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Regulated ribosomal frameshifting by an RNA–protein interaction

HEIKE KOLLMUS,[1] MATTHIAS W. HENTZE,[2] and HANSJÖRG HAUSER[1]

[1] Genetics of Eukaryotes, GBF Gesellschaft für Biotechnologische Forschung mbH., Mascheroder Weg 1, 38124 Braunschweig, Germany
[2] Gene Expression Programme, European Molecular Biology Laboratory, P.O. Box 10.2209, 69012 Heidelberg, Germany

ABSTRACT

Ribosomal frameshifting is a translational mechanism used as an essential step in the replication cycle of retroviruses. Programmed frameshifting in retroviral translation involves two sequence elements: A heptanucleotide slippery sequence which induces a low basal level of frameshifting and a downstream RNA structure as an enhancer of the process. The precise mechanism of function of these downstream elements is still unclear, but their effect does not solely depend on their stability. Likewise, the possibility that frameshifting could be controlled by specific proteins that bind to these elements and enable or modulate their effects has yet not been substantiated. The RNA hairpin of the HIV-1 *gag-pol* frameshift cassette was replaced by the iron-responsive element (IRE) from ferritin mRNA, a stem-loop structure that binds iron regulatory proteins (IRPs) in dependence of the iron status of the cell. When a lacZ/luciferase reporter construct was expressed in transfected BHK-21 cells, the IRE or a point-mutated version that is unable to bind IRPs were found to functionally substitute for the HIV-1 hairpin. When cells were treated with the iron chelator desferrioxamine to stimulate IRP binding to the wild-type IRE, frameshift activity was specifically and strongly augmented by protein binding. Our data establish that frameshifting can be regulated in a reversible fashion by mRNA-binding proteins.

Keywords: iron-responsive element; IRP; ribosomal frameshifting; ribosomal pausing; RNA-binding proteins; RNA secondary structure

INTRODUCTION

The primary, secondary, and tertiary structure of mRNAs play key roles in alternative readings of the genetic code (Gesteland et al., 1992). Examples include the suppression of stop codons by the incorporation of selenocysteine at UGA codons as a prerequisite for the biosynthesis of selenoproteins, as well as programmed frameshifts that are required for the synthesis of transframe proteins (Böck et al., 1991; Hatfield et al., 1992; Berry & Larsen, 1993). Frameshifting in −1 direction is found in retroviruses, corona viruses, retrotransposons, bacterial transposons, and prokaryotic genes (Atkins et al., 1990).

In mammalian retroviruses, −1 frameshifting leads to the suppression of the *gag* termination codon and thereby enables the translation of the *pol* gene (Jacks, 1990). The shift of the reading frame determines the ratio between the structural GAG proteins and the catalytic proteins. The sequence requirements for retroviral −1 frameshifting have been defined: the change of the reading frame occurs on a "slippery" heptanucleotide. Two tRNAs bound to nt 2–7 of this site simultaneously slip onto nt 1–6. Usually, a structured RNA, a simple stem-loop or a pseudoknot, is found downstream of the slippery sequence. The progression of the ribosome may be hindered from further movement by the presence of the adjacent secondary structure. This stalling is thought to contribute to the probability of a reading frame change in the −1 direction (Jacks et al., 1988), consistent with the demonstration that a pseudoknot can cause a translational pause (Tu et al., 1992; Somogyi et al., 1993).

The slippery heptanucleotides of HIV-1 and HTLV-2 alone suffice to mediate a low basic level of frameshifting in vivo, which is clearly above the background of translational errors (Parkin et al., 1992; Reil et al., 1993; Kollmus et al., 1994). The stem-loop structure that is positioned 4–8 nt downstream from the slippery sequence strongly enhances the basic level of frameshift-

Reprint requests to: Hansjörg Hauser, Genetics of Eukaryotes, GBF Gesellschaft fur Biotechnologische Forschung mbH., Mascheroder Weg 1, 38124 Braunschweig, Germany; e-mail: hha@gbf braunschweig.de.

ing. Mutational analysis of the HTLV-2 secondary structure showed that lowering the stability of the hairpin leads to a significant reduction of frameshift efficiency (Falk et al., 1993; Kollmus et al., 1994). On the other hand, mutational alterations of RNA structures predicted to have equal or greater free energy in some cases promote less frameshifting (Brierley et al., 1991; Kollmus et al., 1994). This indicates that specific sequence and/or structural features rather than stability alone are involved in the enhancement of ribosomal frameshifting efficiency. The question of whether or not this observation relates to a requirement for binding of cellular proteins to the downstream structure awaits clarification. Proteins that specifically bind to the secondary structures of frameshift sequences have not yet been identified, although they were often postulated (Jacks, 1990; Ten Dam et al., 1992, 1994) and RNA–protein interactions play central roles in other modes of gene regulation (McCarthy & Kollmus, 1995).

We addressed the question of such a regulatory mechanism by replacing the natural stem-loop sequence of HIV-1 with the iron-responsive element (IRE) from ferritin H-chain mRNA. Either one of two cytoplasmic proteins, iron-regulatory protein-1 (IRP-1) or IRP-2 binds to the IRE with equal affinity in iron-deficient cells, whereas IRE binding of both IRPs is switched off in iron-replete cells (Melefors & Hentze, 1993; Pantopoulos et al., 1995). Therefore, this RNA–protein interaction allows us to study the influence of a heterologous stem-loop structure on frameshifting efficiency and to demonstrate the possible involvement of an RNA–protein interaction. Our results, which have been obtained by using a highly sensitive and quantitative in vivo analysis system for ribosomal frameshifting, confirm that the presence of a hairpin alone is able to stimulate the basal level of ribosomal frameshifting without protein binding. Strikingly, protein binding to this structure strongly augments and thus regulates ribosomal frameshifting.

RESULTS

Basal frameshifting at the HIV-1 *gag-pol* site is enhanced by heterologous stem-loop structures

To study the efficiency of ribosomal frameshifting, we have developed a sensitive quantitative reporter system (Reil et al., 1993). This assay is outlined in Figure 1A. The reporter construct (pBgalluc-1) encodes the bacterial β-galactosidase (lacZ) to which the firefly luciferase is fused in the −1 frame. Nucleotide sequences to be tested for their ability to mediate and enhance ribosomal frameshifting are inserted between the reading frames of β-galactosidase and luciferase. Translation of the mRNA derived from this construct yields mainly β-galactosidase protein. Via frameshifting, a β-galactosidase–luciferase fusion protein (GAL-LUC) with both enzymatic activities is expressed. A control reporter construct (pBgalluc0), where luciferase is fused in frame to the β-galactosidase, serves as a reference (100% enzyme activity). The frameshifting efficiency is calculated by relating luciferase and β-galactosidase expression as enzymatic activities from pBgal-luc1 and pBgalluc0.

Previously, we determined the efficiency of frameshifting from *gag-pol* HIV-1 and HTLV-2 *gag-pro* overlap regions to range between 3 and 5% in several cell lines, including BHK-21 cells (Reil et al., 1993, 1994; Kollmus et al., 1994). Here, we have replaced the stem-loop of the HIV-1 frameshifting site by the IRE from the 5′ UTR of human ferritin H-chain mRNA (Hentze et al., 1987) which forms a moderately stable stem-loop structure with a bulge in the stem (Fig. 1B). The cellular IRPs bind to the wild-type IRE in iron-deficient cells, whereas a point deletion mutation in the loop of the IRE (IREΔC) serves as a negative control that displays similar thermal stability, but to which IRPs cannot bind (Leibold & Munro, 1988; Rouault et al., 1988; Goossen et al., 1990). These two sequences were introduced into the reporter plasmid pBgal-luc1 to replace the HIV-1 stem-loop.

Frameshifting efficiency in BHK-21 cells is given in Figure 2. The shifty sequence alone (pBgalluc-1_{SL}) induces a basal frameshifting activity that is at least 10-fold over the background (pBgalluc-1_{mut}). The downstream HIV-1 hairpin (pBgalluc-1) enhances this basal frameshift activity ~3-fold (frameshifting efficiency 3–4%). Interestingly, the IRE stimulates frameshifting activity (3.5%) as well as the wild-type HIV-1 stem-loop sequence. The mutant IRE (IREΔC) stimulates frameshifting activity, however, to a lesser degree (1.8%) than its wild-type counterpart. In contrast to the wild-type IRE, endogenous IRP binding to IREΔC is negligible. We therefore conclude that the frameshifting activity initiated by the shifty sequence of HIV-1 can be enhanced by heterologous sequences forming stem-loops, such as the IRE or IREΔC.

IRP binding to the downstream IRE specifically regulates frameshifting activity

IRPs repress ferritin and eALAS mRNA translation by binding to the IRE that is located in their 5′ UTRs (Melefors & Hentze, 1993). The IRE-binding activity of IRPs is iron-regulated such that IRPs bind to IREs with high affinity only in iron-deficient cells (Constable et al., 1992; Haile et al., 1992; Emery-Godman et al., 1993; Pantopoulos et al., 1995). By varying the iron content of the culture media, we assessed whether the regulated binding of IRPs to pBgalluc-1_{IRE} mRNA controls ribosomal frameshifting. Transient transfectants were either iron loaded (100 μM hemin) or iron starved (100 μM desferrioxamine). Because both compounds are toxic to the cells, the earliest time of addition is 24 h after transfection and 24 h before harvesting the cells. To monitor the effect of hemin and desferrioxamine on the

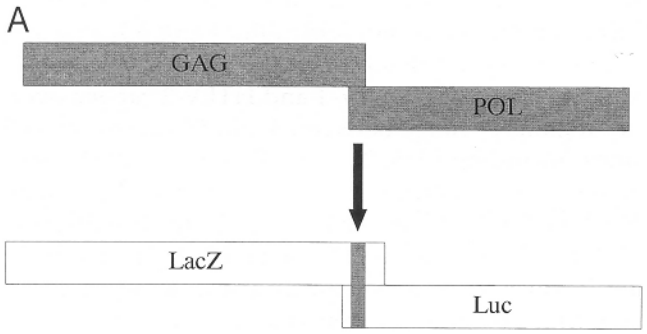

FIGURE 1. Plasmid constructions for the determination of HIV-1 heptanucleotide-mediated frameshift efficiency. **A:** Expression system for the determination of frameshift efficiency in vivo. To quantify frameshift efficiency in mammalian cells, an expression system was used in which the retroviral genes *gag* and *pol* are replaced by the genes encoding the enzymes β-galactosidase (lacZ) and firefly luciferase, respectively. The dashed box symbolizes a cassette, which comprises HIV-1 or HTLV-2 sequences essential for −1 frameshifting. Because luciferase is fused in the −1 reading frame relative to the β-galactosidase start codon, luciferase is only expressed when a −1 frameshift occurs. **B:** Constructs with HIV-1 heptanucleotide used for determination of frameshift efficiency. The respective frameshift cassettes are symbolized on the left. The β-galactosidase open reading frame is depicted as an open box; a hatched box on the right symbolizes the luciferase open reading frame; filled box, HIV-1 heptanucleotide slippery sequence; cross-hatched box, mutated heptanucleotide. The spacer (in nt) between the 3′ end of the slippery sequence and the 5′ end of the stem-loop is indicated. The nucleotide sequence of each frameshift cassette is shown. Sequences derived from HIV-1 are represented by capital letters. The slippery sequence is printed in boldface letters. The flanking stem-loop is marked by inverted arrows. Free energies of secondary structures were calculated with the computer program of Zuker and Stiegler (1981).

B

plasmids	nucleotide sequence	origin of stem-loop	ΔG
pBgalluc-1	**TTTTTTA**GGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAAG	HIV-1 *gag-pol* region	- 12,7
pBgalluc-1$_{IRE}$	**TTTTTTA**GGGAAgatcctgcttcaacagtgcttggacggatcg	IRE	- 4,6
pBgalluc-1$_{IRE\Delta C}$	**TTTTTTA**GGGAAgatcctgcttcaaagtgcttggacggatcg	mutated IRE	- 4,8
pBgalluc-1$_{SL}$	**TTTTTTA**GGGAA	-	-
pBgalluc-1$_{mut}$	cttcctcGGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAAG	HIV-1 *gag-pol* region	- 12,7

cells, CAT expression constructs were transfected in parallel. These constructs contain the IRE in the 5′ UTR of the CAT mRNA such that translation is inhibited upon IRP binding (Hentze et al., 1987; Gray & Hentze, 1994; Stripecke et al., 1994). Iron depletion leads to a strong inhibition of CAT expression of the wild-type IRE construct (IRE.CAT), whereas the mutant IRE construct (IREΔC.CAT) is not affected significantly (Fig. 3B). Iron depletion nearly doubles the frameshifting efficiency, when the frameshift cassette consists of the HIV-1 slippery sequence followed by the wild-type IRE (Fig. 3A). Due to the short half-life time of galactosidase in these cells (data not shown), we can exclude an underestimation of frameshifting efficiency. The specificity of this result is demonstrated by the lack of regulation of frameshift efficiency when the mutant IRE (IREΔC) or the HIV-1 secondary structure sequence are located 3′ to the shifty sequence. We conclude that cellular IRPs mediate iron-regulated ribosomal frameshifting of pBgalluc-1IRE mRNA by a mechanism that requires the presence of a wild-type IRE downstream of the slippery heptanucleotide.

Because the GAL-LUC reporters are driven by the potent SV40 promoter that induces strong expression in BHK-21 cells, the cellular IRP pool might become limiting for saturation of all IRE sequences in the transfected cells. We augmented the cellular IRP content by cotransfection of a human IRP-1 expression plasmid (Fig. 4). The alteration in IRE-binding activity was tested functionally using the IRE.CAT control vectors described in Figure 3. As expected, the mutant IREΔC.CAT construct remains unaffected (compare Fig. 4B with Fig. 3B), whereas the wild-type IRE construct displays more than 20-fold reduced CAT activity as a consequence of IRP-1 overexpression. We consistently noticed that iron regulation of CAT activity was apparently lost in these IRP overexpressing cells and that, even in the hemin-treated cells, CAT translation was repressed. Because IRE-BP is overexpressed, we suspect that the intracellular concentration of iron is not

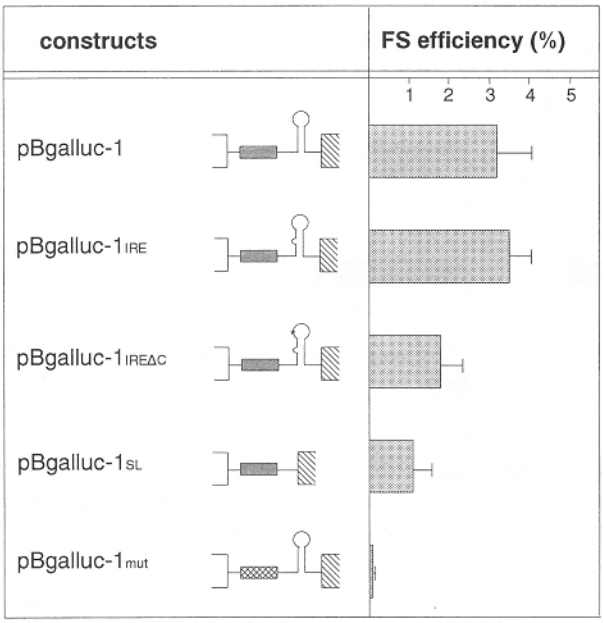

FIGURE 2. Secondary structures determine the efficiency of frameshifting. Frameshifting (FS) efficiency in vivo was determined by measuring enzymatic luciferase and β-galactosidase activity in transient transfection experiments using the indicated plasmids. Luciferase activity was normalized to the expression of β-galactosidase. Values are obtained from six independent transfection experiments in BHK-21 cells. Standard deviations are indicated by error bars. The symbols correspond to those in Figure 1B.

saturating all molecules. This effect would lead to a binding of IRE-BP molecules to the IRE stem-loop. The explanation of this apparent failure of cells to regulate the activity of overexpressed IRP-1 remains to be elucidated. Importantly, overexpression of IRP-1 augments the frameshifting activity of the wild-type IRE frameshift construct 2–3-fold. Because this effect is not observed with the mutant IRE frameshift construct or the HIV-1 construct, we conclude that the higher IRP levels further enhance the translational frameshifting activity, confirming the role of protein binding to the downstream stem-loop structure.

An IRE cannot functionally substitute the stem-loop structure downstream of the HTLV-2 slippery sequence

As shown before, the slippery sequence of HTLV-2 alone is also sufficient to induce a basal level of frameshifting, which is, however, less than that conferred by the HIV-1 heptanucleotide (compare pBgalluc-1_{SL} in Figure 2 and pBgalluc-1HSL1 in Figures 5 and 6; Kollmus et al., 1994). However, the HTLV-2 stem-loop structure is a more efficient enhancer of frameshifting activity than the respective HIV-1 secondary structure. Like the HIV-1 stem-loop structure (Kollmus et al., 1994), the IRE sequence does not augment the frameshifting activity of the HTLV-2 slippery sequence (Fig. 6): the expression background pBgalluc-H_{mut} is about 0.14%, the slippery sequence of HTLV-2, independent of the presence or absence of a downstream IRE sequence, induces frameshifting activity of about 0.6% (pBgalluc-1HIRE and -1HSL in Fig. 6). Furthermore, this efficiency is not improved by IRP overexpression and/or iron depletion (data not shown). Thus, even IRE/IRP complexes fail to boost frameshifting of the HTLV-2 slippery heptanucleotide sequence.

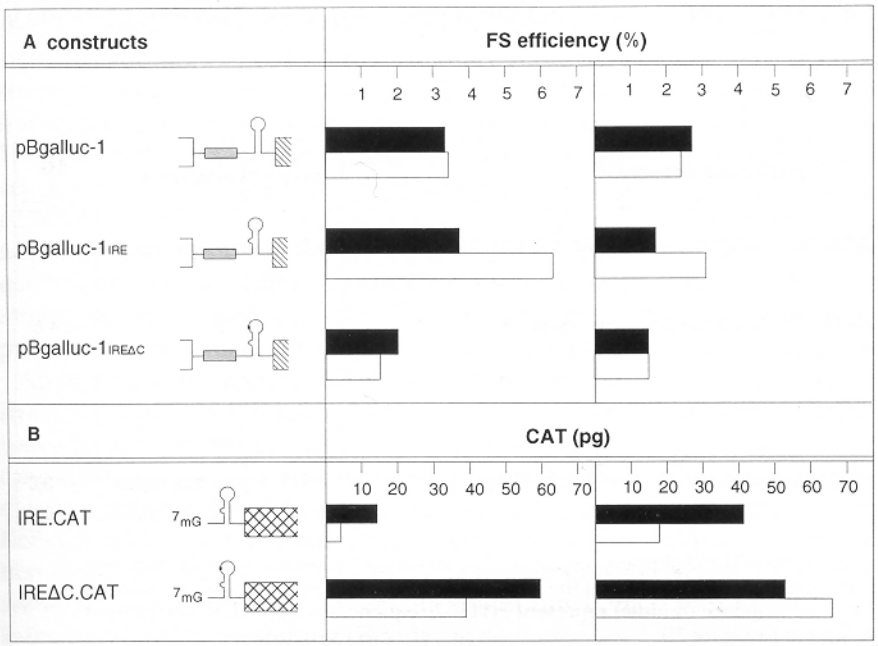

FIGURE 3. Iron-dependent regulation of frameshifting by IRP binding. **A:** The frameshift efficiency of the indicated plasmids after transient transfections in BHK-21 cells was measured in dependence of different concentrations of iron in the culture medium. The results of two independent experiments (left and right) are shown. Filled bars, 100 μM hemin as an iron source; open bars, iron depletion with 100 μM desferrioxamine. **B:** As a control for the iron status of the cell, the CAT plasmids were transfected in parallel and cultivated under the same conditions. The cross-hatched open box symbolizes the CAT open reading frame. The amount of CAT from cells grown in presence of hemin or desferrioxamine is indicated.

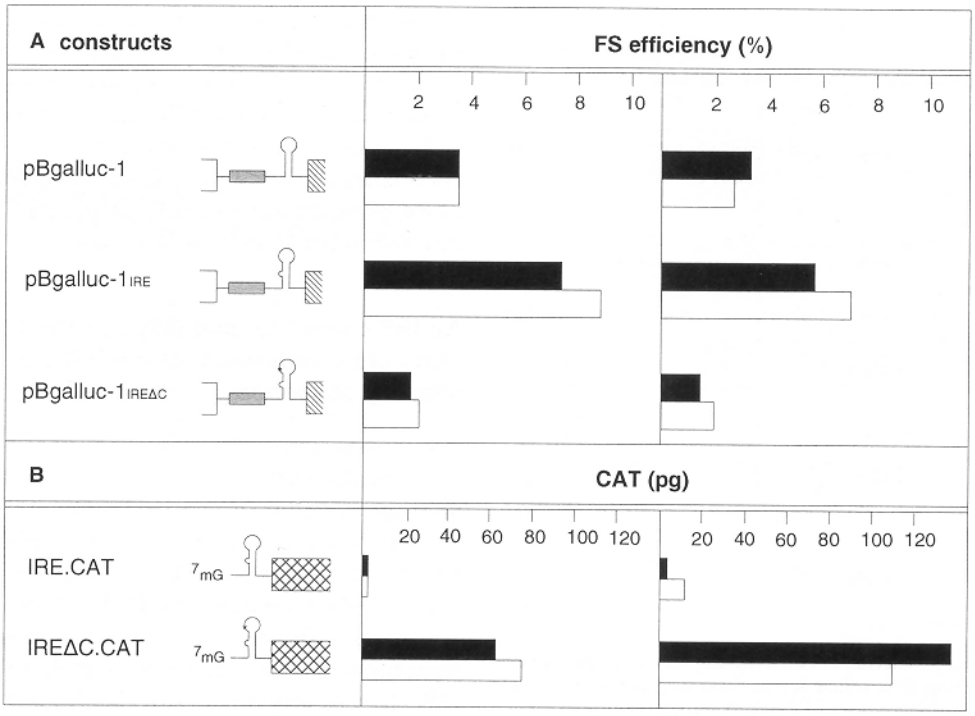

FIGURE 4. Overexpression of IRP-1 further enhances frameshifting. **A,B:** The indicated plasmids were cotransfected with the same amount of the plasmid pSG5hIRF, which encodes the human IRP-1. The symbols and culture conditions correspond to those of Figures 1B and 3.

DISCUSSION

We have devised an experimental strategy to study the influence of protein binding on frameshifting by exploiting the well-studied IRE/IRP interaction. Earlier work had validated this approach by demonstrating that the downstream secondary structures of some, but not all frameshift cassettes can be functionally replaced by heterologous hairpins (Kollmus et al., 1994). The data shown in Figures 2, 3, and 4 extend those findings and show that stem-loop sequences with an apparently unrelated physiological function can act as potent enhancers of the slippery sequence activity of the HIV-1 heptanucleotide.

Even in iron-replete cells, the IRE enhances frameshifting, suggesting that the stem-loop structure alone is sufficient for stimulation of the basic frameshift process from the slippery sequence. This interpretation is

plasmids	nucleotide sequence	origin of stem-loop	ΔG
pBgalluc-1H	**AAAAAAC**TCCTTAAGGGGGGAGATC**TAA**TCTCCCCCCgg	HTLV-2 *gag-pro* region	- 13,0
pBgalluc-1HIRE	**AAAAAAC**TCCTTAAgatcctgcttcaacagtgcttggacggatc	IRE	- 4,6
pBgalluc-1HSL1	**AAAAAAC**TCCTTAAGGGGGGAGATC	-	-
pBgalluc-1H$_{mut}$	gaagaatTCCTTAAGGGGGGAGATC**TAA**TCTCCCCCCgg	HTLV-2 *gag-pro* region	- 13,0

FIGURE 5. Frameshifting plasmids based on the HTLV-2 slippery sequence. The frameshift cassettes localized between the β-galactosidase and luciferase coding regions are symbolized on the left. The HTLV-2 slippery sequence is shown as an open box. Capital letters of the nucleotide sequence (middle) represent HTLV-2 sequences; stop codons are printed in boldface. For further details, see the legend to Figure 1B.

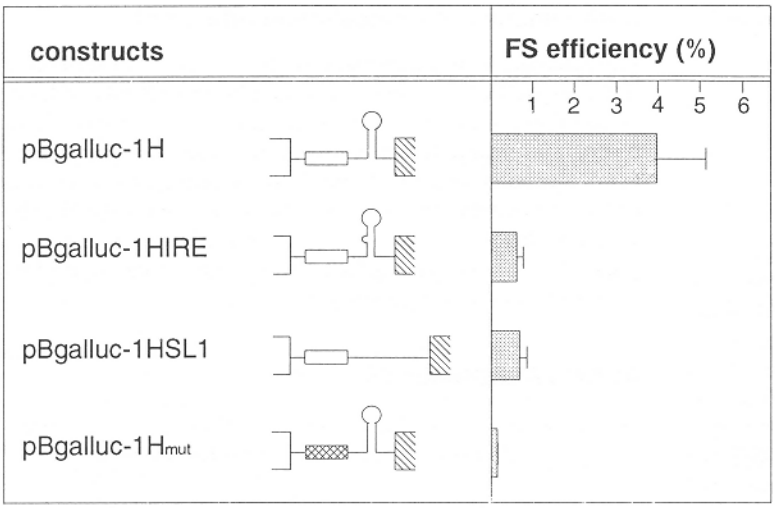

FIGURE 6. The HTLV-2 shifty sequence does not respond to a heterologous downstream secondary structure. The frameshift (FS) efficiency of the plasmids after transient transfection in BHK-21 cells was determined as described in Figure 2.

also supported by the stimulatory effect of the IREΔC mutant, for which IRP binding is negligible (Rouault et al., 1988; Goossen et al., 1990).

In contrast to the HIV-1 heptanucleotide sequence, the IRE does not stimulate the frameshift activity directed by the HTLV-2 slippery sequence. This is likely explained by the low activity of this slippery sequence to initiate frameshifting. The relative instability of the IRE compared to the HTLV-2 secondary structure may also contribute to this inability, which the IRE shares with other secondary structures, including the HIV-1 stem-loop and mutants of the HTLV-2 stem-loop (Kollmus et al., 1994). In the HTLV-2 constructs, the IRE stem-loop is located 7 nt from the slippery sequence, at an identical position, as is the natural HTLV-2 stem-loop to the slippery sequence. This distance is also within the range of 4–8 nt, determined to be the optimum distance between the slippery sequence and stem-loop structure for frameshifting (Kollmus et al., 1994). In addition, specific RNA sequence or tertiary structure determinants may be required to enhance frameshifting across certain heptanucleotide sequences. The combination of incompatible downstream secondary structures with a weak slippery sequence could also account for the results of Chen et al. (1995), who did not observe frameshifting from the *gag-pro* slippery sequence of MMTV with different heterologous secondary structures. This is supported by the fact that the slippery sequences from HTLV-2 and MMTV are identical.

Multiple lines of experimental evidence clearly demonstrate that a possible "roadblock" for approaching ribosomes imposed by the downstream secondary structure cannot be interpreted solely in terms of their thermodynamic stability (Brierley et al., 1991; Falk et al., 1993; Kollmus et al., 1994). In particular, a defined combination of sequence and structure of some pseudoknots seems to be important for the enhancement function in the ribosomal frameshifting (Brierley et al., 1991; Ten Dam et al., 1994; Chen et al., 1995). These observations have prompted models that invoke proteins to stimulate frameshifting efficiency by stabilizing the secondary structure to stall the ribosome or to prevent unwinding of the helix.

This report demonstrates that the binding of cytoplasmic proteins (IRPs) to a stem-loop structure (IRE) positioned downstream of the HIV-1 slippery sequence regulates programmed ribosomal frameshifting in vivo. Regulation occurs as a function of both the intracellular iron concentration as well as the concentration of the binding protein. What is the mechanism by which IRPs regulate frameshifting? The predominantly cytosolic occurrence and lack of ribosomal association of IRPs suggests that a protein that regulates frameshifting does not need to be bound directly to the frameshifting ribosome. Because IRPs do not appear to have evolved to regulate frameshifting physiologically, its mode of action is most likely of sterical nature. Conceivably, IRPs bound to the downstream IRE promote ribosomal pausing, which in turn may favor the frameshifting event. An alternative explanation is that IRPs may stabilize the IRE or influence its refolding in such a way that frameshifting is increased. The results with this experimental model system suggest strongly that ribosomal frameshifting could be regulated physiologically by RNA–protein interactions. However, direct experimental evidence for this is still lacking.

Recently, frameshifting was recognized to be required also for the regulation of cellular genes: the expression of the ornithine decarboxylase antizyme depends on +1 ribosomal frameshifting (Rom & Kahana, 1994; Matsufuji et al., 1995). This process is regulated by polyamines. Although the molecular mechanism is unknown currently, our findings imply the possibility of a regulated interaction between the frameshift region and a binding protein. Finally, the ability to regulate frameshifting in a controlled way offers an experimen-

tal tool to further elucidate the mechanism(s) underlying ribosomal frameshifting.

MATERIALS AND METHODS

Plasmid constructions

The plasmids pBgalluc-1, pBgalluc-1_{SL}, pBgalluc-1_{hepta}, and pBgalluc-1_{mut}, which harbor HIV-1 frameshift sequences, were described earlier (Reil et al., 1993). Plasmid pBgalluc-1_{IRE} was constructed by replacement of the stem-loop of the HIV-1 frameshift cassette (*Bgl* II–*Bam*H I fragment) of pBgalluc-1 with a 33-bp DNA fragment (5′-GATCCTGCTTC AACAGTGCTTGGACGGATCG-3′) that encodes the synthetic IRE structure described earlier (Hentze et al., 1987). For construction of pBgalluc-$1_{IRE\Delta C}$, which harbors the mutated form of the IRE, the DNA fragment, obtained by digestion of DHI.19CAT (named Δ165 in Hentze et al., 1987) with *Bam*H I was inserted into pBT7FSLuc1 (Reil et al., 1993). This plasmid was cut with *Sal* I and *Sma* I, whereby the generated fragment was replaced by the *Sal* I–*Sma* I fragment of pBgalluc-1. The reading frame was identical to the IRE wild-type construct, where five additional nucleotides were inserted 3′ to the stem-loop structure.

The plasmids pBgalluc-1H, pBgalluc-1HSL1, and pBgalluc-$1H_{mut}$, which harbor HTLV-2 frameshift sequences, were described earlier (Kollmus et al., 1994). Plasmid pBgalluc-1HIRE, which has an IRE stem-loop in its frameshift cassette instead of the HTLV-2 wild-type secondary structure, was cloned by digesting the plasmid pBgalluc-2H (Kollmus et al., 1994) with *Afl* II and *Bam*H I. This fragment was replaced by a 29-bp oligonucleotide (5′-TTAAGATCCTGCTTCAACAGT GCTTGGACG-3′). All plasmids were characterized by restriction endonuclease analysis and DNA sequencing. The plasmids IRE.CAT and IREΔC.CAT are the renamed plasmids DH4I.12CAT and DH4I.19CAT, respectivly, which have been described previously (Hentze et al., 1987). Plasmid pSG5hIRF contains the sequence of the human IRP-1 under the control of the SV40 promotor and was kindly provided by Dr. Ö. Melefors.

Cell culture and gene transfer

BHK-21, baby hamster kidney cells (ATCC CC110) were cultivated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transient transfections with plasmids were performed by the calcium phosphate coprecipitation technique as detailed earlier (Reil & Hauser, 1990). Hemin (Sigma) (100 μM) as an iron source or desferrioxamine (Sigma) (100 μM) as an iron chelator were added to the culture media 24 h after transfection, and cells were incubated subsequently for 24 h.

Reporter gene assays

Determination of β-galactosidase and luciferase activity in transient transfectants of BHK21 cells was performed by standard assays detailed previously (Reil et al., 1993). The protein amount of CAT enzyme in the cell extracts was determined by ELISA (Boehringer).

Determination of frameshifting efficiency

The calculation of the frameshift efficiency was described in detail by Reil et al. (1993). In principle, the activity of luciferase expressed following transfection with a construct harboring an inframe fusion between the open reading frames of gal and luc was set to 100%. Frameshifting efficiency was calculated by relating the enzymatic activity expressed by the various constructs to that of the inframe fusion constructs. β-galactosidase enzyme activity from various (fusion) protein constructs is indistinguishable.

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REFERENCES

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

Berry MJ, Larsen PR. 1993. Recognition of UGA as a selenocysteine codon in eukaryotes: A review of recent progress. *Biochem Soc Trans 21*:827–832.

Böck A, Forchhammer K, Heider J, Baron C. 1991. Selenoprotein synthesis: An expansion of the genetic code. *Trends Biochem Sci 16*:463–467.

Brierley I, Rolley NJ, Jenner AJ, Inglis SC. 1991. Mutational analysis of the RNA pseudoknot component of a coronavirus ribosomal frameshifting signal. *J Mol Biol 220*:889–902.

Chen X, Chamorro M, Lee SI, Shen LX, Hines JV, Tinoco I Jr, Varmus HE. 1995. Structural and functional studies of retroviral RNA pseudoknots involved in ribosomal frameshifting: Nucleotides at the junction of the two stems are important for efficient ribosomal frameshifting. *EMBO J 14*:842–852.

Constable A, Quick S, Gray NK, Hentze MW. 1992. Modulation of the RNA-binding activity of a regulatory protein by iron in vitro: Switching between enzymatic and genetic function? *Proc Natl Acad Sci USA 89*:4554–4558.

Emery-Goodman A, Hirling H, Scarpellino L, Henderson B, Kühn LC. 1993. Iron regulatory factor expressed from recombinant baculovirus: Conversion between the RNA-binding apoprotein and Fe-S cluster containing aconitase. *Nucleic Acids Res 21*:1457–1461.

Falk H, Mador N, Udi R, Panet A, Honigman A. 1993. Two *cis*-acting signals control ribosomal frameshift between human T-cell leukemia virus type II *gag* and *pro* genes. *J Virol 67*:6273–6277.

Gesteland RF, Weiss RB, Atkins JF. 1992. Recoding: Reprogrammed genetic decoding. *Science 257*:1640–1641.

Goossen B, Caughman SW, Harford JB, Klausner RD, Hentze MW. 1990. Translational repression by a complex between the iron-responsive element of ferritin mRNA and its specific cytoplasmic binding protein is position-dependent in vivo. *EMBO J 9*:4127–4133.

Gray NK, Hentze MW. 1994. Iron regulatory protein prevents binding of the 43S translation pre-initiation complex to ferritin and eALAS mRNAs. *EMBO J 13*:3882–3891.

Haile DJ, Rouault TA, Harford JB, Kennedy MC, Blondin GA, Beinert H, Klausner RD. 1992. Regulation of interaction of the iron-responsive element binding protein with iron-responsive RNA elements. *Proc Natl Acad Sci USA 89*:11735–11739.

Hatfield DL, Levin JG, Rein A, Oroszlan S. 1992. Translational suppression in retroviral gene expression. *Adv Virus Res 41*:193–239.

Hentze MW, Caughman SW, Rouault TA, Barriocanal JG, Dancis A, Harford JB, Klausner RD. 1987. Identification of the iron responsive element for the translational regulation of human ferritin mRNA. *Science 238*:1570–1573.

Jacks T. 1990. Translational suppression in gene expression in retro-

viruses and retrotransposons. *Curr Topics Microbiol Immunol 157*: 93–124.

Jacks T, Madhani HD, Masiarz FR, Varmus HE. 1988. Signals for ribosomal frameshifting in the Rous Sarcoma Virus *gag-pol* region. *Cell 55*:447–458.

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.

Leibold EA, Munro HN. 1988. Cytoplasmic protein binds in vitro to a highly conserved sequence in the 5′ untranslated region of ferritin heavy- and light-subunit mRNAs. *Proc Natl Acad Sci USA 85*:2171–2175.

Matsufuji S, Matsufuji T, Miyazaki Y, Murakami Y, Atkins JF, Gesteland RF, Hayashi S. 1995. Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. *Cell 80*: 51–60.

McCarthy JEG, Kollmus H. 1995. Cytoplasmic mRNA–protein interactions in eukaryotic gene expression. *Trends Biochem Sci 20*:191–195.

Melefors Ö, Hentze MW. 1993. Iron regulatory factor—The conductor of cellular iron regulation. *Blood Rev 7*:18.

Pantopoulos K, Gray NK, Hentze MW. 1995. Differential regulation of two related RNA-binding proteins, iron regulatory protein (IRP) and IRP_B. *RNA 1*:155–163.

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, Hauser H. 1990. Test system for determination of HIV-1 frameshifting efficiency in animal cells. *Biochim Biophys Acta 1050*: 288–292.

Reil H, Hoexter M, Moosmayer D, Pauli G, Hauser H. 1994. CD4 expressing human 293 cells as a tool for studies in HIV-1 replication: The efficiency of translational frameshifting is not altered by HIV-1 infection. *Virology 205*:371–375.

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

Rom E, Kahana C. 1994. Polyamines regulate the expression of ornithine decarboxylase antizyme in vitro by inducing ribosomal frame-shifting. *Proc Natl Acad Sci USA 91*:3959–3963.

Rouault TA, Hentze MW, Caughman SW, Harford JB, Klausner RD. Binding of a cytosolic protein to the iron-responsive element of human ferritin messenger RNA. *Science 241*:1207–1210.

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

Stripecke R, Oliveira CC, McCarthy JEG, Hentze MW. 1994. Proteins binding to 5 UTR sites: A general mechanism for translational regulation of mRNAs in human and yeast cells. *Mol Cell Biol 14*: 5898–5909.

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

Ten Dam E, Pleij C, Draper D. 1992. Structural and functional aspects of RNA pseudoknots. *Biochemistry 31*:11665–11676.

Tu C, Tzeng TH, Bruenn JA. 1992. Ribosomal movement impeded at a pseudoknot required for frameshifting. *Proc Natl Acad Sci USA 89*:8636–8640.

Zuker M, Stiegler P. 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acids Res 9*:133–148.