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EXPRESSION VECTORS FOR QUANTITATING *IN VIVO* TRANSLATIONAL AMBIGUITY: THEIR POTENTIAL USE TO ANALYSE FRAMESHIFTING AT THE HIV gag-pol JUNCTION

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SUMMARY

Translational errors are necessary so as to allow gene expression in various organisms. In retroviruses, synthesis of *pol* gene products necessitates either readthrough of a stop codon or frameshifting. Here we present an experimental system that permits quantification of translational errors *in vivo*. It consists of a family of expression vectors carrying different mutated versions of the *luc* gene as reporter. Mutations include both an in-frame stop codon and 1-base-pair deletions that require readthrough or frameshift, respectively, to give rise to an active product. This system is sensitive enough to detect background errors in mammalian cells. In addition, one of the vectors contains two unique cloning sites that make it possible to insert any sequence of interest. This latter vector was used to analyse the effect of a DNA fragment, proposed to be the target of high level slippage at the gag-pol junction of HIV. The effect of paromomycin and kasugamycin, two antibiotics known to influence translational ambiguity, was also tested in cultured cells. The results indicate that paromomycin diversely affects readthrough and frameshifting, while kasugamycin had no effect.

This family of vectors can be used to analyse the influence of structural and external factors on translational ambiguity in both mammalian cells and bacteria.

KEY-WORDS: Translation, HIV, Luciferase; gag-pol Junction, Frameshifting, Expression vectors.

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INTRODUCTION

The fidelity of information flow in biological systems is essential. Cells are endowed with mecanisms which control accuracy at each stage of the molecular processes. Translational fidelity is situated at a level which can be considered optimal rather than maximal: this is deduced from the fact that it is possible to increase the natural level by mutation (Ehrenberg *et al.*, 1986). However, some organisms have built into their system of regulation a compulsory level of translational error. Retroviruses are undisputed leaders of this expression mode (for recent reviews see Varmus, 1988; Hatfield and Oroszlan, 1990; Atkins *et al.*, 1990), but it has also been shown that cellular genes can require high translational error levels in order to be expressed (Craigen *et al.*, 1985; Craigen and Caskey, 1986; Tsuchihashi and Kornberg, 1990).

In retroviruses, the *pol* gene lies downstream of the *gag* gene, but the initiation of translation occurs only at the AUG of the gag message and the polyprotein is later cleaved into active products (see Debouck *et al.*, 1987 for an example). Three kinds of arrangements are used: the *gag* and *pol* genes are (1) in frame but separated by a termination codon, (2) in different frames with a small overlap, and (3) a third gene, *pro*, coding for a protease is present in frame with *gag* or *pol*, and overlapping the other (Jacks and Varmus, 1985; Yoshinaka *et al.*, 1985; Jacks *et al.*, 1987; Hizi *et al.*, 1987). A regulation by translational ambiguities is adopted by readthrough of the stop codon in case (1) and by one or two frameshifts in cases (2) and (3) to synthesize the pol products which are required in smaller amounts than the gag protein.

Beside these targeted errors allowing gene expression, a possible role for translational ambiguity during cell differentiation has been suggested by the properties of several ribosomal mutations in the lower eukaryote *Podospora anserina* (Picard-Bennoun, 1982; Dequart-Chablat *et al.*, 1986).

We have undertaken to devise an experimental system that permits the quantification of translational ambiguity *in vivo*. This would make possible not only the analysis of sequences suspected to be targets of enforced translational error, such as gag-pol junctions of retroviruses, but also to detect possible variations in translational accuracy during differentiation and various physiological states in higher eukaryotic cells.

Here we describe a family of expression vectors developed for this purpose. The test is based on the following principle: a stop codon or a frameshift sequence is introduced into the coding frame of a reporter gene. According to the mutation introduced, translational errors can lead to readthrough of a stop codon or frameshift, and an active peptide is thereby synthesised. Such systems have already been described but failed to exhibit a sensitivity level

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RSV = Rous sarcoma virus.

compatible with detection of background errors in mammalian cells (Martin *et al.*, 1989). As a reporter, we chose the *luc* gene from *Photinus pyralis*, since the luciferase assay is the most sensitive for any reporter gene in current use (Schwartz *et al.*, 1990; Williams *et al.*, 1989). With this system, we have been able to measure levels of translational errors as low as 10^{-5}. We have also tested the effect of two antibiotics known to affect translational accuracy: paromomycin (Wilhem *et al.*, 1978; Burke and Mogg, 1985) and kasugamycin (van Bull *et al.*, 1974).

The family of vectors described in this paper can be used to analyse both the *cis*- and *trans*-acting signals that influence translational fidelity. Here, a plasmid containing two unique cloning sites was used to evaluate *in vivo* the frameshifting activity obtained with a DNA fragment from the gag-pol junction of HIV1.

MATERIALS AND METHODS

Bacterial strains and plasmids.

Escherichia coli CM5 α (DH5α/F' *tet*) (Camonis *et al.*, 1990) was used as host for plasmid and M13 propagation; CDJ64 (F⁻/*val*^{r}, Δ *lac-pro*, *nal*^{r}, *rif*^{r}, *thi*, *sup9*) was used as host for the plasmid carrying the UGA mutation (pRSVL74).

Plasmids pRSVL74 and pRSVL121 were derived from pRSVL (de Wet *et al.*, 1987) after *in vitro* mutagenesis (Nakamaye and Eckstein, 1986) with a 27mer oligonucleotide 5'GAAGACGCTAGCTGATCAAACATAAAG3' (pRSVL74) or 25mer 5'GACGCAAAAACATAAAGAAAGGCCC3' (pRSVL121).

pRSVL89 and pRSVL90 were derived from pRSVL74 by digestion with *Nhe*I and *Bcl*I and ligation with two complementary 29mer oligonucleotides 5'CTAGCCA GGCTAATTTTTTAGGGAAGATC3' and 5'GATCGATCTTCCCTAAAAAATT AGCCTGG3' (pRSVL89) or 30mer 5'CTAGCCAGGCTAATTTTTTAAGGG AAGATC3' and 5'GATCGATCTTCCCTTAAAAAATTAGCCTGG3' (pRSVL90).

pCH110 carries a *lacZ* gene under control of the SV40 promoter/enhancer region (Hall *et al.*, 1983).

pRSVLTo5 (kindly provided by O. Bensaude, Institut Pasteur, Paris) was derived from pRSVL after digestion with *Hin*dIII and *Nar*I, which leads to a deletion of the region surrounding the initiator ATG (see fig. 2).

Cells.

NIH3T3 cells were obtained from Dr. M. Sitbon (INSERM U152, Hôpital Cochin, 75014 Paris). They were cultured in modified Ham's F12 medium supplemented with 5 % fœtal calf serum, at 37°C in humidity saturated 7 % CO_2 in air.

Transfection.

One million cells were seeded in a 10-cm diameter Petri dish the day before transfection, transfected with 10 μg of each relevant plasmid and 5 μg of the pCH110 vector using calcium phosphate precipitation, and the medium was changed 24 h later.

Cells were harvested after two days and crude extracts were prepared as previously reported (Camonis *et al.*, 1990).

Enzyme assays.

Luciferase was assayed as described (Nguyen *et al.*, 1988). β-Galactosidase activities were determined according to Miller (1972).

Antibiotic treatment.

Cells were treated with paromomycin (800 μg/ml) (Martin *et al.*, 1989) or kasugamycin (800 μg/ml) the day after transfection, and harvested two days later. After the two days of treatment with kasugamycin, only a 10 % increase in the generation time was observed.

RESULTS

Construction of vectors with mutated *luc* genes.

The parental vector, pRSVL (de Wet *et al.*, 1987), contains the *luc* gene under control of the LTR (long terminal repeat) promoter from the Rous sarcoma virus (RSV) (fig. 1). This allows expression in both mammalian cells and in *E. coli* (de Wet *et al.*, 1987).

The 118-bp fragment between the unique *Hin*dIII and *Xba*I sites from pRSVL was subcloned in M13 mp18 (see fig. 1 and 2). This single-stranded template was used for site-directed mutagenesis. The mutated fragments were sequenced and introduced back into pRSVL in place of the wild-type sequence. Presence of the mutations was confirmed by DNA sequencing of the entire *Hin*dIII-*Xba*I fragment in the final construct.

pRSVL121 contains a deletion of one base at the fourth codon (GCC). This mutation leads to a stop codon (UAA) at the seventh position. Accordingly, a slippage in −1 frame, upstream from this codon (probably at the

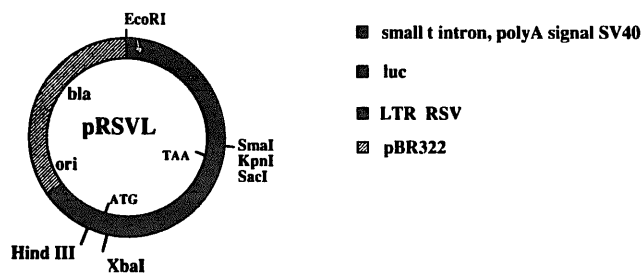

FIG. 1. — *Plasmid pRSVL.*

pRSVL is the base vector for all constructions described in the text.

```
   HindIII  EcoRI
CCAAGCTTGGAATTCCTTTGTGTTACATTCTTGAATGTC

GCTCGCAGTGACATTAGCATTCCGGTACTGTTGGTAAA

ATG GAA GAC GCC AAA AAC ATA AAG AAA GGC
   NarI                 XbaI
CCG GCG CCA TTC TAT CCT CTA GAG GAT GGA

ACC GCT GGA GAG CAA CTG CAT AAG GCT ATG

AAG AGA TAC GCC ...
```

FIG. 2. — *Sequence of the region surrounding the initiator ATG in the wild-type* luc *gene.*

```
                met glu asp ala lys asn ile lys lys gly
pRSVL (wt)      ATG GAA GAC GCC AAA AAC ATA AAG AAA GGC

                met glu asp ala lys thr stop
pRSVL121        ATG GAA GAC GC*A AAA ACA TAA AGA AAG GC
     0 frame    met glu asp ala lys
    -1 frame                    /asn ile lys lys gly

                met glu asp ala ser stop
pRSVL74         ATG GAA GAC GCT AGC TGA TCA AAC ATA AAG AAA GGC
                          NheI  BclI

                met glu asp ala ser glu ala asn phe leu gly arg ile asp gln thr stop
pRSVL89         ATG GAA GAC GCT AGC|CAG GCT AAT TTT TTA GGG AAG ATC|GAT CAA ACA TAA AGA AAG GC
     0 frame    met glu asp ala ser glu ala asn phe leu
    -1 frame                                        /arg glu asp arg ser asn ile lys lys gly

                met glu asp ala ser glu ala asn phe leu arg glu asp arg ser asn ile lys lys gly
pRSVL90         ATG GAA GAC GCT AGC|CAG GCT AAT TTT TTA AGG GAA GAT C|GA TCA AAC ATA AAG AAA GGC
```

FIG. 3. — *Nucleotide sequence and translation of the 5' region of the* luc *gene and mutated derivatives.*

The natural frame translation sequence is shown above the nucleotide sequence. For pRSVL121 and pRSVL89, the deduced amino acid sequence resulting from a −1 shift (see text) is given below the nucleotide sequence. In pRSVL121, the star indicates the deleted nucleotide. In pRSVL74, restriction sites for *Nhe*I and *Bcl*I are underlined. In pRSVL89 and pRSVL90, the vertical bar delineates the insertion site of the HIV slippery sequence (see "Materials and Methods").

sequence CA AAA AC), restores the normal translation frame and allows the synthesis of an active protein (see fig. 3).

To insert other sequences whose translational error potency was to be tested, two additional unique sites, *Nhe*I (GCTAGC) and *Bcl*I (TGATCA), were introduced. This gave rise to pRSVL74 which contains a stop codon (UGA)

from the *BclI* site, in frame with the *luc* gene at the sixth position (see fig. 3). This vector permits measurement of translational readthrough. A 29-bp synthetic DNA fragment containing the sequence of the gag-pol junction, proposed to be the site of frameshifting in HIV (Jacks *et al.*, 1988; Wilson *et al.*, 1988), was introduced at the *Nhe*I-*Bcl*I sites; this led to pRSVL89 (see fig. 3). We introduced, in the same way, a DNA fragment containing the HIV sequence with an insertion of one adenine downstream of the leu codon (UUA): this addition restores the reading frame of the *luc* gene (see fig. 3) and results in the amino acid sequence predicted by the model of slippage at this site (Jacks *et al.*, 1988). This allowed us to test the influence of the added amino acid residues on the specific activity of the resulting protein. The activity of the altered luciferase encoded by this gene (harbored by the plasmid pRSVL90) was about 40 % that of the wild type in mammalian cells (see below). This indicates that the luciferase protein exhibited a certain flexibility at its amino terminal part.

Basal level of translational accuracy in mammalian cells.

To assay for translational errors in mammalian cells, the different vectors were transfected into mouse NIH3T3 cells, together with the pCH110 vector. The latter vector, which carries a *lacZ* gene was used as an internal control for transfection efficiency. Luciferase and β-galactosidase activities were determined two days after transfection. The NIH3T3 line was chosen because of its high transfection efficiency, but similar results were obtained with cells from the L and HepG2 lines (data not shown). With the pRSVL wild-type vector, luciferase activities of up to 2×10^{10} c.p.m./10 μg plasmid/10^6 cells (corresponding to one transfected dish) were obtained; in comparison, the luciferase activity from the mock control (either non-transfected cells or cells transfected with pCH110 and 10 μg of pBR322 DNA) varied between 2×10^4 and 10×10^4 c.p.m. Therefore, the sensitivity of this system is high enough to permit the detection of translational errors as low as 10^{-5}.

Results of transient expression experiments are shown in table I. They are expressed as the ratio of luciferase activities obtained with each vector compared to pRSVL, and standardized for transfection efficiency relative to β-galactosidase activity. Measurable enzyme activities were obtained with all of the vectors. This illustrates the potency of the luciferase system for detecting very low levels of protein synthesis. Strikingly, the level obtained with the readthrough construction (pRSVL74) was 30-fold weaker than that obtained with the frameshift construction (pRSVL121).

We tested the efficiency of the shifty sequence from the gag-pol junction of HIV1 using pRSVL89, which carries the sequence defined previously *in vitro* and in budding yeast (Wilson *et al.*, 1988). This vector gave a frameshifting efficiency of 2.5×10^{-3}, as compared to the activity obtained with the control pRSVL90. This was not significantly different from the value obtained with the pRSVL121 construct and was far lower than the pol/gag ratio ob-

TABLE I. — **Translational error frequency in mammalian cells.**

Vector	Type of error introduced	No antibiotic	Paromomycin 800 μg/ml	Kasugamycin 800 μg/ml
None	–	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$
pRSVL	–	1(*)	1 (±0.2)	1 (±0.2)
pRSVL90	Addition of codons	0.4 (±0.2)	ND	ND
pRSVL74	Stop codon	$6.2\ (\pm 0.6)\times 10^{-5}$	$180\ (\pm 20)\times 10^{-5}$	$5.9\ (\pm 0.7)\times 10^{-5}$
pRSVL121	Frameshift	$1.9\ (\pm 0.2)\times 10^{-3}$	$1.5\ (\pm 0.2)\times 10^{-3}$	$2\ (\pm 0.2)\times 10^{-3}$
pRSVL89	Frameshift	$1\ (\pm 0.2)\times 10^{-3}$	$0.9\ (\pm 0.2)\times 10^{-3}$	$1.1\ (\pm 0.1)\times 10^{-3}$
pRSVLTo5	Deletion of the first AUG	$<10^{-5}$	ND	ND

Luciferase/β-galactosidase ratio compared to pRSVL. Mean of at least 3 independent experiments.
(*) 2×10^{10} c.p.m. for 10^6 cells transfected with 10 μg of vector (see text).
ND = not determined.

served in infected cells (see, for instance, Lightfoote *et al.*, 1986), although no precise quantification has been reported up to now.

To verify that the enzyme activities were not due to reinitiation at an internal AUG, the *Hin*dIII-*Nar*I fragment (see sequence on fig. 2) was deleted: this eliminated the natural initiator codon and made it possible to evaluate a possible reinitiation at the second AUG (30 th codon). In cells transfected with the resulting pRSVLTo5 plasmid, no luciferase activity was detected. This indicates that no active protein was produced from the second AUG.

To eliminate the possibility that the basal activities obtained reflected the presence in the DNA preparation of revertants carrying a wild type *luc* gene, we measured the mutation level at the unique *Nhe*I site of pRSVL89. A plasmid DNA preparation was digested with *Nhe*I and used to transform *E. coli* cells. Any mutation in the recognition site would lead to *Nhe*I-resistant plasmid molecules. Transformants were obtained at a frequency of 10^{-4} as compared to the control. All of the 32 clones obtained possessed plasmid sensitive to *Nhe*I digestion and were likely to result from non-cleaved products of the DNA preparation. In addition, no luciferase activity corresponding to a wild-type gene was found in crude extracts from these clones. This enabled us to estimate the mutation frequency to be lower than 4×10^{-6}, too low to account for the luciferase activities found.

Effect of antibiotics on the translational accuracy level in mammalian cells.

Two antibiotics were tested: paromomycin has been shown to increase readthrough of the three termination codons in mammalian cells (Wilhem *et al.*, 1978; Burke and Mogg, 1985; Martin *et al.*, 1989), but its effect on frameshifting has never been tested; conversely, kasugamycin increases translational fidelity in *E. coli* (van Bull *et al.*, 1974) and has never been evaluated in mammalian systems.

Results presented in table I show that paromomycin increased translational readthrough of the UGA codon by 30-fold. This was consistent with what was already known for the effect of this antibiotic. It is worth noting that paromomycin had no effect on the level of -1 frameshifting. In addition, no effect on translational accuracy was observed with kasugamycin, whatever the vector used (table I). This could reflect a lack of permeation in higher eukaryotes, although lower eukaryotes are sensitive to this compound (Umezawa *et al.*, 1965). More interestingly, this could reflect an idiosyncratic behaviour of the translational apparatus.

Basal level of translational accuracy in *E. coli*.

Although the constructs were not designed for expression in bacteria (lack of a ribosome-binding site upstream from the AUG), we also measured translational accuracy in *E. coli* to determine whether the overall level of errors

was similar in mammalian cells and bacteria. CM5α cells, of an opal suppressor-free strain (Camonis *et al.*, 1990), were transformed with the different plasmids and luciferase-specific activities were determined in crude extracts. Results are expressed as the ratio of specific activities obtained with the different vectors compared to pRSVL (table II). Strain CDJ64, harbouring an opal suppressor tRNA with a tryptophan insertion at the UGA codon, was also transformed with pRSVL74. The luciferase activity found in this strain was 30-fold higher than the basal readthrough, but even with the suppressor-free strain CM5α, significant enzyme activities were obtained with all the mutations (table II). The efficiency of readthrough of the opal codon in pRSVL74 was about 10-fold weaker than that of frameshifting with pRSVL121. Many results obtained in *E. coli* have shown that codon context can affect up to 100-fold the efficiency of readthrough (Ehrenberg *et al.*, 1986; Bossi, 1983; Miller and Albertini, 1983). The difference between pRSVL74 and the other constructions may thus be due to an unfavourable particular codon context.

As in mammalian cells, pRSVLTo5 did not exhibit any significant activity. Therefore, in bacteria as well, no functional protein could be synthesized in the absence of the first AUG.

DISCUSSION

The purpose of the present work was to develop a tool to measure translational accuracy *in vivo* in eukaryotic cells. To achieve this goal, a family of vectors harbouring different mutations in the *luc* gene, used as reporter, were constructed. In these vectors, a stop codon was introduced artificially in the coding frame of the *luc* gene and translational errors led to synthesis of an active peptide. The different mutated derivatives included the insertion of an in-frame UGA codon to measure the readthrough activity of the translational machinery (pRSVL74) and a frameshift mutation (pRSVL121) to test -1 slippage. In addition, the pRSVL74 plasmid possesses an oriented cloning

TABLE II. — **Translational error frequency in *E. coli* CM5α cells.**

Vector	Type of error introduced	Luciferase activity
None	–	$<10^{-5}$
pRSVL	–	1 (*)
pRSVL90	Addition of codons	0.45 (±0.2)
pRSVL74	Stop codon	$1\ (\pm 0.3)\times 10^{-4}$
pRSVL121	Frameshift	$7\ (\pm 3)\times 10^{-4}$
pRSVL89	Frameshift	$5\ (\pm 3)\times 10^{-4}$
pRSVLTo5	Deletion of the first AUG	$<10^{-5}$

C.p.m./mg protein compared to pRSVL. Mean of at least 4 experiments.
(*) 10^{10} c.p.m./mg protein.

sequence (unique *Nhe*I and *Bcl*I sites) to insert any sequence of which the misreading properties are to be tested.

A basal level of translational errors was detected *in vivo* both in mammalian cells in culture and in *E. coli*, for all the constructs. Luciferase activities of around 10^{-5}, as compared to the activity of the wild-type enzyme, were readily detected. We demonstrated that this low basal activity is not due to either the presence of revertants or to translational reinitiation behind the stop codon. These results prove that the luciferase assay is more sensitive than the CAT assays used by Martin *et al.* (1989), who were unable to detect background readthrough level in mammalian cells. In these nucleotidic contexts, the frameshift constructions revealed a higher level of error in eukaryotic cells as well as in *E. coli*.

The effect of the amino acid sequence modification was assessed using pRSVL90 that contained an insertion of 7 codons in the wild type *luc* gene. Only a 2.5-fold decrease in the specific activity of this modified luciferase was observed, as compared to the wild-type enzyme. This indicates that the enzymatic activity of luciferase protein tolerates a certain flexibility in its amino-terminal part, which is encouraging for the use of this model system to insert other sequences of interest.

At the *Nhe*I-*Bcl*I sites of pRSVL74, we inserted the small DNA sequence of the gag-pol junction from HIV to generate pRSVL89. Up to now, all tests to study the HIV gag-pol region responsible for the frameshift have been performed either *in vivo* in yeast or through *in vitro* translation of synthetic RNA in reticulocyte lysates (Jacks *et al.*, 1988; Wilson *et al.*, 1988). With these systems, the frameshift level approached the natural one.

The vectors described here are the first allowing *in vivo* experiments in mammalian cells. As compared to pRSVL90, used as a control, the frameshifting efficiency of this short HIV sequence was found to be weaker (2.5×10^{-3}) than the pol/gag ratio usually observed in retrovirus-infected cells (Weiss *et al.*, 1982). The proximity of the translation initiation codon to the slippery site could decrease the level of frameshift. In yeast, +1 frameshift at the TYA/TYB overlap is inhibited by the proximity (5 codons) of the AUG (Clare *et al.*, 1988; Belcour and Farabaught, 1990); however, no such effect has been observed in *E. coli* for the HIV frameshift sequence (5 codons) (Weiss *et al.*, 1989). In the case of pRSVL89, the shifty sequence is located 9 codons downstream of the AUG. Alternatively, abortive translation can result in a decrease in mRNA stability: it has been shown that a stop-codon-containing message can be very unstable in mammalian cells (Lim *et al.*, 1989). Insertion upstream of the mutated *luc* gene of a sequence whose translation product can be quantified would make it possible to estimate mRNA degradation and the number of ribosomes that shift compared to the number of translating ribosomes, and to move the slippery site away from the AUG. Such constructions are under way.

Two hypotheses specific to the behaviour of HIV can be proposed to account for the low level of frameshifting obtained:

– although we have already tested the pRSVL89 construct in two other cell lines (HepG2 hepatoblastoma cells and C11D cells of the L line, data not shown) and obtained results similar to those with NIH3T3 cells, these cell types are not the normal target of the viral infection (Klatzmann *et al.*, 1984); frameshifting might possibly be higher in T4 lymphocytes;

– it could be that the sequences defined *in vitro* are not sufficient to reach the natural frameshift level; for instance, the palindromic sequence located downstream of the slippery site of HIV (Lee *et al.*, 1989) may be required *in vivo*, although this sequence is dispensable *in vitro* (Jacks *et al.*, 1988); in fact, the presence of palindromic regions has been shown to significantly affect frameshifting at the gag-pol junction of RSV (Jacks and Varmus, 1985) and between open reading frame F1 and F2 in the avian coronavirus infectious bronchitis virus (Brierley *et al.*, 1989).

Whatever the case, the validity of our vectors to detect variations in translational accuracy was proved by the fact that paromomycin affects the accuracy differently depending on the mutation used: it is efficient on readthrough but no effect was found on frameshifting. This probably indicates that different mechanisms are involved, but the results are too preliminary to warrant a detailed discussion.

The experimental system presented in this paper could be used to investigate several problems, both in virology and cell biology. The cloning sites make it easy to introduce any sequence of interest and permit *in vivo* analysis of the structural signals (*cis* factors) responsible for the high level of readthrough or frameshifting in retroviruses. *Trans*-acting factors can also be analysed: we have been able to demonstrate that viral infection does not influence the readthrough level at the gag-pol junction of Moloney murine leukaemia virus (V. Berteaux, unpublished results). In addition, it may be useful for screening drugs potentially affecting the level of slippage directed by the gag-pol junction of HIV, with the aim of interfering with another stage of the virus cycle than those affected by the already known antiviral agents.

Finally, these vectors could be used to detect variations in translational accuracy in several biological processes such as cell transformation with oncogenes, stress response and, as has been suggested, at certain stages of differentiation of eukaryotic cells (Picard-Bennoun, 1982).

RÉSUMÉ

VECTEURS D'EXPRESSION POUR QUANTIFIER LES ERREURS DE TRADUCTION *IN VIVO*: LEUR UTILISATION POTENTIELLE POUR ÉTUDIER LE DÉCALAGE DE CADRE A LA JONCTION gag-pol DU HIV

Les erreurs de traduction sont indispensables à l'expression et à la régulation de gènes dans plusieurs systèmes biologiques. L'expression différentielle des gènes *gag-pro-pol* de nombreux retrovirus est contrôlée par une erreur traductionnelle: « translecture » (readthrough) chez le MoMuLV (Moloney murine leukaemia virus), ou décalage de cadre (frameshift) chez le HIV et le RSV (Rous sarcoma virus). Nous décrivons ici un système permettant de mesurer *in vivo* le taux d'erreurs de traduc-

tion, en particulier les décalages de cadre de lecture observés à la jonction des gènes *gag* et *pol*. Il s'agit d'une famille de vecteurs d'expression portant différentes versions mutées du gène codant pour la luciférase. Les mutations introduisent, soit un codon stop en phase, soit la délétion d'une paire de base; une translecture ou un décalage du cadre de lecture sont alors nécessaires pour obtenir la synthèse d'une protéine fonctionnelle. Ce système est suffisamment sensible pour détecter un niveau basal d'erreurs dans des cellules de mammifère en culture. L'effet de la paromomycine et de la kasugamycine, deux antibiotiques connus pour modifier la précision de la traduction, a été testé. Les résultats indiquent que la paromomycine affecte différemment la translecture et le décalage du cadre de lecture. Un des vecteurs possède deux sites uniques permettant le clonage de séquences potentiellement intéressantes; il a été utilisé pour analyser l'effet de la séquence, cible potentielle du haut niveau de décalage du cadre de lecture, à la jonction des gènes *gag* et *pol* du virus HIV.

L'étude de l'influence des facteurs *cis* et *trans*, sur le niveau d'erreurs traductionelles peut être entreprise à l'aide de cette famille de vecteurs.

MOTS-CLÉS: Traduction, HIV, Luciférase; Jonction gag-pol, Décalage du cadre de lecture, Vecteurs d'expression.

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Note added in proof. — After this work was submitted, Reil and Hauser described vectors using the *luc* gene to test the frameshifting efficiency of the HIV gag-pol junction (Reil H. and Hauser H., 1990, Test system for determination of HIV1 frameshifting efficiency in animal cells. *Biochim. biophys. Acta* (Amst.), **1050**, 288-292). They used an HIV fragment including the palindromic region and found a frameshifting efficiency of approximately 3 %. Although they have not tested the minimal slippery region we used, this result strongly supports the interpretation suggesting that the sequences defined *in vitro* are not sufficient to reach the natural frameshift level.