RNA (1997), 3:1153-1158. Cambridge University Press. Printed in the USA.

In vivo HIV-1 frameshifting efficiency is directly related to the stability of the stem-loop stimulatory signal

LAURE BIDOU,[1] GUILLAUME STAHL,[1] BRIGITTE GRIMA,[1,3] HONGMEI LIU,[1] MICHEL CASSAN,[2,4] and JEAN-PIERRE ROUSSET[1]

[1]Génétique Moléculaire de la Traduction—Équipe d'accueil N° 1831 de l'Université Pierre et Marie Curie, Institut de Génétique et Microbiologie, URA CNRS 2225, Bâtiment 400, Université Paris-Sud, F91405 Orsay cedex, France

[2]Reproduction Sexuée Traduction et Différenciation Institut de Génétique et Microbiologie, URA CNRS 2225, Bâtiment 400, Université Paris-Sud, F91405 Orsay cedex, France

ABSTRACT

In many retroviruses, the expression of reverse transcriptase, protease, and integrase is dependent upon a −1 frameshift event. The frameshift signal is composed of a slippery sequence where the ribosome shifts, and a downstream stimulatory sequence. In most cases, the stimulatory sequence is a pseudoknot, but in some viruses, such as human immunodeficiency virus type 1 (HIV-1), a single stem-loop is involved. Here, we analyzed the precise role of the stem-loop thermodynamic stability. We tested the frameshifting stimulatory activity of a series of HIV-1-derived sequences showing a stepwise increment of the estimated $\Delta G°$. These sequences were introduced at the junction of a *lacZ-luc* fusion gene cloned on a versatile expression vector, and the different constructs were tested in *Saccharomyces cerevisiae* and in mouse NIH3T3 cells. The results showed that the frameshifting efficiency was correlated directly to the stem stability between $\Delta G°$ = −2.5 kcal mol^{-1} and $\Delta G°$ = −19.4 kcal mol^{-1}. This demonstrates the essential role of the stability of the stem-loop and does not support the involvement of a specific RNA-binding protein target sequence. However, increasing further the stem stability led to a diminution of frameshifting efficiency, suggesting that the stem-loop acts through a precise kinetic of pausing. Because the same pattern was observed in both yeast and mouse cells, it is likely that the stimulatory mechanism is conserved through evolution.

Keywords: mammalian cells; retroviruses; *Saccharomyces cerevisiae*; secondary structure; translation

INTRODUCTION

The life cycle of retroviruses necessitates a recoding event, which can be either the read-through of an in-frame stop codon, as in Moloney Murine Leukemia virus, or a −1 frameshift, as in Rous Sarcoma virus and HIV-1 (Atkins et al., 1990; Gesteland et al., 1992). This allows the synthesis, from the same transcript, of the GAG polypeptide and of the *pol* gene products: reverse transcriptase, protease, and integrase enzymes (Atkins et al., 1990; Brierley, 1995). These enzymatic activities are incorporated in virus particles as a GAG-POL multifunctional polyprotein. The rate of production of these crucial products must be controled precisely, because the modification of the GAG-POL/GAG ratio is detrimental to virus propagation (Dinman & Wickner, 1992; Karacostas et al., 1993). During the last years, the −1 frameshift mechanism has been analyzed in vitro and in vivo, leading to the characterization of the *cis*-acting elements responsible for a high frameshifting efficiency (Brierley, 1995). The frameshift signal is composed of two parts: a shifty site and a stimulatory sequence. The shifty site is an X XXY YYZ heptanucleotide (where the 0 frame is indicated), for which different XY associations lead to various frameshifting efficiencies (Brierley et al., 1992). The stimulatory signal is a palindromic sequence that potentially can adopt either a pseudoknot or a stem-loop structure. It must be located 5–8 nt dowstream of the shift site to exert its effect (Kollmus et al., 1994), and probably causes the ribosome to pause (Somogyi et al., 1993).

Reprint requests to: Jean-Pierre Rousset, Génétique Moléculaire de la Traduction—Équipe d'accueil N° 1831 de l'Université Pierre et Marie Curie, Institut de Génétique et Microbiologie, URA CNRS 2225, Bâtiment 400, Université Paris-Sud, F91405 Orsay cedex, France; e-mail: rousset@igmors.u-psud.fr.

[3]Present address: CNRS, IAF, Avenue de la terrasse, F91198 Gif/Yvette cedex, France.

[4]Present address: Centre de Génétique Moléculaire, UPR 9061 CNRS, F91198 Gif/Yvette cedex, France.

In the case of HIV-1, the stimulatory sequence involves a single stem-loop that increases frameshifting efficiency three-to fivefold (Cassan et al., 1990, 1994; Parkin et al., 1992; Reil et al., 1993). This effect has been observed in vitro and in vivo, in both mammalian cells and the yeast *Saccharomyces cerevisiae* (Stahl et al., 1995). The stem-loop structure could act by two mechanisms: either directly, via its thermodynamic stability, or through an interaction with a specific RNA-binding protein. Kollmus and coworkers (1996) have shown that, in a construct where the HIV-1 stem-loop has been replaced by the iron-responsive element from ferritin RNA, frameshifting is increased under conditions that allow binding of iron regulatory proteins. However, no effect on frameshifting efficiency has been observed when increasing amounts of a simian retrovirus-1 (SRV-1) pseudoknot containing RNA transcript were added to a translation reaction programmed with an SRV-1 frameshift reporter (ten Dam et al., 1994). The involvement of a secondary structure-recognizing factor in frameshift mechanism is, therefore, still an open question.

The aim of the present work is to determine whether HIV-1 frameshifting efficiency is dictated directly by the stability of the stem-loop secondary structure or if the nucleotide sequence itself is also involved. The rationale of the experiment is that, if the stem-loop stability is the unique determinant of frameshifting stimulation, there should be a direct relationship between stem-loop stability and frameshifting efficiency. In order to determine whether the mechanisms involved are specific to mammalian cells or conserved in lower and higher eukaryotes, we compared the effect of the mutations in mammalian cells and in yeast *S. cerevisiae*. For that goal, a series of HIV-1-derived *gag-pol* junction mutants have been constructed. All of them contained the wild-type frameshift site, but carried stem-loops of different stability. The mutated sequences were inserted between the *lacZ* and the *luc* coding sequences into a versatile yeast/mammalian cell expression vector, allowing precise quantification of frameshifting efficiency (Stahl et al., 1995). In both organisms, we observed a linear relationship between the frameshifting efficiency and the stability of the stem-loop structure, up to the stability of the wild-type HIV-1 sequence. These data support the hypothesis that the stability of the stem is the most important determinant of the frameshifting efficiency, possibly through a precise kinetic of pausing.

RESULTS

The sequences of the different frameshift signal mutants are shown in Figure 1. They all contained the wild-type slippery site followed either by the wild-type or by a mutated stimulatory sequence. The stability of the stem was modified by three different means: (1) by changing the number of AU versus GC pairings; (2) by changing the number of base pairings; and (3) by changing the sequence of the loop. The $\Delta G°$ were estimated using the Mufold computer program (Jaeger et al., 1989). In mutants 1848, 1851, and 1830, the number of base pairs in the stem was conserved, only the relative number of AU versus GC pairing was modified. Whereas the wild-type potential stem-loop (1789 sequence) contained seven GC and three AU potential pairings (estimated $\Delta G° = -19.4$ kcal mol^{-1}), targets 1848 and 1851 harbored only four GC and one GC, giving rise to estimated $\Delta G°$ of -13.5 and -7.6 kcal mol^{-1}, respectively. Conversely, 1830 stem sequence, which included nine GC, is more stable than the wild-type stem (estimated $\Delta G° = -22.8$ kcal mol^{-1}). For these targets, the sequence of the loop (ACAA) was not changed and the two GU pairings, defining the top and the bottom of the stem, were also conserved. For two other mutants, the secondary structure was either disrupted partially, or removed totally. Construct 1815 contained only five potential nonadjacent pairings (estimated $\Delta G° = -2.5$ kcal mol^{-1}) and construct 89 carried a complete deletion of the stem-loop structure; these two mutants have been described previously (Stahl et al., 1995). Finally, the 1804 sequence only differed from the wild type by the four loop bases, which were replaced by the UGUU complementary sequence, decreasing the stability of the stem (see below).

The frameshift target sequences were inserted at the junction of a *lacZ-luc* fusion gene in the pAC74 expression vector. This vector can be used to analyze frameshifting in mammalian and yeast *S. cerevisiae* cells (Stahl et al., 1995). Upon translation, initiated at the unique AUG of the bicistronic mRNA, each ribosome would give rise to β-galactosidase activity. In contrast, a β-galactosidase–luciferase fusion protein, carrying both activities, would be synthesized only when a -1 frameshift event takes place at the inserted HIV-1 frameshift site. In this reporter, β-galactosidase activity provides an internal control for vector stability, transfection efficiency, transcriptional and translational rates, whereas luciferase activity reflects the frameshifting efficiency (Reil et al., 1993; Stahl et al., 1995).

Because insertion of several amino acids at the β-galactosidase–luciferase junction may disturb the stability or specific activity of the fusion protein, the activity driven by each frameshift-dependent construct was compared to the activity obtained with an in-frame control construct. In these control vectors, one adenine was added after the UUA leucine codon in the slippery sequence (see Fig. 1), leading to the synthesis of a fusion protein identical to that obtained upon frameshifting in the corresponding test construct. Frameshifting efficiency was calculated as the ratio between luciferase activity of the test construct and that of the control construct, after standardization for expression efficiency using β-galactosidase activity. The

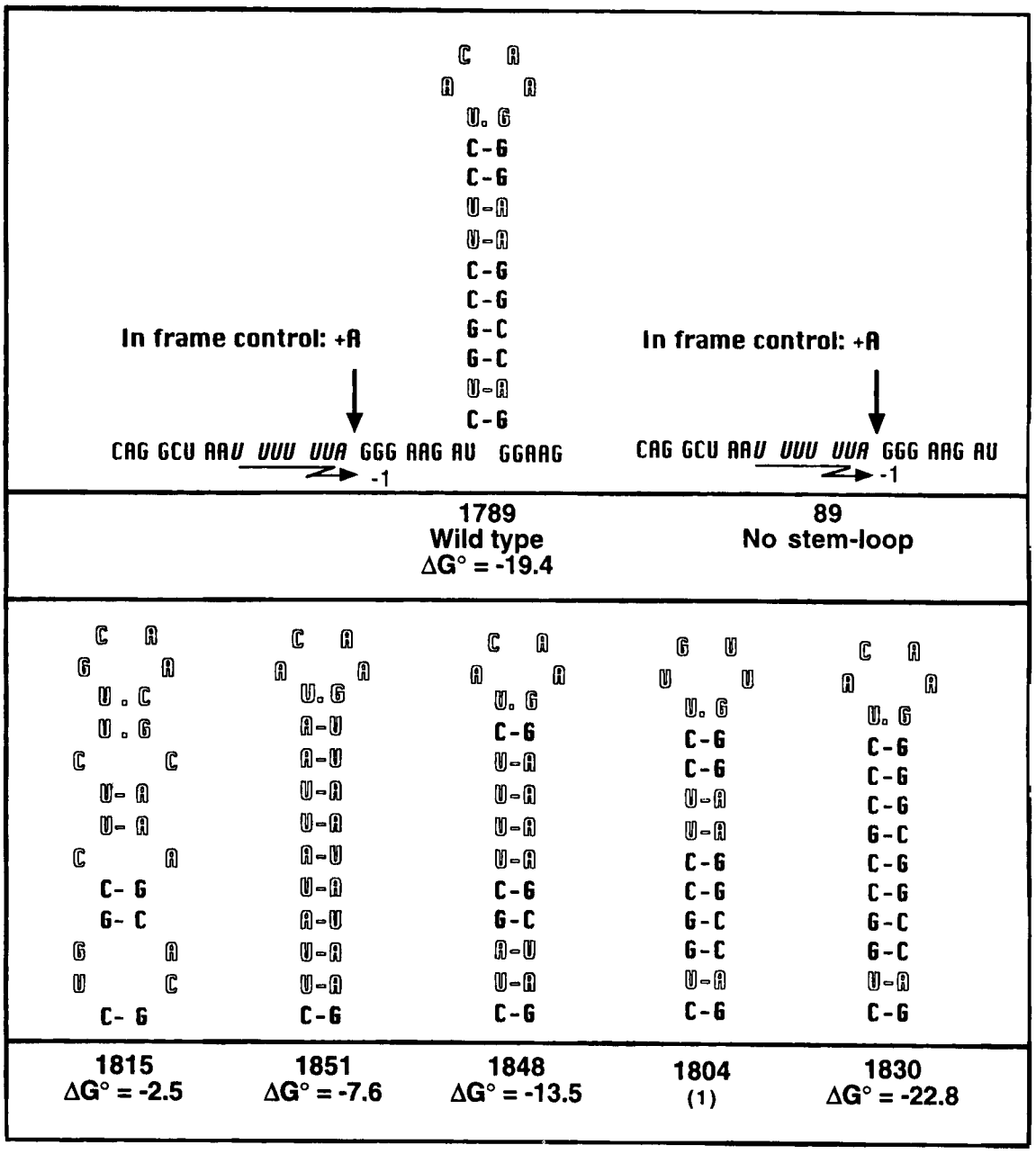

FIGURE 1. Sequence of mutant stimulatory signals. The slippery sequence is in italics. In the stem, the CG pairs are in bold letters. The adenine added after the TTA leucine codon for in-frame controls is indicated. The thermodynamic stability ($\Delta G°$) of each stem loop structure was estimated using the Mufold computer program (Jaeger et al., 1989); values are given in kcal mol^{-1}.

constructs were used in parallel in two sets of experiments: (1) transformation of yeast cells and (2) transfection of mouse NIH3T3 cells.

Data are shown in Table 1 (yeast) and Table 2 (NIH3T3) and illustrated in Figure 2. Although the effect of the mutations were weaker in yeast than in mouse cells, the profiles of the two curves were very similar for both organisms. Frameshifting efficiency increased linearly with the stem loop thermodynamic stability, up to a maximum corresponding to the wild-type sequence. For the targets with a calculated $\Delta G°$ below the wild-type value, the frameshifting efficiency progressively increased from 1.3% (89) to 1.9% (1815), 2% (1851), 3.3% (1848), and 7.3% (1789), in mammalian cells. These values paralleled the $\Delta G°$ of the stem-loop structure. In yeast, there was a similar increase from 1.7% (89) to 2.4% (1815), 3% (1851), 4.4% (1848), and 4.9% (1789). Sequence 1804 differed from the wild-type sequence by the four nucleotides of the loop. This mutation reduced frameshifting efficiency to 75% of

TABLE 1. Frameshifting efficiency in *S. cerevisiae*.[a]

Vectors test-control	Test/control (× 100)[b]	Frameshift efficiency	SD
1830–1831	4.5	4%	0.39
	4.3		
	3.5		
	4.3		
1789–1790	4.5	4.9%	0.38
	5.3		
	4.5		
	5.2		
1804–1805	3.9	3.6%	0.70
	2.4		
	4		
	4.2		
1848–1849	4.4	4.4%	0.25
	4.3		
	4.9		
	4.2		
1851–1852	3.5	3%	0.52
	3.6		
	2.6		
	2.5		
1815–1816	2.3	2.4%	0.16
	2.3		
	2.4		
	2.7		
89–90	1.7	1.7%	0.26
	1.3		
	1.6		
	2		

[a]Frameshifting efficiency was calculated as the ratio of standardized luciferase activity (luciferase activity/β-galactosidase activity) obtained with the test construct, to the standardized luciferase activity obtained with the corresponding in-frame control.
[b]Each value corresponds to an independent experiment.

TABLE 2. Frameshifting efficiency in NIH3T3 cells.[a]

Vectors test–control	Test/control (× 100)[b]	Frameshift efficiency	SD
1830–1831	5.5	5.8%	0.39
	6.3		
	6		
	6		
	5.2		
1789–1790	7.3	7.3%	0.89
	7.5		
	8		
	6.6		
	5.7		
	8.3		
1804–1805	4	5.4%	0.71
	5.5		
	5.8		
	6		
	5.5		
1848–1849	2.6	3.3%	0.75
	3.7		
	2.6		
	4.7		
	2.8		
	3.3		
1851–1852	1.9	2%	0.42
	1.7		
	1.7		
	2.8		
	1.72		
1815–1816	0.86	1.9%	0.53
	2		
	1.5		
	2		
	2.7		
	1.67		
	1.2		
	2.2		
	2		
	2.4		
89–90	1.5	1.3%	0.30
	0.9		
	1.17		
	1.13		
	1.61		
	1.8		
	1		
	1.28		
	0.9		

[a]Same legend as Table 1.
[b]Each value corresponds to an independent experiment.

the wild-type value in both organisms (Tables 1, 2). Finally, when the stability was further increased to a higher value than wild type (sequence 1830), a decreased frameshifting efficiency was observed in both mammalian and yeast cells (80% and 85% of wild-type value, respectively).

DISCUSSION

In this work, we have analyzed the precise involvement of the HIV-1 stem-loop thermodynamic stability in the frameshift stimulatory mechanism. The results showed that there is a tight correlation between frameshifting efficiency and stability of the stem-loop structure, up to the wild-type stem-loop stability. When the stability of the stem-loop increased further, a significant decrease was observed.

The parallel increase of frameshifting and stability obtained in the first part of the curve indicates that nucleotide sequence of the stem is not the main determinant of frameshifting. Two mutants are particularly informative. First, in mutant 1851, the stem contained nine AU pairs, a unique GC pair at the second position from the bottom of the stem, and the two GU pairs that define the ends of the stem. In this mutant, the stability of the stem-loop is much lower than that of the wild-type ($\Delta G° = -7.6$ kcal mol^{-1}, instead of -19.4 kcal mol^{-1}), but the number of pairings is conserved. Strikingly, the frameshifting efficiency obtained with this mutant is also very low. Second, in mutant 1804, only the sequence of the loop was changed. Although the nucleotides in the loop are not involved directly in base pairing, they most probably participate in stem-

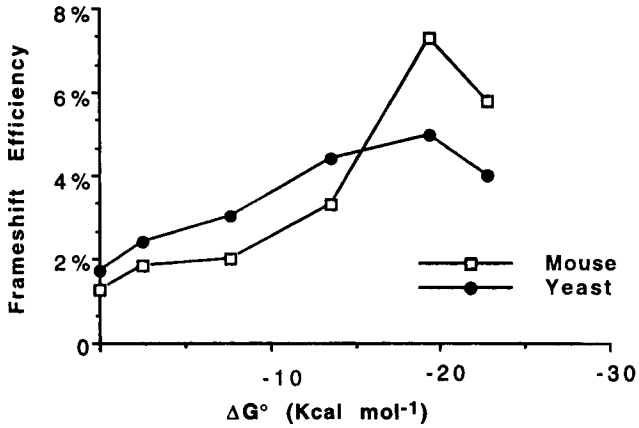

FIGURE 2. Effect of secondary structure stability on frameshifting efficiency. For each mutant, frameshifting efficiency was measured after transformation of yeast *S. cerevisiae* (solid circles) or after transient transfection of mammalian cells (open squares). Each point represents the mean of 4–10 values obtained in independent experiments (see Tables 1 and 2).

loop stability. Indeed, it has been demonstrated that, in RNA tertiary interactions, the stabilization of a stem is optimum when the loop corresponds to a GNRA tetraloop (R = purine and N = purine or pyrimidine) (Antao & Tinoco, 1992). The UGUU sequence of the 1804 loop is more divergent from this consensus than the wild-type ACAA sequence. Therefore, although the decrease of $\Delta G°$ of mutant 1804 compared to the other constructs cannot be estimated precisely by current programs, this mutation is likely to destabilize the secondary structure (Turner et al., 1988). Consistent with this prediction, mutant 1804 gave a frameshifting efficiency that was decreased to about 75% of the wild-type value.

Altogether, these results indicate strongly that thermodynamic stability of the stem is the most important determinant of frameshifting stimulation. However, mutant 1830 demonstrates that another parameter must be involved. Indeed, this mutant has the strongest potential secondary structure ($\Delta G° = -22.8$ kcal mol^{-1}), but shows a 15–20% decrease of frameshifting efficiency compared to wild-type ($\Delta G° = -19.4$ kcal mol^{-1}). Because the role of the stem-loop structure is to slow down or stall the ribosome to allow the two tRNAs to shift, this result may reflect that a precise kinetic of pausing is required for optimal frameshifting. We hypothesize that the time during which the ribosome movement is impeded would be critical for frameshifting efficiency. If the pause is too long, then the tRNAs would have time to realign in the 0 frame, leading to decreased frameshifting efficiency. In the case of mutant 1830, the stability of the stem would be too high, allowing the tRNAs to do an "aller-retour" on the message. This interpretation is reminiscent of the kinetic proofreading mechanism, which modulates amino acid incorporation accuracy (Ninio, 1975). Finally, comparing the results obtained in yeast and mouse cells demonstrates that, as suggested previously (Stahl et al., 1995), the mechanism of −1 frameshifting stimulation is conserved between lower and higher eukaryotes (see Fig. 2).

In conclusion, our results demonstrate that stem-loop stability is the major component of the stimulatory mechanism. This does not exclude that the stem-loop could be recognized and bound by structure-specific binding factors showing no or little sequence specificity. Because the effect of the stem-loop mutations is very similar in yeast and mouse cells, these putative RNA-binding factors should not be specific to mammalian cells, but also exist and be functional in lower eukaryotes. No RNA-binding protein showing such an activity has been characterized up to now. Genetic screens, such as those already developed in yeast, may reveal this type of factor (Dinman & Wickner, 1994).

MATERIALS AND METHODS

Plasmids

The target sequences are shown in Figure 1. For each construct, a pair of oligonucleotides was used to reconstitute a double-stranded frameshift signal, flanked by a 5′ *Nhe* I end and a 3′ *Bcl* I compatible end. For example, for mutant 1804, the sequences of the oligonucleotides were: CTAGCCAGG CTAATTTTTTAGGGAAGATCTGGCCTTCCTTGTTG GGAAGGCCAGGGAAG and GATCCTTCCCTGGCCTTC CCAACAAGGAAGGCCAGATCTTCCCTAAAAAATT AGCCTGG. The sequences of the oligonucleotides used to construct the other mutants can be inferred from sequence data shown on Figure 1.

The mutant sequence was then cloned in the pAC74 vector to reconstitute a *"lacZ-luc"* fusion gene interrupted with the frameshift signal, as described previously (Stahl et al., 1995). A series of in-frame control vectors was constructed following the same scheme. In this case, an adenine was added after the second shifty codon (LEU), to put the *lacZ* and *luc* coding sequences in the same reading frame (see Fig. 1). For each construct, different clones were isolated and the region surrounding the frameshift site was verified by sequencing. Wild-type 1789 construct and mutant constructs 89 and 1815 have been described previously (Stahl et al., 1995).

After amplification in DH5α *Escherichia coli* strain, plasmid DNA was purified using Qiagen-tips procedure. DNA cloning and analysis was performed according to Sambrook et al. (1989).

Yeast transformation and crude extracts preparation

Transformation of the haploid *S. cerevisiae* strain FY 1679-18Bα was performed by the lithium acetate method (Gietz et al., 1992). After transformation, colonies were grown in rich YPD medium (1% yeast extract, 2% bactopeptone, 2% glucose) to early stationary phase. For protein extraction, cultures were diluted 1:20, grown to $OD_{600} = 1.5$, and 4 mL were centrifuged. Then, 150 μL of luciferase assay buffer (Nguyen et al., 1988) and an equal volume of glass beads were added to the pellet. The cells were then broken by

vortexing at 4 °C for 30 min. After removal of cell debris by centrifugation, the crude extract was used immediately to measure the β-galactosidase and luciferase activities (Stahl et al., 1995). For each construct, at least four yeast transformants were analyzed from independent transformations.

Mammalian cell culture, transfections, and crude extracts preparation

NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 7% fetal calf serum (Gibco BRL) and incubated at 37 °C in humidity-satured 6.5% CO_2 in air. Cells were transfected by electroporation. Growing cells were harvested and dissociated as a single cell suspension with trypsin. After low-speed centrifugation, the cell pellet was resuspended in culture medium at a concentration of 4×10^7 cells/mL. For each electroporation, 4×10^6 cells and 18 μg of purified plasmid were transferred extemporaneously to an electroporation cuvette. The cells were exposed to a single electric pulse using a Cellject Electroporation System (Eurogentec) with the following parameters: voltage = 200 V, capacitance = 900 μF, resistance = $+\infty$. In these conditions, the time constant is about 85 ms (±10 ms). Cells were then plated immediately in culture medium. Culture medium was changed 20 h later and cells were allowed to grow for two days. To prepare crude extracts, cells were scraped out, spun at 4 °C, and lysed by repeated pipetting in 150 μL of cold luciferase assay buffer. For each construct, at least five independent transfection experiments were performed with different DNA preparations.

Enzyme assays

Luciferase and β-galactosidase activities were measured from the same crude extract. Luciferase was assayed following the protocol of Nguyen et al. (1988). The reaction buffer was: luciferin 5×10^{-4} M; ATP 5×10^{-4} M in luciferase buffer. Light emission was estimated with a Berthold LUMAT LB 95501 luminometer. Luciferase detection is highly sensitive: a frameshift level as low as 10^{-5} gives a light emission that is still five times over the background (Cassan et al., 1990). β-Galactosidase was assayed as described (Miller, 1972).

ACKNOWLEDGMENTS

The excellent technical assistance of Marie-Claire Fontyne is greatly appreciated. We thank Muriel Grenon and Jean-Marie Rouillard for participating in the initial phase of this work during their training for the "DEA de Génétique Cellulaire et Moléculaire." We are very grateful to Monique Bolotin-Fukuhara and Marguerite Picard for their invaluable support. L.B. was supported by the Fondation Singer-Polignac. H.L. was supported by fellowships from the Fondation KC Wong and from the Fondation pour la Recherche Médicale. This work was granted by Agence Nationale de Recherches sur le SIDA (contract no. 95035), Association pour la Recherche sur le Cancer (contract no. 3065), Ligue Nationale contre le Cancer and Fondation pour la Recherche Médicale.

Received January 27, 1997; returned for revision March 5, 1997; revised manuscript received July 16, 1997

REFERENCES

Antao VP, Tinoco IJ. 1992. Thermodynamic parameters for loop formation in RNA and DNA hairpin tetraloops. *Nucleic Acids Res 20*:819–824.

Atkins JF, Weiss RB, Gesteland RF. 1990. Ribosome gymnastics—Degree of difficulty 9.5, style 10.0. *Cell 62*:413–423.

Brierley I. 1995. Ribosomal frameshifting on viral RNAs. *J Gen Virol 76*:1885–1892.

Brierley I, Jenner AJ, Inglis SC. 1992. Mutational analysis of the "slippery-sequence" component of a coronavirus ribosomal frameshift signal. *J Mol Biol 227*:463–479.

Cassan M, Berteaux V, Angrand PO, Rousset JP. 1990. Expression vectors for quantitating in vivo translational ambiguity: Their potential use to analyse frameshifting at the HIV gag-pol junction. *Res Virol 141*:597–610.

Cassan M, Delaunay N, Vaquero C, Rousset JP. 1994. Translational frameshifting at the gag-pol junction of human immunodeficiency virus type 1 is not increased in infected T-lymphoid cells. *J Virol 68*:1501–1508.

Dinman JD, Wickner RB. 1992. Ribosomal frameshifting efficiency and gag/gag-pol ratio are critical for yeast M1 double-stranded RNA virus propagation. *J Virol 66*:3669–3676.

Dinman JD, Wickner RB. 1994. Translational maintenance of frame: Mutants of *Saccharomyces cerevisiae* with altered −1 ribosomal frameshifting efficiency. *Genetics 136*:75–86.

Gesteland RF, Weiss RB, Atkins JF. 1992. Recoding: Reprogrammed genetic decoding. *Science 257*:1640–1643.

Gietz D, St. Jean A, Woods RA, Schiestl RH. 1992. Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res 20*:1425.

Jaeger JA, Turner DH, Zuker M. 1989. Improved prediction of secondary structures for RNA. *Proc Natl Acad Sci USA 86*:7706–7710.

Karacostas V, Wolffe EJ, Nagashima K, Gonda MA, Moss B. 1993. Overexpression of the HIV-1 Gag-Pol polyprotein results in intracellular activation of HIV-1 protease and inhibition of assembly and budding of virus-like particles. *Virology 193*:661–671.

Kollmus H, Hentze MW, Hauser H. 1996. Regulated ribosomal frameshifting by an RNA-protein interaction. *RNA 2*:316–323.

Kollmus H, Honigman A, Panet A, Hauser H. 1994. The sequences of and distance between two *cis*-acting signals determine the efficiency of ribosomal frameshifting in human immunodeficiency virus type 1 and human T-cell leukemia virus type II in vivo. *J Virol 68*:6087–6091.

Miller JH. 1972. *Experiments in molecular genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Nguyen VT, Morange M, Bensaude O. 1988. Firefly luminescence assays using scintillation counters for quantitation in transfected mammalian cells. *Anal Biochem 171*:404–408.

Ninio J. 1975. Kinetic amplification of enzyme discrimination. *Biochimie 57*:587–595.

Parkin NT, Chamorro M, Varmus HE. 1992. Human immunodeficiency virus type 1 *gag-pol* frameshifting is dependent on downstream mRNA secondary structure: Demonstration by expression in vivo. *J Virol 66*:5147–5151.

Reil H, Kollmus H, Weidle UH, Hauser H. 1993. A heptanucleotide sequence mediates ribosomal frameshifting in mammalian cells. *J Virol 67*:5579–5584.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: A laboratory manual*. New York: Cold Spring Harbor Laboratory Press.

Somogyi P, Jenner AJ, Brierley I, Inglis SC. 1993. Ribosomal pausing during translation of an RNA pseudoknot. *Mol Cell Biol 13*:6931–6940.

Stahl G, Bidou L, Rousset JP, Cassan M. 1995. Versatile vectors to study recoding: Conservation of rules between yeast and mammalian cells. *Nucleic Acids Res 23*:1557–1560.

ten Dam E, Brierley I, Inglis S, Pleij S. 1994. Identification and analysis of the pseudoknot-containing *gag-pro* ribosomal frameshift signal of simian retrovirus-1. *Nucleic Acids Res 22*:2304–2310.

Turner DH, Sugimoto N, Freier SM. 1988. RNA structure prediction. *Annu Rev Biophys Biophys Chem 17*:167–192.