechanism of frameshifting has been relatively well established—it is clear that the ribosome can be perturbed in many ways to produce shifts in either direction or translational hops of dozens of codons. Although the overwhelming impression is of diversity, a few common themes occur over and over among these sites. First is the necessity for a translational pause. Translational pauses are essential because the frameshift event always occurs in competition with a normal elongation event, whether that be continued translation or termination. Frameshift events are at a distinct kinetic disadvantage and rarely occur at random sites in the genome. At a frameshift site, the alternative canonical translation event is slowed to a rate which is much closer than normal to the rate of the frameshift event. With this adjustment, frameshifting ceases to be a kinetically unfavorable outcome, allowing the ribosome to efficiently shift frames.

The second common theme is that frameshifting requires the "recoding" or "rephasing" of translation. In nearly all cases, frameshifting occurs because a tRNA which has been selected by the ribosome as a cognate codon for an normal frame codon shifts position to another cognate or near-cognate codon. Interestingly, in the early literature, it was thought that frameshifting would occur by the misplacement of incoming aminoacyl-tRNA in the ribosomal A site (see the discussions of possible frameshift mechanisms in references 2 and 13). Recent work with the Ty3 retrotransposon in *S. cerevisiae* has reintroduced the idea of efficient out-of-frame binding of aminoacyl-tRNA as a mechanism of programmed frameshifting. The rat ornithine decarboxylase antizyme gene may provide a second example of this phenomenon in a higher eukaryote.

The next phase of research on programmed translational frameshift will probably diverge into two paths. One focus of research will be on the factors responsible for promoting these events. For example, several laboratories are currently attempting to identify a factor which stimulates −1 simultaneous-slippage frameshifting. The ultimate goal of these groups may be to use this factor to develop novel antiviral agents which could target this essential step in the expression of prominent disease-causing viruses, including HIV-1. The success of this line depends on the existence of such a factor, which still is in doubt. The second focus will be to use programmed frameshift systems as probes of the frame maintenance mechanism. The +1 frameshift and translational hop systems are more likely candidates for this path, since −1 simultaneous-slippage frameshifting appears to be a truly noncanonical event which may be, like suppression of UGA codons by the selenocysteine-incorporation system (16), a specially encoded alternative event. However, even these frameshifts may depend on bending the molecular rules of frame maintenance. The next few years should reveal whether either route will provide new insights into the mechanisms of programmed translational frameshifting.

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