YEAST VOL. **11**: 1115–1127 (1995)

Ribosomal Frameshifting in Yeast Viruses

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Received 5 May 1995; accepted 8 May 1995

Proper maintenance of translational reading frame by ribosomes is essential for cell growth and viability. In the last 10 years it has been shown that a number of viruses induce ribosomes to shift reading frame in order to regulate the expression of gene products having enzymatic functions. Studies on ribosomal frameshifting in viruses of yeast have been particularly enlightening. The roles of viral mRNA sequences and secondary structures have been elucidated and a picture of how these interact with host chromosomal gene products is beginning to emerge. The efficiency of ribosomal frameshifting is important for viral particle assembly, and has identified ribosomal frameshifting as a potential target for antiviral agents. The availability of mutants of host chromosomal gene products involved in maintaining the efficiency of ribosomal frameshifting bodes well for the use of yeast in future studies of ribosomal frameshifting.

KEY WORDS — *Saccharomyces cerevisiae*; ribosomes; ribosomal frameshifting; L-A dsRNA virus; Ty; retrotransposon; retrovirus; hungry codons; polyamines

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INTRODUCTION

Maintenance of correct reading frame is fundamental to the integrity of the translation process and, ultimately, to cell growth and viability. However, a number of cases of directed frameshifting have been identified. Frameshifting events produce fusion proteins, in which the N- and C-terminal domains are encoded by two distinct, overlapping open reading frames (ORFs). These are, for the most part, seen in viruses, e.g. retroviruses, coronaviruses, the yeast L-A dsRNA virus, the dsRNA virus of *Giardia lamblia*, the Ty family of viruses in yeast, (+) ssRNA viruses of plants, bacteriophage T7, and a number of bacterial transposons, as well as in a few bacterial cellular genes and the ornithine decarboxylase antizyme gene in mammals (for reviews, see references 1, 13, 29, 30, 35). Ribosomal frameshifting is different from frameshift suppression in that these events are

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CCC 0749–503X/95/121115–13

directed by specific mRNA sequences and structures, rather than being a consequence of mutations in host gene products, e.g. tRNAs containing four base anticodon loops. The study of these ribosomal frameshifts is important both because of their critical role in animal and plant pathogens, and because of the information they provide about the mechanisms by which the reading frame is normally maintained.

Most of the cases of ribosomal frameshifting seen to date are found in viruses which use their (+) strands as (1) mRNAs encoding multiple protein products, (2) the species of RNA that is packaged into nascent viral particles and (3) the template for replication of the viral genetic material. Production of multiple protein products could be achieved by mRNA splicing or editing. These mechanisms could lead to the production of altered (+) strands, resulting in the production of mutant viral genomes, unless splicing or editing removed an RNA site required for packaging or replication of the genomic RNA. Perhaps for this reason, (+) ssRNA and dsRNA viruses are not known to use splicing or mRNA editing and retroviruses remove the packaging site (Ψ) when they splice their RNA.[42,56] All of these classes of viruses use ribosomal frameshifting and/or readthrough of termination codons to make fusion proteins. Neither of these mechanisms alters the template and so neither packages mutant viral genomes.[33]

Ribosomal frameshifting in the − 1, or 5′ direction in retroviruses, (+) ssRNA viruses and dsRNA viruses results in the production of Gag–pol fusion proteins. It requires a special sequence, X XXY YYZ (the 0-frame is indicated by spaces) called the ‘slippery site’.[34] The simultaneous slippage of ribosome-bound A- and P-site tRNAs by one base in the 5′ direction still leaves their non-wobble bases correctly paired in the new reading frame. A second promoting element,[34] usually an mRNA pseudoknot, is located immediately 3′ to the slippery site.[7,18,53] It is thought that the role of the mRNA pseudoknot is to induce elongating ribosomes to pause over the slippery site. Both of these elements have been found to be required for the promotion of efficient − 1 ribosomal frameshifting in the L-A virus of yeast.

In eukaryotes, frameshifting in the +1, or 3′ direction has been observed in the Ty retrotransposable elements in yeast (for review, see reference 25), and in the ornithine decarboxylase antizyme gene in mammalian cells.[43,49] In Ty1 and Ty3, +1 ribosomal frameshifting between the *TYA* and *TYB* genes in Ty1 and the *GAG3* and *POL3* genes in Ty3 also results in the production of Gag–Pol fusion proteins. Although heptameric sequences and the induction of a ribosomal pause are required to promote efficient frameshifting, the actual mechanisms involved are very different from those used in − 1 ribosomal frameshifting.

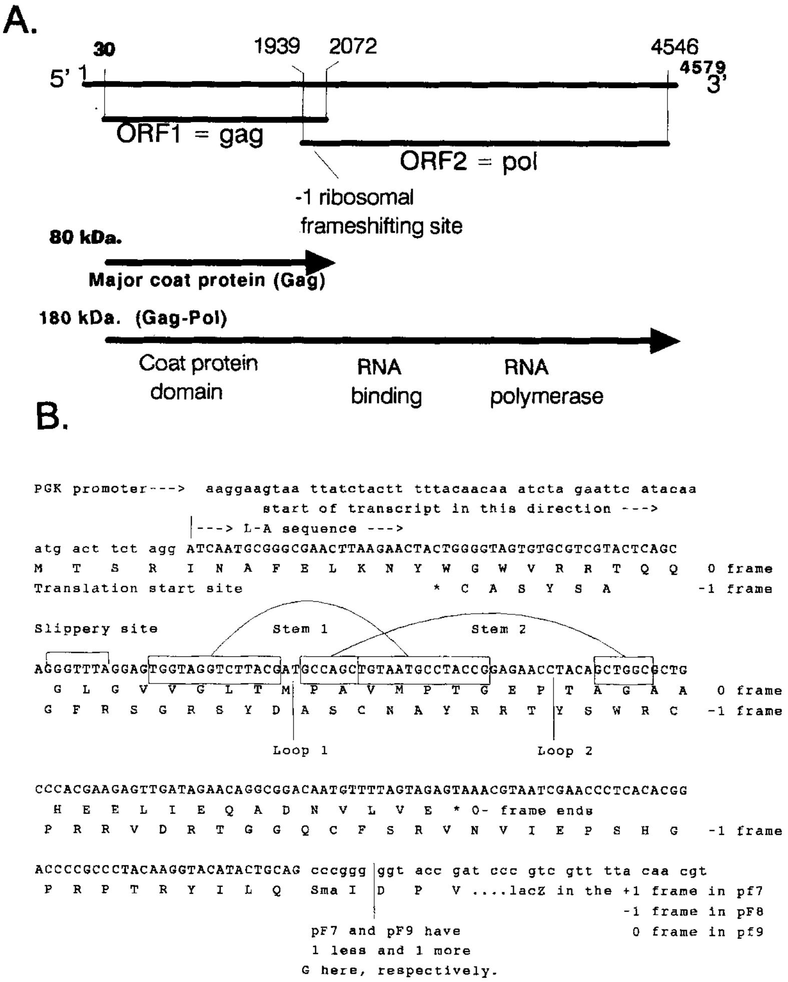
Here, I will present a review of ribosomal frameshifting research in yeast. The elements involved in promoting − 1 and +1 ribosomal frameshifting will be discussed. The importance of the efficiency of ribosomal frameshifting for the production of the correct ratios of viral proteins, and the consequences for viral propagation when these ratios are perturbed, will be considered. Research in yeast focused on the generation and genetic characterization of yeast strains in which mutations in unique chromosomal genes result in cells having ribosomal frameshifting efficiencies significantly greater than normal cells will be examined, and a prospectus considering the implications of these classes of mutants upon the fields of translational control and virology will be presented.

− 1 RIBOSOMAL FRAMESHIFTING: THE L-A dsRNA VIRUS OF YEAST

The 4.6 kb dsRNA L-A virus of *Saccharomyces cerevisiae* has two ORFs (Figure 1). The 5′ *gag* gene encodes the major coat protein and the 3′ *pol* gene encodes a multifunctional protein domain which includes the RNA-dependent RNA polymerase and a domain required for viral RNA packaging.[27,32,33,48] A − 1 ribosomal frameshift event is responsible for the production of the Gag–Pol fusion protein.[18,26,33] M_1, a satellite dsRNA virus of L-A which encodes a secreted killer toxin (reviewed in reference 12), is encapsidated and is replicated in L-A encoded particles.

Elements involved in − 1 ribosomal frameshifting: the slippery site

According to the ‘simultaneous slippage’ model[34] (Figure 2), − 1 ribosomal frameshifting occurs at a heptameric ‘slippery site’, X XXY YYZ (0-frame indicated by spaces). This can occur when ribosome bound A- and P-site tRNAs unpair from the 0-frame on the mRNA, and then re-pair their non-wobble bases in the − 1 frame of the mRNA. Oligonucleotide site-directed *in vitro* mutagenesis



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PGK promoter--->  aaggaagtaa ttatctactt tttacaacaa atcta gaattc atacaa
                       start of transcript in this direction --->
                 |---> L-A sequence --->
atg act tct agg ATCAATGCGGGCGAACTTAAGAACTACTGGGGTAGTGTGCGTCGTACTCAGC
M   T   S   R   I  N  A  F  E  L  K  N  Y  W  G  W  V  R  R  T  Q  Q   0 frame
Translation start site                   *  C  A  S  Y  S  A        -1 frame

Slippery site            Stem 1                Stem 2

AGGGTTTAGGAGTGGTAGGTCTTACGATGCCAGCTGTAATGCCTACCGGAGAACCTACAGCTGGCGCTG
  G  L  G  V  V  G  L  T  M| P  A  V  M  P  T  G  E  P| T  A  G  A  A   0 frame
 G  F  R  S  G  R  S  Y  D |A  S  C  N  A  Y  R  R  T |Y  S  W  R  C   -1 frame

                        Loop 1                    Loop 2

CCCACGAAGAGTTGATAGAACAGGCGGACAATGTTTTAGTAGAGTAAACGTAATCGAACCCTCACACGG
  H  E  E  L  I  E  Q  A  D  N  V  L  V  E  * 0- frame ends
 P  R  R  V  D  R  T  G  G  Q  C  F  S  R  V  N  V  I  E  P  S  H  G   -1 frame

ACCCCGCCCTACAAGGTACATACTGCAG cccggg |ggt acc gat ccc gtc gtt tta caa cgt
 P  R  P  T  R  Y  I  L  Q   Sma I  |D   P   V ....lacZ in the +1 frame in pf7
                                    |                     -1 frame in pF8
                             pF7 and pF9 have              0 frame in pf9
                             1 less and 1 more
                            G here, respectively.
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Figure 1. (A) Gene organization of the L-A (+) strand (from reference 33). ORF1 encodes the major coat protein (Gag). ORF2 overlaps ORF1 by 130 nucleotides and is expressed as a fusion protein, the 180-kDa minor coat protein (Gag-Pol). (B) Partial sequence of the vectors used to assay for − 1 ribosomal frameshifting. L-A sequences start at base 1905 and end at 2122 of the L-A sequence. The L-A sequence is in upper-case letters and the vector sequences are in lower-case letters. The 'slippery site' (GGGTTTA), and stems 1 and 2 of the mRNA pseudoknot are indicated.

has been extensively used to dissect the slippery site in L-A, retroviruses and (+) ssRNA viruses. Experiments altering the sequence of the first triplet (X XX), which corresponds to tRNAs slipping between the 0- and − 1 frames at the ribosomal P-site, show that disruption of the identity of the three bases significantly reduces the ability of ribosomes to shift reading frame. Substitution of any three identical nucleotides in this position is sufficient to direct efficient − 1 ribosomal frameshifting. Different combinations yield different efficiencies of frameshifting, with pyrimidines promoting the most efficient frameshifting, followed by purines, such that UUU>CCC>AAA>GGG.[18] In the second triplet (corresponding to the ribosomal A-site), only triplets of A and U promote efficient levels of − 1 ribosomal frameshifting. The seventh base can be A, U or C, but not G.[18,19]

The simultaneous slippage model stresses the ability of the non-wobble bases of ribosome-bound tRNAs to be able to re-pair to the − 1 frame. However, ribosome-bound tRNAs must first unpair from the 0-frame before they can re-pair to

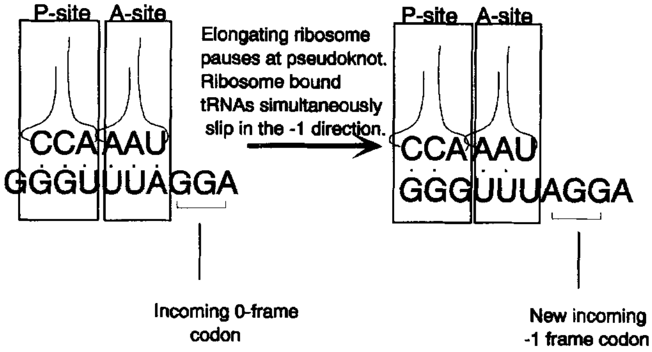

Figure 2. The simultaneous slippage model[34] as applied to the L-A slippery site. Peptidyl-tRNA and aminoacyl tRNA occupy the ribosomal P- and A-sites respectively as the ribosome pauses at the mRNA pseudoknot. Both tRNAs can simultaneously slip one nucleotide in the 3′ (− 1) direction, in such a way that their non-wobble bases can repair to the codons in the − 1 reading frame. After peptidyl transfer and translocation, incoming tRNA recognizes the AGG codon in the new (− 1) reading frame.

the − 1 frame. The A and U restriction in the second triplet suggests that tRNA–mRNA pairing is stronger at the ribosomal A-site than at the P-site because the A·U base pair contains one less hydrogen bond than the G·C base pair.[18] This was tested by examining the efficiencies of − 1 ribosomal frameshifting using constructs having A-site triplets with fewer 0-frame hydrogen bonds (i.e. A·U rich and better able to un-pair), but capable of forming fewer hydrogen bonds in the − 1 frame (i.e. less able to re-pair), as opposed to those having more 0-frame hydrogen bonds (i.e. G·C rich, less capable of un-pairing), but having a greater potential of re-pairing to the − 1 frame. The A·U rich 0-frame triplets were more capable of promoting efficient − 1 ribosomal frameshifting than those having more 0-frame hydrogen bonds (i.e. G·C rich), even when they were less well suited to base pairing in the − 1 frame.[19] These data reinforce the notion that tRNA–mRNA pairing is stronger at the ribosomal A-site than at the P-site, although it is also possible that there are specific tRNAs that are particularly good at frameshifting in the context of the slippery site.

The mRNA pseudoknot

A second element, an mRNA pseudoknot, is required to promote efficient − 1 ribosomal frameshifting.[6,7,18] An mRNA pseudoknot is a stem-loop whose loop can base pair to a sequence 3′ to the stem. These elements are commonly referred to as stem 1 (S1) – loop 1 (L1) – stem 2 (S2) – loop 2 (L2) (Figure 3B). The mRNA pseudoknot of L-A is particularly interesting in that it can potentially assume a variety of conformers, ranging from a long stem 1/short stem 2 to a short stem 1/long stem 2. Oligonucleotide site-directed mutagenesis was used to determine the biologically active mRNA pseudoknot conformation.[19] In all cases that allowed for maximization of base pairing in stem 1, frameshifting was at or near wild-type levels, whereas changes which maximized stem 2 showed decreased frameshifting efficiencies comparable to mutants in which base pairing was entirely disrupted. Changes which strengthened stem 1 tended to increase the ability of the pseudoknot to promote efficient frameshifting, a finding which was also noted in coronaviruses.[7]

The mRNA pseudoknot induces elongating ribosomes to pause over the slippery site,[51,54] and this is thought to increase the probability of 5′ ribosomal movement. Remarkably, energetically equivalent stem-loop structures will not substitute.[7,51] The spacing between slippery sites and pseudoknots is also critical.[7,8,19,45] For efficient frameshifting, the ribosome must pause with the slippery site precisely positioned in its A and P sites.

How are mRNA pseudoknots particularly able to promote efficient − 1 ribosomal frameshifting? One idea invokes a ‘pseudoknot recognizing factor’. However, evidence for the existence of such a factor has not been forthcoming, either by

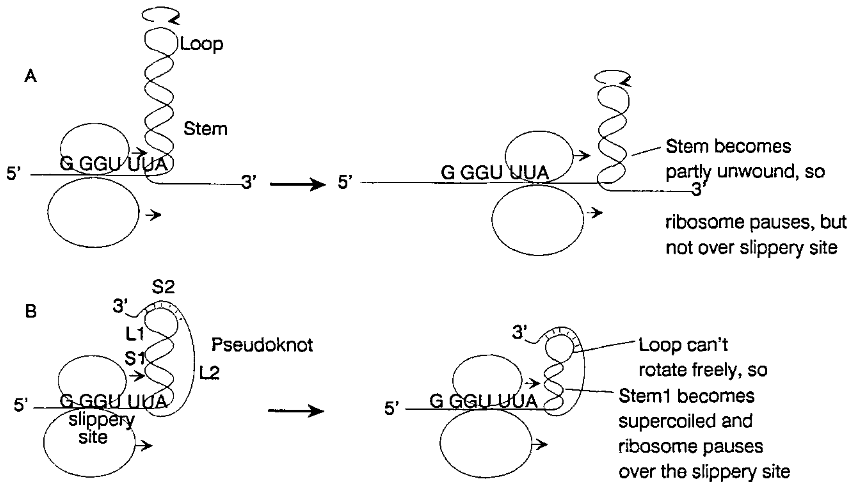

Figure 3. The mRNA pseudoknot imposes torsional resistance on ribosome movement. (A) The ribosome can relatively easily unwind a simple stem-loop because there is no restriction on the rotation of the loop, even with a long stem. There is no unique pause site. (B) The ribosome meets added resistance to unwinding stem 1 of an mRNA pseudoknot, because loop 1 cannot easily rotate. If the mRNA pseudoknot is properly placed, the ribosome pauses over the slippery site and frameshifting occurs more often. S1, Stem 1; S2, Stem 2; L1, Loop 1; L2, Loop 2.

competition assays in *in vitro* translation systems (ten Dem, cited in reference 24) or by gel retardation assays (J. Dinman, unpublished data). Another suggestion is that, since the 5′ and 3′ ends of the pseudoknot are not contiguous, a ribosome-associated helicase has greater difficulty unwinding a pseudoknot than a simple stem loop,[52] or perhaps some unique structural feature is what makes mRNA pseudoknots less resistant to unwinding.

Here we propose a 'Torsional Resistance Model' for how RNA pseudoknots promote efficient −1 ribosomal frameshifting (Figure 3). Addition or deletion of only three nucleotides in the spacer between the slippery site and stem 1 prevents efficient frameshifting.[7,8,19,45] Thus, the five to eight nucleotide spacing between slippery sites and pseudoknots is critical. If elongating ribosomes were able, before pausing, to unwind just one extra codon, i.e. one third of a helical turn of stem 1, the ribosomal A- and P-sites would not be correctly positioned over the slippery site, and −1 ribosomal frameshifting would not proceed efficiently. A simple stem-loop structure, no matter how long, does not force the ribosomes to stop at one special point, and so cannot efficiently promote −1 ribosomal frameshifting (Figure 3A). How then do RNA pseudoknots make elongating ribosomes pause in the right place?

As the ribosome unwinds a stem loop it forces loop 1 to rotate. If this loop is anchored or restrained, as in a pseudoknot by stem 2, then stem 1 cannot be unwound. The ribosome is thus forced to pause at a special point in stem 1 (Figure 3B). A simple stem-loop is not restrained, and can rotate freely; only the base pairs at the bottom of the stem resist ribosome movement. The pseudoknot has both these base pairs and those of stem 2 resisting ribosome motion. Thus, as the ribosome tries to unwind stem 1, stem 2 forces the supercoiling of stem 1, providing extra resistance to ribosome movement.

Several predictions of this model are borne out by the experimental data:

- Disrupting the first three base pairs of stem 1 would allow the ribosome to elongate beyond the slippery site, eliminating frameshifting.[45]
- Destabilizing stem 2 would allow it to be unwound more readily, decreasing the efficiency of frameshifting.[6,54]
- Replacing bulges in stem 1 with base pairs would increase the energy required to unwind its first three base pairs. A longer ribosomal pause over the slippery site would follow, yielding increased efficiencies of −1 ribosomal frameshifting.[6,19]

A weak point of this model is that, in some mRNA pseudoknots, stem 1 is only five or six base pairs in

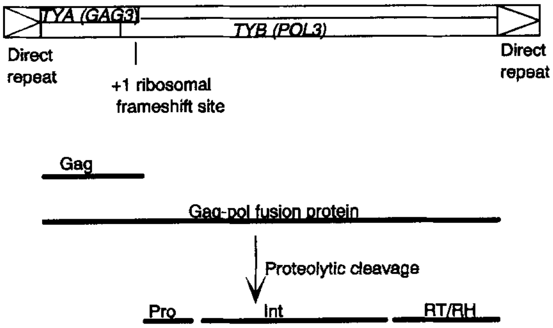

Figure 4. The general structure of Ty*1*–Ty*4*. Each Ty element contains two open reading frames flanked by direct repeats (triangles). The *TYA* (*GAG3* in Ty*3*) open reading frame encodes the major structural gag analogue proteins. The TYB (*POL3* in Ty*3*) open reading frame encodes the Gag-pol fusion protein, which is subsequently processed into proteins having enzymatic functions. Pro, protease; Int, integrase; RT/RH, reverse transcriptase/RNase H.

length, i.e. less than a full helical turn. In these mRNA pseudoknots, unwinding of just one extra codon, i.e. one third of a helical turn of stem 1, would be sufficient to completely open up stem 1, rendering useless the contribution of stem 2. At this juncture, no definitive experiments have been designed to test these theories. Perhaps the identification and characterization of yeast chromosomal mutants capable of either efficient −1 ribosomal frameshifting in response to a simple stem loop, or conversely, incapable of efficient −1 ribosomal frameshifting through an RNA pseudoknot, will provide the necessary tools to definitively address the question of how RNA pseudoknots promote efficient −1 ribosomal frameshifting.

+1 RIBOSOMAL FRAMESHIFTING: Ty*1* AND Ty*3*

The Ty retrotransposable elements of *S. cerevisiae* are all approximately 5 kb in length and are flanked by direct repeats (Figure 4; for reviews, see references 7, 50). They have the same general genomic organization as do viruses that use −1 ribosomal frameshifting, i.e. a 5′ *gag* ORF, followed by a *pol* ORF. The 5′ ORF in Ty*1*, Ty*2* and Ty*4* is called *TYA* and in Ty*3* it is called *GAG3*. The 3′ ORFs are called *TYB* and *POL3*, respectively. *TYB* and *POL3* are expressed as protein fusions to the product of the upstream genes in these elements.[15,38,44] In the Ty elements, however, *pol* is in the +1 reading frame relative to *gag*, and +1 ribosomal frameshifting is used to form Gag–pol fusion proteins.[4,23] Ty*5* is different, in that it has a single long ORF (D. Votyas, cited in reference 25).

+1 Ribosomal frameshifting in Ty*1* is directed by a heptanucleotide sequence CUU AGG C (0-frame indicated by spaces).[4] In Ty*3*, it is promoted by the heptameric sequence GCG AGU U.[23] A ribosomal pause is required in each case, and sequence downstream helps to promote efficient +1 ribosomal frameshifting. At this point, however, the similarities between +1 and −1 ribosomal frameshifting end.

Although both +1 and −1 ribosomal frameshifting occur at heptameric ‘slippery sites’, the nature of these sites are completely different. Unlike −1 ribosomal frameshifting, the simultaneous slippage of ribosome-bound A- and P-site tRNAs from the 0-frame to the +1 frame would not allow their non-wobble bases to repair. Also, in −1 ribosomal frameshifting, the downstream sequence that is required to promote efficient frameshifting is the mRNA pseudoknot. Although a potential pseudoknot structure can be inferred in Ty*1*, the structure is not required, and no such structure can be inferred from the Ty*3* sequence.[25] The purpose of the downstream sequences in the Ty elements is not understood, but they do not involve pseudoknots.

In −1 ribosomal frameshifting, the RNA pseudoknot promotes a ribosomal pause. In +1 ribosomal frameshifting in the Ty elements, the ribosomal pause is promoted by ‘hungry codons’, in the 0-frame A-site (i.e. nucleotides 4–6 of the slippery site). Hungry codons correspond to tRNAs that are not abundant in the cell. Elongating ribosomes pause at the slippery site with their P-sites occupied by peptidyl-tRNAs, awaiting the arrival of the cognate tRNA that should base pair to the hungry codon at the ribosomal A-site. It is during this pause that the shift in reading frame occurs.

Figure 5 shows how +1 ribosomal frameshifting in Ty*1*, Ty*2*, Ty*3* and Ty*4* is thought to occur. In the Ty*1*, Ty*2* and Ty*4* elements, the slippery Leu tRNA_{UAG} occupying the P-site of the +1 ribosomal frameshift signal recognizes its cognate codon CUU by two out of three decoding.[55] The normal decoding of the in-frame A-site AGG is slow because of the low availability of the cognate Arg tRNA_{CUU}, causing a translational pause, during which +1 ribosomal frameshifting occurs. Two

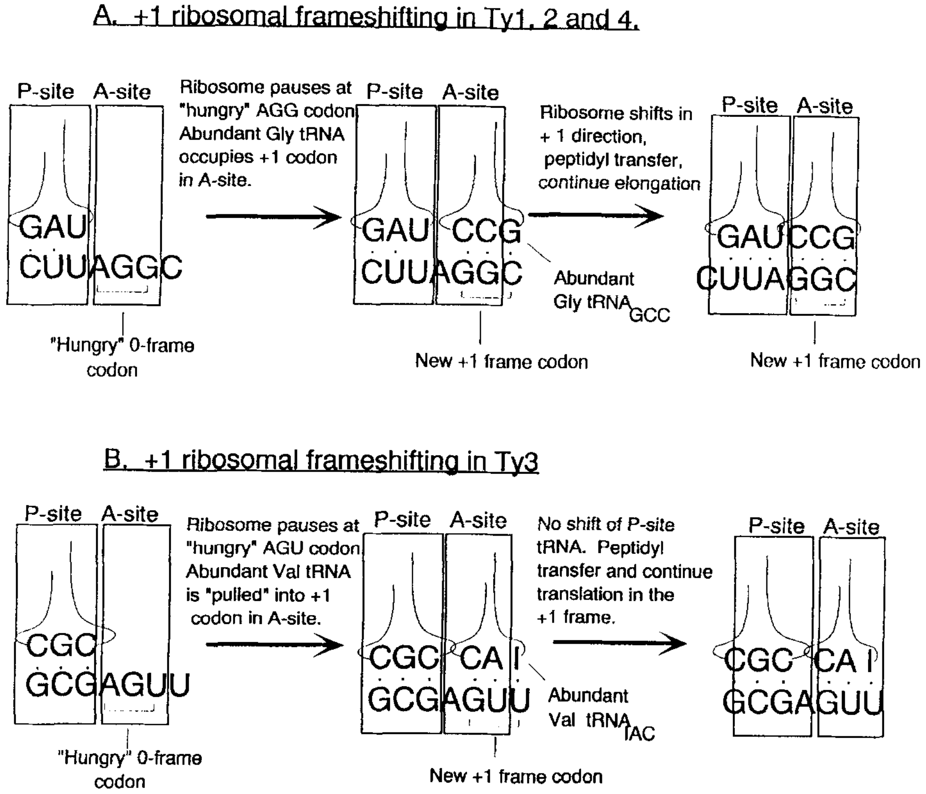

Figure 5. +1 Ribosomal frameshifting in Ty1, 2 and 4, and in Ty3: two distinct mechanisms. In Ty1, Ty2 and Ty4, the slippery Leu tRNA$_{UAG}$ recognizes its cognate codon CUU by two out of three decoding. The ribosome pauses at the AGG 'hungry codon' due to the low availability of its cognate Arg tRNA$_{CCU}$. During this translational pause, Gly tRNA$_{GCC}$ likely binds transiently to the +1 frame codon GGC, followed by slippage of the Leu tRNA$_{UAG}$ to the UUA codon. In Ty3, after recognition of the GCG codon by Ala tRNA$_{CGC}$, AGU serves as the 'hungry' codon, corresponding to the low abundance Ser tRNA$_{GCU}$. This allows for the recognition of the +1 frame codon GUU by Val tRNA$_{IAC}$. The body of the peptidyl-Ala tRNA$_{CGC}$ (shown as 'bent to the right') allows the out-of-frame tRNA to be accepted by the ribosome, allowing peptide transfer to occur, shifting the ribosome into the +1 reading frame.

slightly different models have been proposed. In the first, the P-site Leu tRNA$_{UAG}$ first slips in the +1 direction, followed by binding of Gly tRNA$_{GCC}$ to the +1 frame A-site.[4] In the second mechanism, the Gly tRNA$_{GCC}$ transiently binds to the +1 frame codon GGC in the ribosomal A-site, followed by the slippage of Leu tRNA$_{UAG}$ in the +1 direction to the UUA codon.[46] The second model is currently favored because, since the amount of the Gly tRNA$_{GCC}$ is important,[46] the +1 tRNA must bind before slippage, assuming that the elongating ribosome is not in equilibrium. Further, this model would unify the +1 ribosomal frameshifting mechanisms used by Ty*1*, Ty*2* and Ty*4* with that of Ty*3* (see below). Thus, this is the model depicted in Figure 5A.

In Ty*3* (Figure 5B), Ala tRNA$_{CGC}$ is bound to the GCG codon in the 0-frame P-site and the 0-frame A-site AGU codon, corresponding to the rare Ser tRNA$_{GCU}$, provides the pause. However, in this case, the Ala tRNA$_{CGC}$ cannot slip onto the +1 CGA codon but, rather, it is thought to force a Val tRNA$_{IAC}$ into the +1 GUU codon at the ribosomal A-site. Saturation mutagenesis of the frameshift site of Ty*3* has demonstrated that there is no correlation between the ability of a peptidyl-tRNA to slip and its ability to promote efficient +1 ribosomal frameshifting.[55] Eight different tRNAs were shown to be capable of promoting frameshifting, four of which cannot slip. Some other aspects of these tRNAs must allow them to promote frameshifting by directing out-of-frame binding of the incoming aminoacyl-tRNA. Substitution of tRNA bodies with different anticodons showed that it is the body of the P-site tRNA that promotes forcing of the incoming A-site tRNA into the +1 frame.[46] These data imply that interactions between the P-site tRNA and the incoming ternary complex mispositions the aminoacyl tRNA onto the +1 codon. Thus, Ty*3* frameshifting occurs without tRNA slippage: a special tRNA in the P-site is able to promote A-site tRNA binding to the +1 codon, provided that there is a translational pause provided by a hungry codon.[25,55]

THE IMPORTANCE OF THE EFFICIENCY OF FRAMESHIFTING FOR VIRAL PROPAGATION

We have determined that the efficiency of − 1 ribosomal frameshifting in the naturally occurring L-A slippery site is 1.8–2.0%.[18,19] The 39 nm L-A viral particle contains 120 Gag proteins,[11,22] and the 1.9% efficiency of frameshifting can be interpreted as providing 1 Gag-pol molecule for every 59 Gag proteins made, i.e. each viral particle contains two Gag-pol molecules. Genetic and geometric considerations from the frameshifting data led us to hypothesize that Gag-pol functions as a dimer in the viral particle.[19] Reconstructions of the L-A virus particle from cryoelectron microscopic observations show that Gag is also a dimer.[14]

Changing the efficiency of − 1 ribosomal frameshifting would change the ratio of Gag to Gag-pol. This might in turn affect viral particle assembly, and therefore the ability of the cell to propagate the virus. Changing the slippery site sequence affects the efficiency of − 1 ribosomal frameshifting.[18,19] The efficiency of − 1 ribosomal frameshifting can also be affected by mutations in the cellular gene products that presumably interact with these tRNA and mRNA factors.[19,20] Using both molecular and genetic methods, we demonstrated that the 1.9% efficiency of ribosomal frameshifting yields the optimum ratio of structural Gag to enzymatic Gag-pol proteins. Changing frameshifting efficiencies more than two- to three-fold greater (or 70% less) than wild-type levels results in the loss of the M_1 satellite virus, whether the virus is supported by L-A cDNA clones containing altered slippery sites,[19] or by the wild-type L-A virus in host cells containing chromosomal mutations which result in cells having higher efficiencies of − 1 ribosomal frameshifting in response to the L-A frameshift signal (see below).[19,20] Even slight changes in − 1 ribosomal frameshifting efficiencies significantly lower M_1 copy numbers. A +1 frameshifting signal derived from Ty*1* can substitute for a − 1 signal in maintaining M_1 as long as frameshifting efficiencies fall within this acceptable 'frameshift window'.[19]

Analogously, the importance of the efficiency of +1 ribosomal frameshifting in determining the relative ratios of Gag to Gag-pol have been tested in Ty*1*. Ty elements transpose through an RNA intermediate using the same replication and integration strategy employed by the metazoan viruses.[6,28,44] Thus, in order to transpose they must go through a viral intermediate. Increasing the abundance of the Arg $tRNA_{CUU}$ critical for inducing the translational pause by providing it *in trans* on a high copy vector nearly abolishes +1 ribosomal frameshifting,[37] and dramatically reduces Ty*1* transposition frequencies. Similarly, deleting the single-copy gene for this tRNA gene, called *HSX1*[36] promotes extremely high levels of frameshifting and also results in loss of Ty*1* transposition.[60] We have found that starvation for the polyamine spermidine and the consequent elevation of intracellular concentrations of putrecine also increases the efficiency of +1 frameshifting in Ty*1*.[2,3] Loss of the ability of a *HIS3*-marked Ty*1* cDNA clone to transpose into the yeast genome paralleled the increase in +1 ribosomal frameshifting in polyamine-starved *spe2* cells.[3] Taken together, these findings support the hypothesis that the efficiency of ribosomal frameshifting is critical for viral propagation, and that agents which affect ribosomal frameshifting efficiency may have antiviral activities.[2,3,19,20]

CHROMOSOMAL MUTATIONS WHICH AFFECT THE EFFICIENCY OF − 1 RIBOSOMAL FRAMESHIFTING

Recently a number of host chromosomal mutants which affect the efficiency of − 1 ribosomal frameshifting have been described.[19–21,39] The mutants isolated in our laboratory are called *mof* (*M*aintenance *Of F*rame). To date, nine such *mof* mutants have been characterized. These mutations show differential effects on various frameshifting signals and have numerous secondary phenotypes (Table 1). These mutants appear to be affecting the elongation phase of protein synthesis.

Table 1. Summary of properties of *mof* mutants

Cells	Frameshifting (fold WT)	M1 dsRNA	ts	Arrest phenotype	pet	Upf phenotype
WT	1	+				
mof1-1	2·7	−				
mof2-1	8·9	−	ts	Dumbbell	pet	Weak Upf$^-$
mof3-1	2·8	+				
mof4-1	4·4	−				Strong Upf$^-$
mof5-1	3·5	−	ts	Mulit-bud.	pet	Weak Upf$^-$
mof6-1	3·3	−	ts	Large, unbudded		
mof7-1	2·9	+				
mof8-1	3·3	+				Weak Upf$^-$
mof9-1	2·5	+				

The fold increase in frameshifting efficiency is from reference 20. M_1 dsRNA denotes the ability of the mutant cells to maintain the M_1 satellite virus of L-A. ts, temperature sensitive. pet, ability to grow on glycerol, a non-fermentable carbon source. Upf phenotype denotes the amount of endogenous un-spliced CYH2 precursor mRNA in these cells.

The mof4-1 mutation is an allele of the UPF1 gene

In normal cells, nonsense mRNAs, e.g. unspliced mRNAs which escape into the cytoplasm, or mis-transcribed mRNAs which contain premature termination signals, are rapidly degraded. A class of genes, called *UPF* (*UP F*rameshift) and *NMD* (*N*onsense *M*ediated *D*ecay), are involved in the degradation of nonsense mRNAs. The half-lives of nonsense mRNAs are increased in this class of mutants, and they also have frameshift suppressor phenotypes (reviewed in reference 47). The constructs that we originally used for the detection of *mof* mutants have the *lacZ* gene downstream of the L-A −1 ribosomal frameshift signal in the −1 reading frame relative to a translational start site (see Figure 1B). To elongating ribosomes, this would present a long mRNA containing a short 5′ ORF that is quickly interrupted by a termination codon, i.e. a nonsense mRNA. The assay that was designed to detect *mof* mutants relied upon finding cells expressing increased amounts of β-galactosidase (β-gal) as a result of an increase in the efficiency of −1 ribosomal frameshifting. However, the same result could also be observed if the cells were *upf* or *nmd* mutants, because a longer half-life of the 'nonsense' reporter mRNA would result in the greater accumulation of the frameshifted β-gal gene product. Thus, mutants in the nonsense-mediated mRNA decay pathway could be mistaken for *mof* mutants on the basis of their higher β-gal activities.

We have identified *mof4-1* as an allele of the *UPF1* gene. The *UPF1* protein contains a putative zinc finger domain and a predicted helicase domain.[40] The original *upf1-2* mutation has a tryptophan-to-termination nonsense mutation at amino acid residue 205. We have sequenced the *mof4-1* mutation and determined that it consists of a cystine (Cys)-to-tyrosine missense mutation at amino acid 62, the first Cys residue in the putative zinc finger.[17]

Although the half-lives of the −1 ribosomal frameshifting reporter mRNAs are increased in *upf* and *nmd* mutants, the ribosomes translating them would continue to frameshift with the same efficiency. Thus, although *upf*/*nmd* mutants should be indistinguishable from *mof* mutants by the β-gal assay, *upf*/*nmd* mutants should be able to maintain the M_1 virus because the ratio of Gag to Gag-pol would remain unaffected. True *mof* mutants, by virtue of their effect upon −1 ribosomal frameshifting efficiency, should not be capable of propagating M_1, i.e. they should have Mak$^-$ phenotypes (*MAK* = *MA*intenance of *K*iller; for review, see reference 58). *mof-1-1, mof2-1, mof4-1, mof5-1* and *mof6-1* have the Mak phenotype and are thus true *mof* mutants. *mof4-1* has a strong nonsense mRNA decay mutant phenotype, and *mof2-1*, *mof5-1* and *mof8-1* also have weak Upf$^-$ nonsense mRNA decay mutant phenotypes (Table 1). *upf1-2*, *UPF1::URA3*, *upf2-1*, *UPF2::URA3*, *upf3-1* and *upf4-1* are all capable of maintaining M_1, indicating that *mof4-1* is a unique *mof* allele of

UPF1.[17] Recent studies examining *UPF1* show that mutations in the zinc finger domain affect frameshift suppression, whereas mutations in the helicase domain affect the nonsense-mediated mRNA decay phenotype.[57] Our results with *mof4-1* complement these findings, in that the *mof4-1* mutation, which is in the zinc finger domain, has a specific translational defect that results in an increased efficiency of −1 ribosomal frameshifting.

Recently, Lee *et al.*[39] have identified two *I*ncreased *F*rame*S*hift (*ifs*) mutants in yeast. Using a construct containing the yeast *CUP1* gene downstream of a −1 ribosomal frameshift signal from the mouse mammary tumor virus *Gag-Pol* junction, *ifs* mutants were identified by loss of copper sensitivity in *cup*Δ cells. Both of these had −1 ribosomal frameshifting efficiencies approximately two-fold greater than wild-type cells, as measured by β-gal activities. They cloned and sequenced one of these, *ifs1*. Comparison of *IFS1* to *UPF2 (NMD2)* sequence[16,31] shows that they are identical. We have also determined that *ifs2* and *mof4-1* fall into the same complementation group and that the Ifs⁻ phenotype of *ifs2* can be corrected with a clone of *UPF1*. Both *ifs1* and *ifs2* mutants are able to propagate M_1. This could be due to the fact that a two-fold increase in −1 ribosomal frameshifting efficiency is not large enough to affect the propagation of M_1 in these cells. Alternatively, there could be no change in the efficiency of −1 ribosomal frameshifting in these mutants *per se*, but rather the ability of these mutants to grow in the presence of copper might be due to the increased half-lives of the nonsense reporter mRNAs.

These data demonstrate that the *mof4-1* is a unique maintenance of frame allele of *UPF1*. It has both the Mak$^-$ and Upf$^-$ phenotypes, and promotes a greater efficiency of −1 ribosomal frameshifting in response to a specific viral signal. As such, it represents the first time that a single protein has been linked to both the processes of translation and mRNA decay. This demonstrates that there is a connection between the phenomena defined by the *mof* and *upf*/*nmd* mutants, illuminating the continuity of the translational process, from mRNA stability through the translation of the complete protein product.

mof9: 5S rRNA is involved in fidelity of translational reading frame

Yeast ribosomes are composed of at least 77 ribosomal proteins and four ribosomal RNAs (rRNAs; for a review, see reference 59). Slightly more than half of the cloned ribosomal protein genes are represented by two isoforms in yeast, whereas there are over 100 copies of the rRNA genes in the genome. One would expect that the *mof* mutants would be associated with ribosomal protein genes rather than rRNAs, because a mutation in only one copy of an rRNA gene would be expected to be masked by the presence of over 100 copies of the wild-type gene. Surprisingly, *mof9-1*, which increases the efficiency of ribosomal frameshifting 2.5- to 3-fold, is complemented by a clone of 5S rRNA on either a single or high copy vector.[21] The *mof9-1* mutation maps to the yeast rDNA locus, and two other independent mutations of 5S rRNA at that locus also have the Mof9$^-$ phenotype and can be complemented by wild-type 5S rRNA. Mutant 5S rRNAs expressed from episomal vectors as 20–50% of total cellular 5S rRNA also have the Mof9$^-$ phenotype. The *mof9* mutants also increase the efficiency of +1 ribosomal frameshifting directed by a Ty*1* frameshift signal, but have no effect upon readthrough of UAG or UUA termination codons, indicating that not all translational specificity is affected. There is no detectable increase in the amount of steady-state *lacZ* mRNA transcribed from the −1 ribosomal frameshift test plasmid. Therefore the increased amount of β-gal activity is the direct result of an increase in the efficiency of ribosomal frameshifting, and is not due to a defect in the nonsense-mediated mRNA degradation pathway. Prior to these studies, no specific role had been assigned to 5S rRNA, aside from a vague 'scaffold' function. The *mof9* data suggest a role for 5S rRNA in maintaining reading frame in translation.

CHROMOSOMAL MUTATIONS AFFECTING +1 RIBOSOMAL FRAMESHIFTING

As noted above, +1 ribosomal frameshifting in Ty*1* depends upon the low abundance Arg $tRNA_{AGG}$, encoded by the single-copy *HSX1* gene. Deletion of this gene results in extremely high levels of +1 ribosomal frameshifting as directed by a Ty*1* +1 frameshifting signal, and the loss of the ability of Ty*1* cDNA clones to transpose. Conversely, overexpression of this tRNA gene on high copy plasmids decreases +1 ribosomal frameshifting efficiency, and results in the loss of the ability of Ty*1* cDNA clones to transpose.

A second class of mutants which affect the efficiency of +1 ribosomal frameshifting are involved in the biosynthesis of polyamines.[2,3] Upon starvation for spermidine, the efficiency of +1 ribosomal frameshifting directed by a Ty*1* frameshift signal increases dramatically in deletion mutants of *SPE2* (S-adenosyl decarboxylase). Paralleling the increase in +1 frameshifting efficiency is a decrease in transposition frequency of Ty*1*. Interestingly, deletion of *SPE1* (orinithine decarboxylase, which produces putrescine, a biosynthetic precursor of spermidine) can reverse the increase in +1 ribosomal frameshifting efficiency in *spe2*Δ cells depleted of spermidine. The high level of +1 ribosomal frameshifting efficiency in *spe2*Δ cells is the result of the combined effects of both spermidine deprivation and the large increase in the level of intracellular putrescine resulting from the derepression of the *SPE1* gene in spermidine-deficient strains. Since the overexpression of Arg tRNA_{AGG} suppressed the increase of +1 ribosomal frameshifting in spermidine-depleted *spe2*Δ cells, the results from these studies suggest that spermidine may be required for selection and/or insertion of cognate tRNA at the ribosomal A-site.

HOW NOT FRAMESHIFTING CAN BE INSTRUCTIVE: Ty5 AND Tf1

As noted above, Ty5 consists of a single ORF and ribosomal frameshifting does not appear to be involved. Likewise, all of the proteins in the retrotransposable element of the fission yeast *Schizosaccharomyces pombe*, Tf1, are derived from a single primary translation product.[41] How do these elements regulate the relative ratios of structural Gag proteins to those having enzymatic functions, i.e. integrase (Int), reverse transcriptase (RT), protease (Pro) and RNAse H? The ratio of structural (Gag) proteins to those having enzymatic functions in Tf1 particles is 30:1. Although there has been no demonstration that Tf1 particles require this 30:1 ratio to transpose, DNA blot results indicate that the bulk of mature cDNA is produced by particles having this ratio of structural to enzymatic proteins (H. Levin, personal communication). The implications for viral particle assembly and replication in these 'non-frameshifting' retrotransposable elements are very exciting.

SUMMARY

The studies on − 1 and +1 ribosomal frameshifting in the L-A and Ty viruses of yeast serve as an example of how such pairings can further our understanding of translational elongation, nonsense-mediated mRNA decay, the control of viral and cellular gene expression and the dynamics of viral capsid assembly and RNA packaging. The molecular and biochemical characterization of the host chromosomal mutants affecting the efficiency of ribosomal frameshifting will provide a unique set of tools for these investigations. That these mutants affect the ability of cells to propagate viruses which use ribosomal frameshifting suggests that the characterization of the *mof* mutations and Mof gene products may serve to identify targets for the rational design of antiviral agents. The yeast cellular host constitutes an ideal system for drug screening along these lines.

ACKNOWLEDGEMENTS

I would like to acknowledge Phil Farabaugh, Henry Levin, Jim Umen, Susanna Lee, Ying Cui and Stuart Peltz for sharing strains, manuscripts and information prior to publication. I also want to thank Reed Wickner for all that he has done to make my postdoctoral days rich, rewarding and fun.

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