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Ribosomal frameshifting, jumping and readthrough

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New examples of high-level ribosomal frameshift and readthrough events have been described over the past year and a half. These include −1 frameshifiting at tandem codons and +1 frameshifiting at neighboring slow codons. Several bizarre examples of ribosome jumping and multiple stop-codon readthrough continue to perplex investigators in this field.

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Introduction

Our knowledge of the alternative paths followed by ribosomes through open reading frames (ORFs) is rapidly growing. Examination of translational events that lead to changes in reading frame, and readthrough or bypass of stop codons, continues to reveal unexpected intricacy in both the mechanisms used and the reasons for their existence. A general guide to following the path of ribosomes through alternative translation frames or stop codons is shown in Fig. 1. The field is also beginning to show signs of maturity, in that the models for −1 and +1 frameshifting are now well established and are strengthened with each new example found. Advancement to the stage where investigators can be cognizant of the rules governing these various events is already leading to correct anticipations and predictions of these alternative modes of gene organization and expression. This level of knowledge is apparent in may of the recent experiments reviewed here, and is especially evident in the plethora of genes that are being isolated using the tandem slippery codon rule of −1 frameshifting. Common themes are also emerging for +1 frameshifting, with the demonstration that the *Saccharomyces cerevisiae* retrotransposon Ty1 frameshift obeys rules similar to bacterial high-level +1 frameshift sites. But what is also currently evident is the existence of certain events that still defy categorization. The most prominent members of this group include high-level ribosome jumping and a new case of multiple TGA codons specifying selenocysteine within a single ORF. The occurrence of such translational oddities hinders our elucidation of just how complex these translational events are.

Frameshifting: −1

The predominant class of −1 frameshifting events continues to be of the tandem slippery codon variety. Evidence accumulated in the past year indicates that the expansion of this list is still in an explosive period. Recent results provide details of the −1 mechanism in the *Escherichia coli dnaX* gene, bacterial insertion sequences (ISs), bacteriophage T7, yeast double-stranded RNA, and the retroviruses and coronaviruses. In many cases, not only are the interesting minutiae of the mRNA sequence requirements understood, but the different rationales for these types of ORF arrangements are becoming evident.

The mystery of the production of two co-amino terminal polypeptides from a single ORF of the *E. coli dnaX* gene has been solved in triplicate [1,2,3]. The *dnaX* gene products, the τ and γ subunits of DNA polymerase III holoenzyme, are produced in a ratio of one-to-one. Normal ORF translation yields the τ (71-kD) subunit, and −1 frameshifting at an internal A-AAA-AAG heptameric motif produces the γ (47-kD) subunit. This motif has been shown to elicit high-level −1 shifting in *E. coli* in the context of retroviral −1 shift sites [4], and conforms to the 'simultaneous slippage model' of Jacks *et al.* [5]. The *dnaX* shift region also makes use of a 3′ stimulator, which is possibly a stem-loop [6].

The members of the large IS3 family of bacterial transposable elements and the unrelated IS1 element display a common arrangement of their two main ORFs: the shorter ORFA overlaps ORFB such that a −1 frameshift will generate an ORFA–B fusion polypeptide. ORFA is though to be involved in sequence-specific DNA-binding at the ends of the IS element, whereas ORFB encodes activities necessary for DNA cleavage and ligation during transposition [7]. Genetic evidence [8] supports the hypothesis that −1 frameshifting in the ORFA–B overlap of IS1 determines the ratio of the two translation products: a transposition inhibitor (ORFA) and an active transposase (ORFA–B). The level of frameshifting in IS1 has been established as approximately 1% [9•] and the mRNA motifs required for the event include an A_6C frameshift and a weak 3′ stimulator nearby. This puts the mechanism squarely in the tandem slippery class, and changes in transposition levels resulting from alteration of the ORFA–B–ORFA ratio [9•,10] suggest that −1 frameshifting can set the rate of transposition.

Abbreviations

carA—carbamoylphosphate synthetase; **IS**—insertion sequence; **ORF**—open reading frame; **RF**—release factor; **TMV**—tobacco mosaic virus; **UTR**—untranslated region.

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More recent analysis of an IS3 group element, IS911 [11••], uncovers another example of A_6G motifs in bacterial −1 frameshifting. The level of frameshifting in the IS911 ORFA–B overlap is substantially higher than in IS1 and the nature of the flanking sequence appears to be more complicated, requiring perhaps both 3′ and 5′ stimulatory elements. The IS911 story is further complicated by expression of ORFB from an AUU initiation codon overlapping the frameshift region, suggesting an intricate relationship between the expression of these three polypeptides (ORFA, ORFA–B and ORFB) and the control of transposition. From experiments monitoring A_6G sequences, it is known that 5′ sequences can have inhibitory effects on −1 frameshifting at tandem slippery codons. The Shine–Dalgarno sequence, which is known to stimulate +1 frameshifting, inhibits −1 frameshifting when placed three–five nucleotides 5′ of an A_6G sequence [12]. Analysis of the natural context found in the above examples should lead to a more comprehensive view of how ribosomes sense mRNA sequence and structure in the mRNA track.

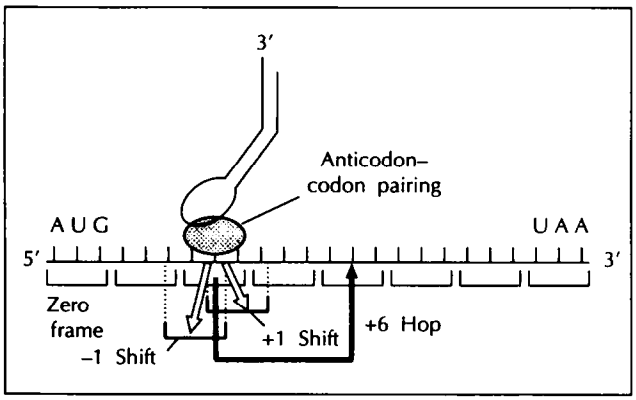

Fig. 1. The common vernacular for describing the alternative paths that ribosomes may follow into and out of open reading frames. A single tRNA is shown bound to mRNA via triplet anticodon–codon pairing. Most high-level ribosomal frameshifts are characterized by the formation of stable anticodon–codon pairing in alternative reading frames. The arrows show the displacement of the anticodon–codon pairing from the original starting frame (the zero frame) to new frames displaced either −1, +1 or +6 nucleotides from the zero frame.

Another long-standing example of −1 frameshifting, that of the bacteriophage T7 gene 10B coat protein, joins the class of tandem slippery codons. Solid evidence for the frameshift junction was obtained by peptide mapping [13] and direct protein sequencing [14]. The junction coincides in T7 gene 10B with a G-GUU-UUC heptamer and, by analogy, with C-CCA–AAG in phage T3. In addition, 3′ flanking sequences are required to stimulate these sites to the observed 10% level of frameshifting [14].

Evidence of both tandem slippery codons and 3′ hairpin-loop-type pseudoknot stimulation is observed in the −1 frameshifting of the L-A double-strand RNA virus from *S. cerevisiae* [15•]. A *gag–pol*-like 180-kD protein is produced from fusion of ORF1 and ORF2. Several combinations of slippery codons at the heptameric shift site give levels of frameshifting substantially higher than the naturally occurring 2%, and compensating mutations suggest the involvement of a 3′ pseudoknot. Yeast should prove to be a particularly fruitful system for investigating the mechanism of 3′ pseudoknot stimulation of frameshifting on a eukaryotic ribosome.

Examples of pseudoknot stimulation at tandem slippery codons in coronaviruses continue to accumulate. Sequence analysis of gene 1 of mouse hepatitis virus reveals an organization similar to the avian coronaviruses, with an overlapping region between ORF1a and ORF1b containing a U-UUA-AAC sequence six nucleotides 5′ of a predicted pseudoknot [16]. Experiments have shown that this region directs high-level −1 frameshifting in rabbit reticulocyte lysates. Strangely, these viruses attempt to produce an ORF1a–1b fusion protein by translating 7193 zero-frame codons, while frameshifting with 50% efficiency at a single internal site. Another example among coronaviruses is found in the ORF1a–1b overlap of equine arteritis virus [17]. In this case, the predicted tandem slippery codons are G-UUA-AAC. This motif is undoubtedly suboptimal, but it is demonstrably shifty at a level of 20% in reticulocyte lysates.

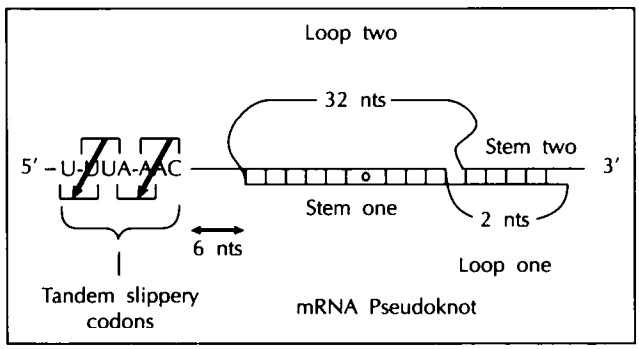

Fig. 2. Sequence elements comprising a coronaviral −1 frameshift site. Mutational analysis suggests that optimal spacing of 6 nucleotides (nts) between a tandem slippery codon frameshift site and 3′-pseudoknot structure is required for −1 frameshifting at the 50% level (see [18••]).

An extensive mutational analysis of the avian coronavirus infectious bronchitis virus has been directed at the pseudoknot requirement for stimulating −1 frameshifting [18••]. The key sequence elements of the frameshift site are shown in Fig. 2. Both loop one (two nucleotides) and loop two (32 nucleotides) can tolerate insertions, up to a point, and loop two can be deleted to a minimum of eight nucleotides in length with no ill-effects. Extensive changes in the stem structures revealed no other requirement besides base-pairing, and also showed that precise co-axial stacking, or at least a standard base-pairing, might not be required between stem one and stem two. The analysis culminates with an elegant demonstration that a simple hairpin structure consisting of the same nucleotides as the pseudoknot will not stimulate −1 frameshifting. This implies that it is the geometry of the pseudoknot that is of paramount importance for initiating the event.

Proof of a pseudoknot as the 3′ stimulator in a high-level retroviral *gag–pro* overlap was obtained by a mutational

analysis of the mouse mammary tumor virus *gag–pro* overlap [19•]. Small loop sizes and GC-rich stems characterize this compact structure. The analysis shows that the native A_6C motif cannot be substituted with A_6G, A_6A or A_6U, demonstrating the subtlety of anticodon–codon pairing at these sequences, as well as highlighting the apparent preference for terminal wobble mis-pairing in the shifted position. The effect of this mis-pair and its relationship to the pseudoknot stimulation, if any, is not yet known.

Frameshifting: +1

The *S. cerevisiae* retrotransposon Ty1 governs the +1 frameshift that couples TyA and B. The sequence elements of Ty1 are functionally analogous to retroviral *gag* and *pol*, and are shown to consist of a simple seven-nucleotide sequence: CUU-AGG-C [20••]. Using mutational and protein sequence analysis, the frameshift is inferred to occur at the CUU leucine codon. A model that explains the Ty1 frameshift includes two of the three elements known to underlie the other well known high-level +1 frameshift (the *E. coli* release factor 2 site: CUU-UGA): a slippery CUU-decoding peptidyl-tRNA and a 'slow' codon presented in the A site. What is most striking about the Ty1 case is the high level of frameshifting (upwards of 50%) achievable with only seven nucleotides. In addition, 5′-stimulator sequences, such as those used by release factor (RF)2, do not seem to be required to reach these impressive levels. Yeast is also unusual in that it decodes CUU leucine codons with a tRNA containing an unmodified uracil in its anticodon wobble base, allowing it to decode both CUU and UUA. The requirement in the Ty1 sequence of a specific seventh base is not yet understood, but could reflect a 3′ context effect on the recognition of an AGG by an arginine tRNA, or a need for decoding in the +1 frame by a specific glycine tRNA. Confirmation of one basic feature of the model, the slow rate of AGG decoding, has been provided by the isolation of a gene which, when present in multiple copies, inhibits the frequency of Ty1 transposition by decreasing the ratio of TyA–B to TyA [21•]. This gene encodes the AGG-reading arginine tRNA.

In earlier experiments, it was shown that suppressor tRNAs could decrease the RF2 frameshift rate and that, with a sense codon substituted for the stop codon, an inverse correlation existed between codon usage and the rate of frameshifting [22]. The +1 shifts seem to be cases of peptidyl-tRNA slippage, stimulated by an unoccupied A site. The common themes emerging from the comparison of these two +1 frameshifts suggest a fundamental conservation of mechanism between *E. coli* and yeast ribosomes. The use of a CUU-decoding leucine tRNA in both instances may be coincidental, but the demonstration that the *E. coli* RF2 window elicits a moderate level of frameshifting in reticulocyte lysates [23] extends this comparison further and suggests that CUU codons may be likely suspects for +1 frameshifts. The finding that the *Bacillus subtilis* RF2 gene contains a potential CUU-UGA frameshift site (RB Weiss, unpublished data) re-enforces this suspicion. Although tangential, it is worth mentioning the finding by Kawakami and Nakamura [24•] that the UGA suppressor, *supK*, is the result of an in-frame UGA mutation in the *E. coli* RF2 gene which, of course, encodes a protein involved in termination of UGA codons. The up-regulation of the rate of +1 frameshifting in the *supK* mutant demonstrates that RF2 auto-regulates the rate of frameshifting *in vivo*, and explains how these cells manage to survive, despite having to produce RF2 by sequential frameshift and readthrough events.

Ribosome jumping

The single translation system involving a high-level ribosome jump, that of the bacteriophage T4 gene *60* coding gap, has been broken down into components by the mutational analysis of gene *60*–lacZ fusions in *E. coli* [25••]. A 'smash-and-bash' genetic approach defines the black box that expedites the ribosome's excursion past the intervening 50 nucleotides, but only several of the components are recognizable in terms of other known events. A stop codon at the 5′-junction is required, but bypass can still occur at a 10% rate in it's absence, suggesting analogy to a P site event stimulated by an unoccupied A site. The stop is enfolded in a short stem-loop, also required for maximal bypass, although how and when it may form in the A site is unclear. The codons flanking the 50 nucleotides need to be virtually identical, fostering the view that the gene *60* jump is an example of high-level tRNA hopping [26]. The lack of specific sequence requirements within the 50 nucleotides also effectively rules out alternative models that postulated base-pairing between the two ends of this sequence [27,28]. The remaining unexplained components are the optimality of the 50-nucleotide distance for high-level jumping, and the unexpected finding that a strict specific sequence of amino acids within the nascent peptide chain is critical for bypass.

The gene *60* case remains enigmatic as the sole example of a high-level ribosome jump. Another potential example is reported for the carbamoylphosphate synthetase (*carA*) gene for *Pseudomonas aeruginosa* [29]. Comparison of the amino-terminal of the *carA* protein with both DNA and cDNA sequences reveals that the amino acids encoded by codons five–eight are absent from the mature polypeptide. The only overt similarity to gene *60* is a direct repeat which would be consistent with a tRNA hop. Definition of this case as 'ribosome jumping' may require the elimination of alternative explanations. For instance, a recent paper describes the production of polypeptides containing amino acid deletions via a protein splicing mechanism [30].

Stop codon readthrough

Several new sequence contexts that stimulate readthrough of stop codons in eukaryotes have been uncovered. The leaky UAG stop codon found in tobacco mosaic virus (TMV) is shown to require *in vivo* a minimal sequence of UAG-CAR-YYA [31]. Extensive analysis also ruled out a tRNA hopping mechanism for TMV

readthrough. Several reports have attempted to unravel the context of the leaky UAG at the *gag–pol* border of Moloney murine leukemia virus, known to promote glutamine insertion at a 5–10% level. The extent of flanking sequence required to stimulate readthrough *in vitro* has been narrowed down to 57 nucleotides 3′ of the UAG [32], and the critical sequences have been defined [33•,34]. Solid evidence for a pseudoknot located eight nucleotides 3′ of the UAG codon has been provided by an exhaustive use of compensating mutations [33•], thus confirming an earlier prediction of its existence [35]. A sequence requirement in the eight-nucleotide spacer is also inferred from a separate study [34]. The underlying mechanism of this novel use of a 3′-pseudoknot to influence a readthrough versus stop choice in the A site is not obvious, but its elucidation will certainly be revealing.

An unusual case involving selenocysteine incorporation at multiple UGA codons to produce a single polypeptide is emerging from the cloning and sequencing of a cDNA for rat selenoprotein P [36••]. Selenoprotein P is a plasma selenoprotein of unknown function that has been estimated to contain 7.5 +/− 1.0 selenocysteine residues per polypeptide. It constitutes approximately 8% of total body selenium, and has a half-life of approximately 4 h. The cDNA sequence encoding this protein has 10 in-frame UGA codons, corresponding to amino acids 40, 245, 263, 304, 316, 338, 352, 354, 361 and 363 within the predicted polypeptide. Three sections of the predicted amino acid sequence were verified by amino acid sequencing of isolated peptide fragments, and selenocysteine incorporation at UGA codons 263 and 304 was confirmed.

From recent work on the mRNA sequence required for selenocysteine insertion into formate dehydrogenase of *E. coli*, it is postulated that a considerable 3′ stem-loop is necessary for directing the selenocysteyl-tRNA^{Sec}, and the alternate elongation factor SELB, to insert selenocysteine at the specific UGA codon [37•]. Recently, the insertion of selenocysteine at a single UGA codon in mammalian Type 1 iodothyronine 5′-deiodinase has been shown to require a 3′-untranslated region (UTR) [38••]. Intriguingly, the 3′-UTR from rat glutathione peroxidase can restore activity when it replaces the natural 3′-UTR of rat iodothyronine 5′-deiodinase. How a 3′-UTR can govern readthrough of the UGA codon and whether a unifying principle exists between selenocysteine insertion at single or multiple UGA codons remains to be seen.

Conclusions

The past year has seen an impressive increase in the number of examples of alternative paths which ribosomes may follow during the elongation and termination phases of protein synthesis. This is probably a reflection of both the expanding database of known ORF sequences and the increasing sophistication of the physical and genetic analyses used to investigate the control of gene expression. As this knowledge continues to expand, predictive methods for determining whether a sequence may or may not stimulate an alternative translation event should become more powerful. On another front, the variety of events that are being uncovered will provide a powerful new tool for examining in more detail the physical mechanism of protein synthesis and ribosome movement.

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