Comparative mutational analysis of *cis*-acting RNA signals for translational frameshifting in HIV-1 and HTLV-2

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) and human T cell leukemia virus type II (HTLV-2) use a similar mechanism for −1 translational frameshifting to overcome the termination codon in viral RNA at the end of the *gag* gene. Previous studies have identified two important RNA signals for frameshifting, the slippery sequence and a downstream stem–loop structure. However, there have been somewhat conflicting reports concerning the individual contributions of these sequences. In this study we have performed a comprehensive mutational analysis of the *cis*-acting RNA sequences involved in HIV-1 *gag–pol* and HTLV-2 *gag–pro* frameshifting. Using an *in vitro* translation system we determined frameshifting efficiencies for shuffled HIV-1/HTLV-2 RNA elements in a background of HIV-1 or HTLV-2 sequences. We show that the ability of the slippery sequence and stem–loop to promote ribosomal frameshifting is influenced by the flanking upstream sequence and the nucleotides in the spacer element. A wide range of frameshift efficiency rates was observed for both viruses when shuffling single sequence elements. The results for HIV-1/HTLV-2 chimeric constructs represent strong evidence supporting the notion that the viral wild-type sequences are not designed for maximal frameshifting activity but are optimized to a level suited to efficient viral replication.

INTRODUCTION

Programmed ribosomal framshifting modulates the expression of two open reading frames (ORFs) in many retrovirus, plant virus, coronavirus and protozoan genes (reviewed in 1,2). In human immunodeficiency virus 1 (HIV-1) −1 translational frameshifting of its mRNA leads to synthesis of the Gag–Pol fusion protein which gives rise to the viral protease, reverse transcriptase and integrase (3). Without frameshifting only the precursor of structural proteins, Gag, is expressed. The ratio of Gag to Gag–Pol proteins is highly regulated and critical for viral propagation (4,5). Similarly, in human T cell leukemia virus type II (HTLV-2) two −1 ribosomal frameshift events result in

synthesis of the fusion proteins Gag–Pro and Gag–Pro–Pol (6,7). It has been demonstrated by *in vitro* and *in vivo* studies that in both systems two *cis*-acting sequence elements located within the overlapping region of the *gag* and *pol* genes are critical for translational frameshifting to occur (reviewed in 1,2). One is a slippery heptamer sequence (U UUU UUA in HIV-1, A AAA AAC in HTLV-2) at which the frameshift takes place and the other is a structural RNA motif downstream that in retroviruses assumes either a stem–loop or pseudoknot structure. The general slippery sequence is X XXY YYZ, where spaces indicate the codons before shifting and X can equal Y. The simultaneous slippage model (8) proposes that the secondary structure of the second RNA signal stimulates the actual frameshift at the slippery site by representing a barrier to the mRNA translocation machinery inducing the two ribosome-bound tRNAs in the P and A sites to slip backwards in the 5′ direction simultaneously from their initial positions in the zero frame. This leaves two of the three codon–anticodon interactions unchanged if X ≠ Y. In the case of HIV-1 and HTLV-2 X equals Y and all three codon–anticodon interactions in the P site and two out of three in the A site are maintained.

For HIV-1 and HTLV-2 it has been demonstrated that a simple stem–loop structure promotes frameshifting at the slippery site *in vitro* and *in vivo*, however, the slippery site of HIV-1 alone is sufficient to mediate a basal level of frameshifting (9,10). When chimeric constructs of HIV-1 and HTLV-2 stem–loop sequences and slippery sequences were tested for frameshifting activity, conflicting results were obtained with regard to the individual contributions to frameshifting efficiency of the RNA elements (9,10). Kollmus *et al.* (9) came to the conclusion that the slippery sequence of HIV-1 combined with the stem–loop of either HIV-1 or HTLV-2 is more efficient in promoting −1 frameshifting than the HTLV-2 slippery site. However, Honda *et al.* (10) reported that the HTLV-2 slippery sequence is much more potent in inducing frameshifting when placed upstream of the HIV-1 stem–loop.

These discrepancies suggest that the slippery sequence and the stem–loop motif are not isolated components in determining frameshifting efficiency, but it is likely that the context in which they appear is also of importance. In order to address this question and to clarify the contradicting reports, we have performed a detailed and systematic analysis of RNA signals within the frameshifting regions of HIV-1 and HTLV-2. In addition to the slippery site and stem–loop motif we include in

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our study the region upstream of the slippery sequence as well as the spacer element that is located between the slippery site and the stem–loop. By shuffling individual or multiple elements of the HIV-1 and HTLV-2 wild-type sequences we were able to investigate the individual contributions of the RNA signals to frameshifting efficiency. Further, we show that the degree by which frameshifting efficiency is altered on exchange of the various RNA elements is strongly dependent on the nature of the particular stem–loop sequence.

MATERIALS AND METHODS

### Template construct for frameshifting assay

The green fluorescent protein (GFP) gene amplified from the pEGFP-c2 vector (Clontech) by PCR was ligated to an *Eco*RI/*Bam*HI-digested pGEM-3Z vector (Promega) with a T7 promoter sequence upstream of the *Eco*RI site. Subsequently, the glutathione *S*-transferase (GST) gene, PCR-amplified from the pGEX-5X1 vector (Amersham Pharmacia), was inserted between the *Pst*I and *Hin*dIII sites. For generation of the individual reporter constructs the *Bam*HI and *Pst*I sites of the resulting plasmid vector were used for cloning –1 frame-shifting elements from either HIV-1 or HTLV-2. The respective sequences were obtained using annealed duplex DNA oligomers. To generate the stem–loop deletion mutants the internal *Bgl*II and *Pst*I restriction sites were used. The accuracy of all wild-type and mutant constructs was confirmed by dideoxy DNA sequencing.

The UAG termination codon of the GFP ORF is located immediately after the inserted frameshifting region. If a –1 frameshift occurs at the slippery sequence, the termination codon is not read and translation proceeds through the GST gene, resulting in the production of a GFP–GST fusion protein.

Frameshifting assay

All plasmids were isolated and purified as described (11). The lyophilized DNA was dissolved in TE buffer (Tris–HCl, pH 8.0, and 1 mM EDTA). The T_NT Quick coupled T7 tran-scription/translation system (Promega) was used according to the manufacturer's protocol. We compared this system with the previously used T_NT coupled T7 transcription/translation system (Promega) (11) and noticed no substantial difference in intra- and inter-assay variability except for a slightly lower product yield with the T_NT Quick coupled T7 transcription/translation system. Aliquots of 400 ng template DNAs were used in a 20 μl reaction containing 10 μl reticulocyte lysate and 0.8 μl of 10 μCi/μl ^{35}S-labeled methionine (NEN).

The GFP–GST fusion product yields a protein of 58 kDa that contains 18 methionine residues, whereas the non-frameshifting GFP protein product is 30 (HIV-1) or 28 kDa (HTLV-2) with six methionines. The high number of methionines in GST enhances the sensitivity for measuring frameshifting rates since the levels of frameshifting efficiency in HIV-1 and HTLV-2 are low compared to other viral systems such as BWYV and PLRV (11,12). In order to separate the GFP–GST fusion protein from the non-frameshifting product (GFP) the samples were separated through 12% SDS–polyacrylamide gels (Fig. 2B). After electrophoresis, gels were dried and exposed to a PhosphorImager screen (Molecular Dynamics). Quantitation of signal intensities was done using
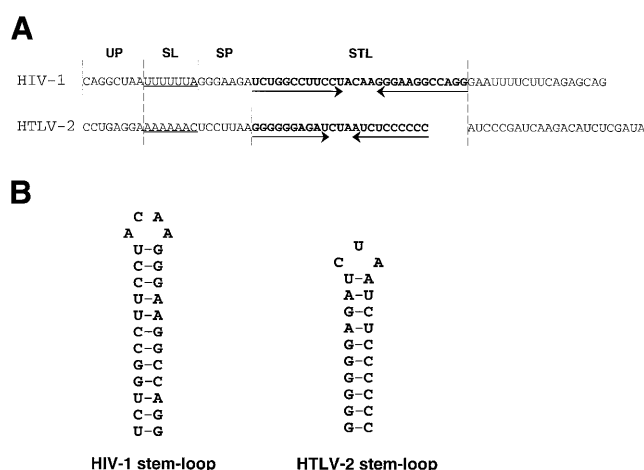
Figure 1. (**A**) Alignment of the HIV-1 and HTLV-2 frameshifting regions. Individual sequence elements are separated by dotted lines and the stem–loop regions (STL) are in bold. The base pairing residues of the stem–loop are indicated by arrows. UP, upstream sequence; SL, slippery sequence; SP, spacer element. (**B**) Predicted secondary structure of the stem–loop element from the frameshifting regions of the HIV-1 and HTLV-2 mRNAs.

PhosphorImager software (Molecular Dynamics). Frameshifting efficiencies were calculated using the formula $(I_{FS}/18)/[(I_{FS}/18) + (I_{NFS}/6)]$, where I_{FS} is the signal intensity of the frameshifting product and I_{NFS} is the signal intensity of the non-frameshifting product. All individual *in vitro* assays were accompanied by HIV-1 wild-type controls and repeated three times or more to determine average frameshifting efficiencies. The mean ± standard deviation frameshifting efficiency of the HIV-1 wild-type reactions in this study was 5.6 ± 0.4%.
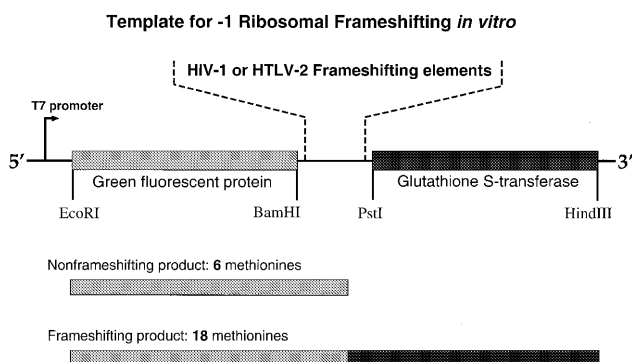
RESULTS

Experimental strategy

Figure 1A compares the sequence regions of HIV-1 and HTLV-2 involved in translational frameshifting of the viral *gag–pol* or *gag–pro* genes, respectively. The predicted secondary structure of the stem–loop motif is shown in Figure 1B. For quantitative *in vitro* analysis of frameshifting activity promoted by wild-type and mutant HIV-1 or HTLV-2 RNA elements we inserted them between the ORFs of GFP and GST (Fig. 2). The non-frameshifted product yields a protein of 30 (HIV-1 constructs) or 28 kDa (HTLV-2 constructs) with six internal methionines. Upon –1 frameshifting a fusion protein of 58 kDa containing 18 methionines is produced in reticulocyte extracts. To determine frameshifting efficiencies of HIV-1 and HTLV-2 constructs the ratios of frameshifting to non-frameshifting product were determined from at least three independent reactions each. Figure 3 shows an example of an autoradiogram obtained from electrophoretically separated *in vitro* translation products from incubations with HIV-1 and HTLV-2 wild-type reporter constructs and selected mutants. The HIV-1 wild-type construct yielded a frameshifting activity of ~5.6%, whereas the HTLV-2 wild-type sequence yielded ~9.3%. Deleting the stem–loop of the HIV-1 or HTLV-2 wild-types drastically reduced frameshifting (0.8% for HIV-1, 1.3% for HTLV-2) without eliminating it completely (Fig. 3), in accordance

Table 1. Frameshifting efficiencies of HIV-1 (I) and HTLV-2 (T) wild-type constructs and chimeras

	UP	SL	SP	STL		
HIV-1	CAGGCUAA	UUUUUUA	GGGAAGA	UCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAG		
HTLV-2	CCUGAGGA	AAAAAAC	UCCUUAA	GGGGGGAGAUCUAAUCUCCCCCCAUCCCGAUCAAGACAUCUCGAUA		
	UP	SL	SP[a]	STL	Composition	Efficiency (%)
1	CAGGCUAA	UUUUUUA	GGGAA	I-STL	IIII	**5.6 ± 0.4 (WT)**
2	CCUGAGGA	UUUUUUA	GGGAA	I-STL	TIII	4.5 ± 0.3
3	CAGGCUAA	AAAAAAC	GGGAA	I-STL	ITII	9.1 ± 0.6
4	CAGGCUAA	UUUUUUA	UCCUU	I-STL	IITI	3.2 ± 0.2
5	CCUGAGGA	AAAAAAC	GGGAA	I-STL	TTII	2.3 ± 0.7
6	CCUGAGGA	UUUUUUA	UCCUU	I-STL	TITI	2.2 ± 0.1
7	CAGGCUAA	AAAAAAC	UCCUU	I-STL	ITTI	10.9 ± 0.4
8	CCUGAGGA	AAAAAAC	UCCUU	I-STL	TTTI	1.9 ± 0.3
9	CCUGAGGA	AAAAAAC	UCCUU	T-STL	TTTT	**9.3 ± 0.9 (WT)**
10	CAGGCUAA	AAAAAAC	UCCUU	T-STL	ITTT	14.3 ± 0.5
11	CCUGAGGA	UUUUUUA	UCCUU	T-STL	TITT	17.0 ± 0.6
12	CCUGAGGA	AAAAAAC	GGGAA	T-STL	TTIT	11.0 ± 1.0
13	CAGGCUAA	UUUUUUA	UCCUU	T-STL	IITT	17.9 ± 1.1
14	CAGGCUAA	AAAAAAC	GGGAA	T-STL	ITIT	18.9 ± 0.8
15	CCUGAGGA	UUUUUUA	GGGAA	T-STL	TIIT	15.5 ± 0.5
16	CAGGCUAA	UUUUUUA	GGGAA	T-STL	IIIT	12.8 ± 0.7
17	CAGGCUAA	GGGAAAC	GGGAA	I-STL	IGII	0.5 ± 0.1
18	CCUGAGGA	GGGAAAC	UCCUU	I-STL	TGTI	0.2 ± 0.1
19	CCUGAGGA	GGGAAAC	UCCUU	T-STL	TGTT	2.7 ± 0.1

UP, upstream sequence; SL, slippery site; SP, spacer element; STL, stem–loop (underlined).
[a]First 5 bases of spacer sequence.

Template for -1 Ribosomal Frameshifting *in vitro*

Figure 2. Schematic representation of reporter constructs and resulting protein products used for *in vitro* ribosomal frameshifting measurements. Boxed regions indicate ORFs for GFP (shaded light gray) and GST (dark gray).

with earlier *in vitro* and *in vivo* results from other groups (10,13,14).

Contribution of slippery sites to frameshifting in HIV-1 and HTLV-2 is context dependent

The slippery sequences in HIV-1 and HTLV-2 have been shown to be essential for frameshifting (9,10), with an optimal repetition of A_6 or U_6 within the consensus X_6Y. If the HIV-1 slippery site U_6A is replaced by G_3A_3C, frameshifting is almost eliminated (Table 1). In the case of HTLV-2 the same slippery sequence (G_3A_3C) leaves a residual frameshifting activity of 2.7%. However, when in addition the stem–loop is replaced by the HIV-1 stem–loop frameshifting is nearly abolished.

The seemingly conflicting results of Kollmus *et al.* (9) and Honda *et al.* (10) concerning the influence of the slippery sequence on frameshifting efficiencies of HIV-1 and HLTV-2 prompted us to investigate the contribution of the slippery sequence on frameshifting within the different sequence backgrounds of the HIV-1 and HTLV-2 mRNAs. As shown in Figure 3, frameshifting increases by 63% (from 5.6 to 9.1%) when the wild-type slippery sequence UUUUUUA is replaced by the slippery site AAAAAAC of HTLV-2. Furthermore, an HIV-1 construct lacking the stem–loop but with the HTLV-2 slippery site still had higher frameshifting activity than the complete HIV-1 wild-type sequence (7.8% compared to 5.6%; Fig. 3).

Intriguingly, changing the wild-type slippery sequence of HTLV-2 from A_6C to the U_6A sequence of HIV-1 also greatly increased frameshifting efficiency, from 9.3 to 15.3%. Taken together, these results strongly argue for a modulatory role of the upstream sequences and/or the spacer element on

A

B
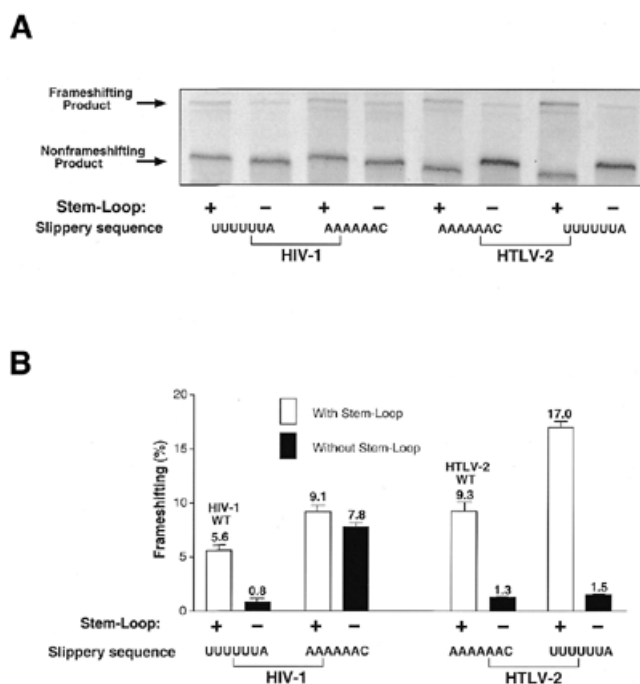
Figure 3. (**A**) SDS–PAGE analysis of [^{35}S]methionine-labeled translation products from the *in vitro* ribosomal frameshifting assay of wild-type HIV-1 and HTLV-2 constructs as well as selected mutants. The positions of the frameshifting and non-frameshifting products are indicated by arrows. The slight shift in positions of the bands is due to small size differences between the wild-type and mutant translation products. The constructs for HIV-1 and HTLV-2 harbor the wild-type sequences with (+) or without (−) the stem–loop and with either the HIV-1 slippery site (UUUUUUA) or the HTLV-2 slippery sequence (AAAAAAC). (**B**) Quantitative analysis of frameshifting results from the autoradiogram in (A). The average percentages of frameshifting from at least three independent experiments are depicted, including error bars.

frameshifting function, since these represent the only non-constant components within the described reporter constructs.

The upstream sequences and spacer elements in HIV-1 and HTLV-2 modulate frameshifting efficiency

In order to analyze the influence of the sequence residing immediately upstream of the slippery site we tested the frameshifting activities of HIV-1 and HTLV-2 constructs with switched upstream regions (Table 1). Exchanging the upstream sequence CAGGCUAA of HIV-1 for the HTLV-2 sequence CCUGAGGA slightly reduced frameshifting activity (5.6 versus 4.5%), whereas in HTLV-2 insertion of the HIV-1-derived upstream sequence led to an ~50% increase in frameshifting rate (9.3 versus 14.3%). Interestingly, in HIV-1 frameshifting activity was further reduced when in addition to the HTLV-2 upstream sequence either the slippery sequence or the spacer was replaced by the corresponding sequence of HTLV-2 (see Table 1). Exchanging all these elements with HTLV-2 sequences, leaving only the stem–loop of HIV-1, further decreased frameshifting. Equally, transferring the HTLV-2 upstream sequence and spacer elements individually compromised frameshifting efficiency when placed in the HIV-1 background. The exception was the HTLV-2 slippery sequence, which enhanced the frameshifting described above.

In this case the HTLV-2 A_6C slippery sequence was extended to an A_8C motif because of the HIV-1 upstream sequence. A stimulatory effect on frameshifting through an increasing number of adenines in the slippery sequence is consistent with the earlier results of Honda *et al.* (10).

In HTLV-2 double replacement of the upstream and slippery sequences or the upstream sequence and the spacer both increased frameshifting rates (17.9 and 18.9%, respectively; Table 1). Individual exchange of the slippery sites boosted frameshifting to 17%, while spacer replacement yielded 11% (Table 1). However, exchanging all three elements resulted in 12.8% frameshifting.

Frameshiftings mediated by the HIV-1 or HTLV-2 stem–loops show different sensitivities to changes in surrounding sequences

Of the several RNA elements examined, the stem–loop sequence, in concert with a slippery site, is certainly the most important component of the frameshifting region, as has been demonstrated by several groups (9,10,13). This was confirmed in our *in vitro* experiments (see Fig. 3). Further, it has been shown for HIV-1 that frameshifting rate correlates with the thermodynamic stability of the stem–loop (15).

We asked whether frameshifting mediated by the HIV-1 and HTLV-2 stem–loops would be similarly influenced by changes in the other *cis*-acting RNA elements, a behavior that would indicate a purely additive contribution from each element to frameshifting function. As shown in Figure 4A, the changes in the extent of frameshifting brought about by changes in the slippery sequence were different for the HIV-1 and HTLV-2 stem–loops. The range of frameshifting rates in HIV-1 was much smaller (1–9%) than the range for HTLV-2 stem–loop constructs (2–18%). This behavior might be a direct conse-quence of the inherently different thermodynamic stabilities of the two stem–loop structures at 37°C, which for HTLV-2 was calculated to be $\Delta G = -18.3$ kJ/mol, while for HIV-1 $\Delta G = -20.9$ kJ/mol, as estimated using the Mufold program (16). In order to examine this property more directly we introduced mutations into the HTLV-2 stem–loop that only changed the orientation of base interactions but did not substantially influence the calculated thermodynamic stability of the stem–loop structures. As documented in Figure 4B, only minor changes in frameshifting efficiency were observed with mutants M1–M4 in combination with the HTLV-2 wild-type slippery sequence. However, when introducing the HIV-1 slip-pery site, 2-fold differences in frameshifting rates were meas-ured between mutants (Fig. 4B). The most drastic change was apparent after inversion of the terminal three G-C base pairs of the stem, which might alter base stacking within the helix. Similarly, inverting the medial three G-C base pairs decreased frameshifting substantially, whereas inversion of all six G-C base pairs yielded frameshifting activities close to wild-type levels (Fig. 4B). In line with the results described above, an analogous mutational analysis with the HIV-1 stem–loop resulted in only minor differences in frameshifting efficiencies (data not shown).

DISCUSSION

The frameshifting regions of HIV-1 and HTLV-2 mRNA contain several RNA elements, of which the slippery sequence

A

B
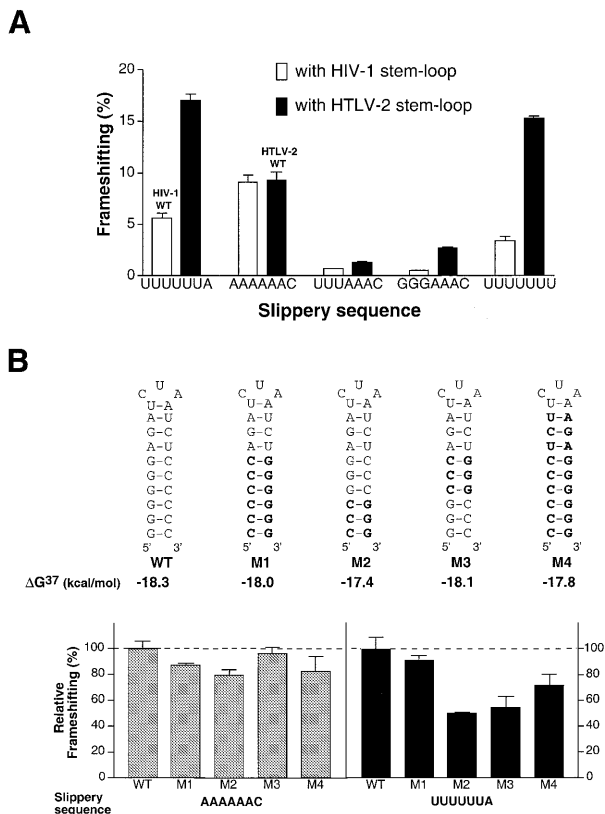
Figure 4. (**A**) Influence of mutated slippery sequences on –1 frameshifting in HIV-1 and HTLV-2. The percentage of frameshifting obtained from constructs with altered slippery sites is analyzed when placed in the HIV-1 compared to HTLV-2 sequence background. (**B**) Sensitivity of frameshifting rates to changes in the slippery sequence is altered in HTLV-2 stem–loop mutants. The wild-type (WT) HTLV-2 stem–loop secondary structure is depicted, with base changes in mutants M1–M4 in bold. The results from *in vitro* translation assays are shown as columns with error bars. The relative extent of frameshifting is determined as the ratio between each mutant and the wild-type stem–loop. All mutants were tested in combination with the HTLV-2 slippery sequence (AAAAAAC, left) and the HIV-1 slippery site (UUUUUUA, right).

and the stem–loop have been studied extensively with respect to their importance for frameshifting function. Here we have extended the analysis of *cis*-acting RNA signals to the neighboring upstream region and the spacer sequence. We have examined their functional interrelationships by shuffling HIV-1- and HTLV-2-derived elements. Our results from interchanging the slippery sites provide an explanation for the apparently contradictory results obtained by other groups (9,10). As reported by Honda *et al.* (10) and confirmed in our experiments, the combination of the HIV-1 stem–loop and the HTLV-2 slippery sequence results in higher frameshifting activity than the HIV-1 stem–loop combined with the HIV-1 slippery site. However, if the surrounding RNA elements, the upstream sequence and the spacer, are also exchanged for the respective HTLV-2 sequences, then we obtain the opposite result. In fact, an HTLV-2-based construct with the HIV-1 stem–loop was used in the study by Kollmus *et al.* (9) and this explains how they came to the conclusion that the HIV-1 slippery sequence is more efficient in mediating –1 frameshifting.

If we compare all possible combinations of HIV-1/HTLV-2 chimeras that differ only in the slippery sequence (mutant nos 1/3, 2/5, 4/7, 6/8 and 11/9), then three out of five combinations give higher frameshifting with the HIV-1 slippery site. Clearly, the frameshifting activity induced by a given combination of RNA elements is not predictable from the individual contributions of single components, but rather is the result of a complex interplay between each sequence region within the mRNA and probably their interaction with the translational machinery.

Interestingly, the construct with the highest frameshifting activity (Table 1, mutant no. 14) was a mosaic of alternating RNA elements from HIV-1 and HTLV-2. Also, the second best frameshifting chimera (mutant no. 13) contained two RNA elements from each virus. The question arises, why did the frameshifting region evolve to harbor at least four *cis*-acting RNA signals even though one or two of these elements, optimally combined, can yield the same level of frameshifting? It is widely known that the efficiency of translational frameshifting is regulated during the viral life cycle (for reviews see 17–19). Such mechanisms require additional sequences that interact with viral and/or host factors. A number of *trans*-acting factors that positively or negatively influence the efficiency of frameshifting have already been genetically identified (20–23). Within viral RNA the candidate region most likely to be subject to regulatory control is the stem–loop. It is well known that during translation of the frameshift region the secondary structure has to be unfolded for the ribosome to proceed along the mRNA. The stem–loop is believed to collide with the moving ribosomal machinery causing it to stall (24,25). This pausing is a prerequisite for efficient frameshifting and it has recently been shown that the average ribosomal pause time is greater for that fraction of ribosomes that proceed in the –1 frame (26). Cellular or viral *trans*-acting factors that interact with the stem–loop region could thus positively or negatively influence frameshifting by stabilizing or destabilizing the secondary structure. In fact, Kollmus *et al.* (27) have demonstrated that when replacing the HIV-1 stem–loop by the iron-responsive element, frameshifting rates increase under conditions that allow binding of iron regulatory proteins.

When comparing HIV-1 and HTLV-2 constructs, mutants of the frameshifting region had a more profound effect on frameshifting in the context of the HTLV-2 stem–loop than with the HIV-1 sequence, indicating a higher sensitivity of this element to changes in the surrounding sequences. These results are in line with earlier work demonstrating that the HTLV-2 stem–loop is much more sensitive to changes in the length of the spacer element than the HIV-1 stem–loop (9,28). The differences might be solely dependent on the thermodynamic stability of the stem–loop structure. However, when we tested mutants of the HTLV-2 stem–loop that do not change the overall thermodynamic stability of the secondary structure but only alter base pair orientation, the frameshifting efficiencies of individual mutants still varied depending on the nature of the slippery sequence. The HIV-1 slippery site proved much more sensitive to mutations in the stem–loop than that derived from HTLV-2. Taken together, these findings also argue for the involvement of additional *cis*- and/or *trans*-acting factors.

It has recently been shown that the introduction of an upstream or downstream termination codon relative to the slippery site also influences frameshifting efficiency (29,30).

An upstream termination codon in the –1 frame located at various positions impairs frameshifting via an unknown mechanism (29). However, a downstream termination codon in the –1 frame (30) enhances frameshifting, probably because it represents another pausing element for the translating ribosomes acting in concert with the RNA secondary structure. The translational termination signal probably leads to sequestration of protein factors to the frameshifting region either positively or negatively interfering with the frameshifting process.

Although the mechanics of ribosomal frameshifting are largely unknown, we should not be surprised to find that frameshifting efficiency is affected by the upstream sequences and spacer sequences, as well as the more thoroughly studied slippery sequence and downstream structural motif, in this case a stem–loop. The spacer element usually has 6 or 7 nt. It is likely that they are normally in a stacked configuration with ~3.4 Å per base. A number of nuclease digestion experiments have been done and they reveal that the number of nucleotides found between the coding site and the outside of the ribosome where the nuclease acts is 12–15 nt in prokaryotes (31,32) and 20 nt in eukaryotes (33). This clearly suggests that the spacer segment undergoes considerable elongation before ribosomal frameshifting occurs. The power behind this extension is the translocational mechanism of the ribosome, which moves the mRNA–tRNA complex one codon (probably ~10 Å) associated with tRNA translocation from the A site to the P site. This translocation moves in the upstream direction and presumably elongation of the spacer segment is due to the fact that the downstream secondary structure, in this case the stem–loop, cannot enter the ribosomal mRNA channel. We do not as yet know the mechanism behind the translocation process and mRNA movement, but it is likely that a significant component consists of pressures to move the tRNA itself. It is this movement in the upstream direction which, when faced with an extended mRNA and a secondary structural element that does not unravel, leads to sliding of the tRNA by –1 nt in the upstream direction. The detailed sequence of bases in the spacer segment will determine both its initial stacking energy and its gradual loss through unstacking and extension, as well as the extent to which it interacts with other ribosomal components forming the mRNA channel. In the same way, the upstream sequence of the message must continue to move through the ribosome during the translocational process. Thus, it too may have an opportunity to be influenced by contacts with those elements that make the ribosomal mRNA channel. However, it has recently been shown that specific inhibition of EF-2-mediated translocation by pokeweed antiviral protein does not change the efficiency of –1 ribosomal frameshifting. Therefore, the frameshift must occur before completion of the peptidyl-transferase reaction (34).

An important element is, of course, the interaction of the stem–loop structure with the ribosome, which helps to determine whether it will unravel, and therefore not frameshift, or maintain its structure, leading to frameshifting. Here it is not a matter of the stabilizing energy of the isolated stem–loop. Instead, it is the energy of the stem–loop as it abuts the ribosome. As seen by the variation in frameshifting associated with changes in the stem–loop structure (Fig. 4B), it seems clear that a string of CG base pairs are important for stability near the end of the stem–loop structure. However, there is a curious destabilization associated with mutants M2 and M3 in

which the six CG base pairs have been changed to blocks of three with the adjacent block inverted. These lose considerable frameshifting ability with the HIV-1 slippery sequence, but much less so with HTLV-2. This may reflect the fact that interaction of the stem–loop with the ribosome is a much more important component of frameshifting in HIV-1 than in HTLV-2.

Discussions of the mechanics of ribosomal frameshifting will shortly undergo an abrupt change due to the recent publication of high resolution X-ray crystallographic studies of both the large and small ribosomal subunits (35–37). At present it is possible to locate the position of the two tRNAs in the A and T sites and give a precise description of that environment. In the near future, with further developments in this area, we will be able to transform the discussion of ribosomal frameshifting from generalities to highly specific suggestions about which structural elements and interactions may be important in understanding this process.

ACKNOWLEDGEMENTS

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