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Retroviral *gag* Gene Amber Codon Suppression Is Caused by an Intrinsic *cis*-Acting Component of the Viral mRNA

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In some type C retroviruses, translation of the *pol* gene appears to require translational suppression of the proximal *gag* amber codon. To identify the region of the viral nucleic acid responsible for synthesis of the *pol* gene products, a 300-base-pair DNA fragment containing the stop codon from a type C murine virus (AK virus) was inserted into the *Escherichia coli lacZ* gene such that the translational reading frame was maintained. Introduction of the resulting fusion gene into cells resulted in the suppression of the viral stop codon. As measured by β-galactosidase production, suppression occurred at a frequency of approximately 10%. Suppression could occur in at least several vertebrate cell types and was not augmented by virus replication or the expression of viral gene products. This indicates that *gag* amber codon suppression does not require augmented levels of suppressor tRNA species.

Translational termination codon suppression is used in several procaryotic and eucaryotic systems to modulate expression of distally located genes in polycistronic mRNAs. In bacteriophages such as Qβ and lambda, readthrough of a stop codon is required for the synthesis of the IIb and *Q* gene products, respectively (25, 36, 39). Similarly, for some type C retroviruses and for tobacco mosaic virus, amber codon suppression appears to be used for the translational expression of enzymes involved in viral nucleic acid replication (18, 28, 29). Here, the presence of a proximal suppressible stop codon provides the means to consistently produce differential amounts of specific viral proteins; those proteins needed only in small amounts can be encoded by genes downstream from suppressible stop codons.

The overall spatial array of retroviral genes is diagrammed in Fig. 1. Transcription of the viral DNA yields at least two discrete mRNA species, one a spliced subgenomic message encoding the envelope glycoprotein and the second a full-length RNA which is believed to serve both as a source of mRNA for the *gag* and *pol* open reading frames and for packaging into progeny virus particles. It is this second RNA species that is believed to exhibit amber codon suppression during translation. DNA sequence analysis of several type C retroviruses, Moloney murine leukemia virus (32), AK virus (AKV) (11), baboon endogenous virus (33), and spleen necrosis virus (K. Talbot and A. Panganiban, unpublished data), has revealed that a single amber codon separates the *gag* and *pol* open reading frame. During infection, translation of the *gag-pol* RNA most frequently results in termination at this stop codon, generating a *gag* precursor polypeptide (7, 35). However, at an efficiency of about 2 to 5%, a long *gag-pol* precursor polypeptide is synthesized. The *gag* and *gag-pol* precursor proteins are then both proteolytically cleaved to the mature virion proteins (6, 41) (Fig. 1). Thus, the four *gag* proteins located upstream from the amber codon are synthesized profusely, while the protease, reverse transcriptase, and IN protein, which all require amber codon suppression for their expression, are produced in diminished amounts. The processing protein responsible for cleaving the peptide bonds of the *gag* precursor is encoded by both the *gag* and *pol* open reading frames.

In vitro translation experiments with virion RNA reveal that increased amounts of the *gag-pol* precursor can be generated by the addition of yeast amber suppressor tRNA (29). Subsequently, direct sequencing of the N terminus of the protease encoded at the junction revealed that the amino acid glutamine is inserted at the position corresponding to the amber codon (40). The insertion of glutamine might be due to the presence of one or more natural amber suppressor tRNAs or, alternatively, might be effected by anomalous recognition of the amber codon by the normal $tRNA^{Gln}$. Examination of the various $tRNA^{Gln}$ species in mouse cells indicates that there is a small amount of a natural amber suppressor $tRNA^{Gln}$, consistent with the possibility that readthrough is due to the presence of an amber suppressor tRNA (19). Moreover, infection of cells with Moloney murine leukemia virus results in an apparent increase in the amount of this suppressor species, leading to a model in which efficient *pol* gene expression requires an increase in the suppressor tRNA pool brought about by infection (19).

This report describes an in vivo assay for translational amber codon suppression which can be used in a variety of cell types. Suppression of the *gag* amber codon appears to be due to an intrinsic feature of the viral RNA and can occur in cells from several vertebrate species. Furthermore, suppression is not dependent on, or facilitated by, *trans*-acting functions encoded by the virus. The data indicate that a short *cis*-acting segment of the RNA at the *gag-pol* junction is both necessary and sufficient for suppression. There is no indication that elevated levels of a suppressor tRNA are required for efficient suppression.

MATERIALS AND METHODS

Construction of *lacZ* fusion plasmids. A deletion derivative of the plasmid RSV cat (9) (designated pRSV c) was first generated by digestion with the enzymes *Pvu*II and *Bam*HI, treatment with DNA polymerase (Klenow) and deoxynucleotides to make the *Bam*HI end blunt ended, and treatment with T4 DNA ligase to generate pRSV c. This manipulation resulted in the loss of most of the chloramphenicol acetyltransferase-coding region and the regeneration of a *Bam*HI site at the site of *Pvu*II and *Bam*HI ligation. A *Bam*HI-*Bgl*II fragment containing the *lacZ* region from pRZ305 (15) was inserted into *Bam*HI-cleaved pRSV c by ligation with T4 DNA ligase to yield a plasmid designated pRSV *lac*. Plasmid RZ305 is a derivative of pMC1403 (3) with the *lacZ* region

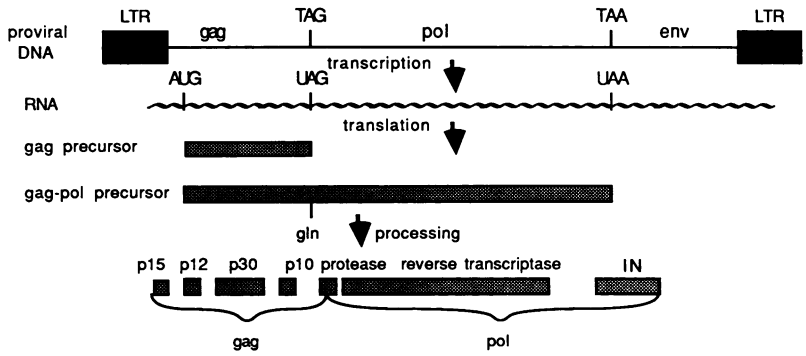

FIG. 1. Expression of the murine retrovirus *gag* and *pol* open reading frames. The *gag* and *pol* genes are translated from the same mRNA, resulting in a predominant *gag* precursor and a less abundant *gag-pol* precursor. LTR, Long terminal repeat.

inserted into the transposon Tn*5*. The simian virus 40 poly(A) addition site (on an *Hpa*I-*Apa*I fragment from pRSV c) was substituted into the *Sma*I-*Apa*I region of pRSV *lac* to give pRSV c-*lac*. A 300-base-pair (bp) fragment containing the AKV *gag-pol* junction was excised from pMC19 (J. Horowitz and R. Risser, unpublished data) and inserted into the *Bam*HI site of pRSV c-*lac* to generate pRSV c-*gp-lac*. An 18-base self-complementary oligonucleotide containing an amber codon was synthesized and provided by the University of Wisconsin Biotechnology Center and introduced by ligation into the *Bam*HI site of pRSV c-*lac* to yield pRSV c-*am-lac*. All DNAs were introduced into, and maintained in, *Escherichia coli* JM109 *supE*. To examine expression in cells lacking an amber suppressor, I introduced DNAs into strain JM83.

Transfection of cells and measurement of β-gal. Cells were plated at a density of 10^5/100-mm-diameter plate and grown to a density of approximately three-fourths confluence in Eagle medium supplemented with 5% calf serum. Chicken embryo fibroblasts were grown with the addition of 5% fetal bovine serum and 5% calf serum. Several plates of cells were rinsed with phosphate-buffered saline (PBS), trypsinized, concentrated by centrifugation, and resuspended in PBS to a concentration of about 10^8 cells per 0.5 ml. Cells were electroporated at 5 A with a rise time of 70 nsec and ψ = 20 msec, replated at a density of about one-fourth confluence, and grown for an additional 24 to 48 h. Cells were prepared for the β-galactosidase (β-gal) assay by being rinsed three times with PBS, treated with 0.5 ml of 40 mM Tris (pH 7.9)–1 mM EDTA–150 mM NaCl per 100-mm plate, and pooled in a single tube. The cells were centrifuged for 15 s in a microcentrifuge, suspended in β-gal assay buffer, and disrupted by sonication (five 20-s bursts at maximum setting), and debris was removed by a 10-min centrifugation in a microcentrifuge at 0°C. The supernatant was recovered and assayed for β-gal activity (24) by incubation in β-gal assay buffer (0.1 M sodium phosphate [pH 7.0] 1 mM Mg_2SO_4, 0.1 M β-mercaptoethanol) containing 0.8 mg of *o*-nitrophenyl-β-D-galactopyranoside per ml at 30°C for 30 to 180 min. The reaction was terminated by the addition of sodium carbonate to 0.33 M, the optical density at 420 nm was measured, and the amount of active enzyme was determined by comparison with parallel reactions containing known amounts of purified β-gal. For analysis of β-gal with anti-β-gal monoclonal antibody, 50 μg of protein from transfected cells was spotted onto a nitrocellulose filter prewet with PBS by using a Schleicher & Schuell dot blotter. The filter was treated for 2 h at room temperature with PBS containing 1% dry milk (PBSM), transferred to 15 ml of the same buffer containing 5 μl of monoclonal anti-β-gal (Promega Biotec, Madison, Wis.), and incubated for 30 min. The filter was rinsed sequentially for 20 min each time with PBSM (twice), PBSM plus 0.05% Tween 20 (once), and PBS (once), incubated for 60 min with 15 ml of PBSM containing 2.7 μl of goat anti-mouse immunoglobulin G conjugated with alkaline phosphatase (Promega Biotec), and rinsed in a manner identical to that performed after treatment with the primary antibody. Finally, the filter was incubated in 15 ml of 100 mM Tris (pH 9.4)–100 mM NaCl–5 mM MgCl–0.33 mg of Nitro Blue Tetrazolium per ml–0.165 mg of 5-bromo-4-chloro-3-indolyl phosphate per ml for 40 min and treated with 20 mM (pH Tris 7.9)–5 mM EDTA. Bradford protein assays were performed with Bio-Rad reagents (1).

RNA preparation and S1 analysis. RNA was isolated from D17 (dog) cells 48 h posttransfection and purified by the CsCl method (30). DNA probes for S1 analysis were prepared by labeling the 3′ ends with [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase followed by isolation of the appropriate DNA fragment on polyacrylamide gels. RNA-DNA hybridization reactions contained 10 or 1 μg of total cellular RNA, 35 μg of tRNA, and end-labeled probe in a total volume of 50 μl of formamide hybridization buffer. Samples were denatured by heating at 65°C for 15 min and then incubated for 16 h at 50°C. S1 digestions were done as described previously (22), and DNAs were analyzed on 5% denaturing polyacrylamide gels.

RESULTS

Strategy for detecting amber codon suppression. Catalysis by the bacterial enzyme β-gal (encoded by the *E. coli lacZ* gene) is not dependent on the N-terminal end of the molecule, so it is possible to alter the first few amino acids without markedly disrupting the active domain of the protein (26). This property has enabled the construction of N-terminal fusion proteins that retain biological activity in *E. coli* (31), yeast (5), and higher eucaryotic cells (27). To examine the determinants of retroviral amber codon suppression, I constructed a fusion gene comprising the *lacZ* gene and the *gag-pol* junction. Although several retroviruses are known to contain *gag* and *pol* reading frames separated by a single amber codon (11, 32, 33; K. Talbot and A. Panganiban, unpublished data), AKV was selected because the amber codon could be isolated on a small restriction fragment and molecularly cloned in the proper reading frame into a vector for examining suppression (described in detail below). AKV is a murine retrovirus derived from an endogenous virus harbored by AKR mouse strains (20). The

overall strategy was to generate a recombinant DNA molecule containing the *gag* amber codon upstream and in the same reading frame as the *lacZ* gene. Thus, enzymatically active β-gal would be attained only by successful suppression of the amber codon (Fig. 2A). Detectable enzyme activity following introduction of the DNA into cells and transcription of the chimeric gene would indicate that suppression of the amber codon had taken place transiently in vivo. Transcription initiation and termination were effected by the Rous sarcoma virus long terminal repeat (9) and the simian virus 40 polyadenylation sites (10), respectively.

An initial fusion gene was constructed in which the first 38 codons of the bacterial chloramphenicol acetyltransferase gene (*cat*) were substituted for the first 8 codons of *lacZ* (pRSV c-*lac*) (Fig. 2B). In this alignment there is no functional chloramphenicol acetyltransferase activity, but proficient β-gal activity is still retained (see below). In addition, there is a unique *Bam* recognition sequence at the site of fusion which facilitated subsequent insertion of a DNA fragment carrying the AKV *gag-pol* amber codon (pRSV c-*gp-lac*) (Fig. 2B). The 300-bp DNA fragment (11) contains the AKV amber codon beginning 111 nucleotides from the end of the fragment, so transcription of the fusion gene would be expected to result in an RNA with 37 codons from *gag*, the *gag* amber codon, and 62 codons from *pol* all in frame with the distally situated *lacZ* gene. An additional fusion gene was constructed with a synthetic 18-bp DNA fragment containing an in-frame amber codon (pRSV c-*am-lac*) (see Materials and Methods).

The fusion proteins are enzymatically active. It was feasible to assess activity of the fusion proteins in *E. coli* cells harboring the recombinant plasmids as the Rous sarcoma virus promoter-enhancer contains a fortuitous constitutive procaryotic promoter (23) and the fusion genes all contain an appropriately spaced ribosome-binding site derived from the *cat* gene. When either pRSV c-*gp-lac* or pRSV c-*am-lac* was introduced into *E. coli* cells containing the *supE* amber tRNA suppressor and a deletion in the cellular *lacZ* gene, colonies were light blue when grown on plates containing 5-bromo-4-chloro-3-indoyl-β-galactoside (X-gal), indicating that enzymatically active β-gal was synthesized. The level of suppression in this strain was approximately 2% of that found for the fusion protein derived from the plasmid lacking an in-frame termination codon (pRSV c-*lac*) (data not shown). In contrast, in cells without a suppressor tRNA, no apparent β-gal activity was observed. These results indicate that the fusion proteins retain activity in *E. coli* and that translational expression of β-gal from RNA derived from pRSV c-*gp-lac* or pRSV c-*am-lac* requires amber codon suppression. Further, there was no apparent functional difference between the AKV and synthetic amber codons in *E. coli*, indicating that the 300 nucleotides from the *gag-pol* junction cannot effect suppression in this procaryotic system.

AKV *gag* amber codon is suppressed in vertebrate cells. In contrast to *E. coli*, significant levels of readthrough of the AKV amber codon were seen in a variety of vertebrate cells, while no apparent suppression was seen in cells containing the nonviral amber codon. For this experiment, DNA was introduced into mouse, chicken, dog, and human cells by electroporation and β-gal activity was measured after incubation and transient expression. Transfection with pRSV c-*lac* resulted in relatively high, easily detectable levels of β-gal expression; for the dog cell line, five separate assays gave an average of 32 Miller units/μg of protein (24) (Table 1) with measured values ranging from 28 to 35 units in individual assays. In the mammalian cells containing pRSV c-*gp-lac*, β-gal activity was consistently higher than for those cells with pRSV c-*am-lac*; cells containing the latter DNA produced activity no higher than that of background (Table 1). Suppression occurred with a frequency of about 10% in all

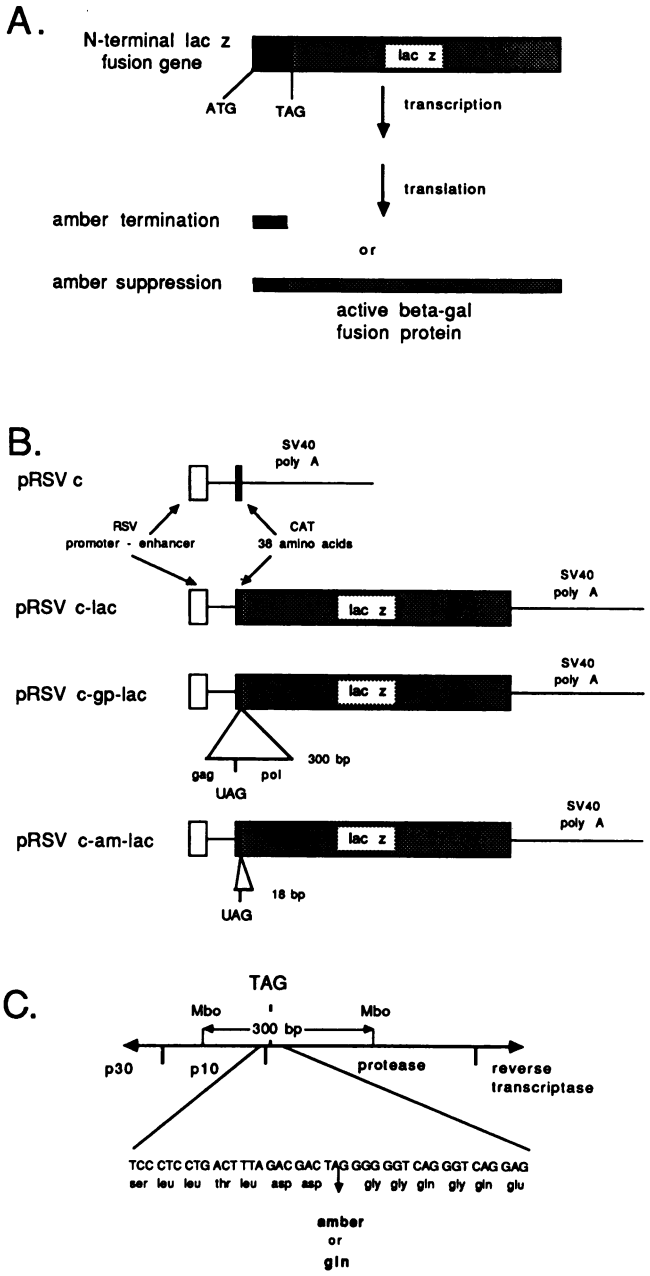

FIG. 2. Strategy for assaying amber codon suppression. (A) Insertion of an amber codon into the 5′ region of the *E. coli lacZ* gene and subsequent suppression might lead to the translational expression of a N-terminal β-gal fusion protein. (B) Pertinent region of the fusion constructs used in the assay. All plasmids contain the Rous sarcoma virus (RSV) promoter-enhancer region and the simian virus 40 (SV40) transcriptional termination and poly(A) addition signals (9). pRSV c-*gp-lac* and pRSV c-*am-lac* contain in-frame amber codons as shown and as described in the text. CAT, Chloramphenicol acetyltransferase. (C) Origin of the *Mbo* fragment from AKV that was used to construct pRSV c-*gp-lac*. The coding sequence surrounding the *gag* amber codon is also shown.

TABLE 1. β-Gal activity following transfection of vertebrate cells

Cell type	Fusion gene	Relative activity[a]	Activity/c-*lac* Activity[b]	No. of assays/no. of transfections
D17 (dog)	c	<0.01	<0.01	5/3
	c-*lac*	1.0	1.0	5/3
	c-*gp-lac*	0.11	0.11	5/3
	c-*am-lac*	<0.01	<0.01	5/3
HeLa (human)	c	<0.01	<0.02	3/2
	c-*lac*	0.48	1.0	3/2
	c-*gp-lac*	0.04	0.08	3/2
	c-*am-lac*	<0.01	<0.01	3/2
NIH 3T3 (mouse)	c	<0.01	<0.02	5/3
	c-*lac*	0.40	1.0	5/3
	c-*gp-lac*	0.05	0.12	6/3
	c-*am-lac*	<0.01	<0.02	4/3
NIH 3T3 (mouse; infected)	c	<0.01	<0.02	2/2
	c-*lac*	0.46	1.0	3/2
	c-*gp-lac*	0.03	0.06	3/2
	c-*am-lac*	<0.01	<0.02	2/2

[a] The relative activity of each extract was normalized relative to the activity following transfection of dog cells with pRSV c-*lac*. A value of 1.0 indicates a specific activity of 32 nmol of *o*-nitrophenyl-β-D-galactopyranoside hydrolyzed per min per μg of protein (Miller units per microgram of protein) (29).

[b] The activity of each extract was normalized to that of pRSV c-*lac* for each independent cell type.

the mammalian cell types containing pRSV c-*gp-lac*. The chicken cells contained a high level of endogenous β-gal-like enzyme activity that obscured any β-gal activity that may have originated from the introduced plasmids (data not shown). However, it was possible to physically detect β-gal expression and concomitant amber codon readthrough with a monoclonal antibody to bacterial β-gal (see below).

Virus infection does not increase suppression efficiency. The 300-bp AKV DNA fragment does not fully encode any of the known murine retroviral gene products, so expression of β-gal in the assay did not appear to depend on any expressed viral gene product. To determine whether any *trans*-acting viral gene product might augment suppression efficiency of the AKV amber codon or of amber codons in general, I used cells expressing viral gene products in the transient assay. The 3T3 cells were infected with an amphotropic murine virus capable of supplying *trans*-acting functional gene products required for replication in a variety of retroviral species including AKV. Subsequently (5 days postinfection), the plasmids bearing the various fusion genes were introduced and β-gal activity was again assayed. The prior expression of viral gene products in the mouse cells had no apparent effect on amber codon suppression (Table 1; Fig. 3). Thus, RNA transcribed from the 300-bp fragment appears to be sufficient for effective readthrough of the amber codon.

The results of the previous experiments are consistent with the hypothesis that the viral RNA possesses an intrinsic property that selectively allows termination codon readthrough and that suppression is not augmented by virus infection. However, alternative hypotheses could also account for the observed results. The different fusion proteins might possess grossly different enzymatic activities in vertebrate cells even though they are all active in *E. coli* cells carrying amber suppressor tRNA molecules. Additionally, differential RNA stability might lead to artificially high RNA pools from pRSV c-*gp-lac* relative to pRSV c-*am-lac*. To

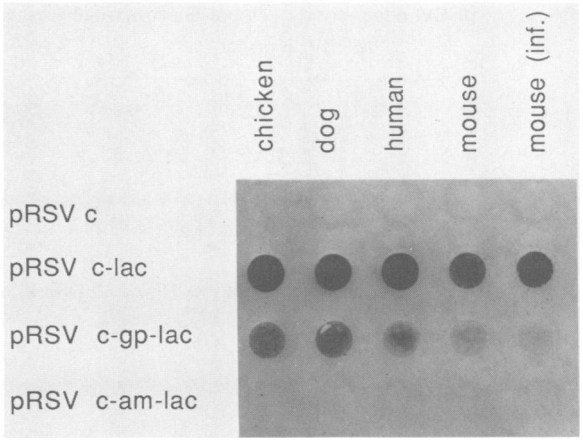

FIG. 3. Monoclonal antibody detection of β-gal following the transfection of vertebrate cells. Cell extracts from transfected cells were prepared, spotted on nitrocellulose, and detected by anti-β-gal antibody as described in Materials and Methods. inf., Infected.

test these alternative hypotheses, I performed experiments to detect the physical presence of the β-gal peptide irrespective of enzyme activity and to measure mRNA levels encoding the different fusion proteins.

The plasmids encoding fusion proteins were again introduced into cells by electroporation, but following transient expression, the presence of β-gal was assessed by binding with a monoclonal antibody rather than by measuring enzyme activity. The rationale for the experiment was that antibody binding might be expected to occur even if the enzymatic activity of the fusion proteins from pRSV c-*lac*, pRSV c-*gp-lac*, and pRSV c-*am-lac* differed markedly. The results of this antibody binding assay paralleled the results of the initial experiment in which enzyme activity was measured. Expression of DNA lacking an in-frame termination codon (pRSV c-*lac*) produced easily detectable quantities of cross-reactive material in chicken, dog, human, and mouse cells. Transient expression of the construct bearing the in-frame amber codon from the viral origin (pRSV c-*gp-lac*) also produced detectable β-gal, but as with the enzymatic assay, the overall amount was diminished relative to that of the recombinant without a stop codon (pRSV c-*lac*). The presence of the nonviral amber codon (pRSV c-*am-lac*) blocked production of any detectable β-gal, indicating that this nonviral amber codon is not efficiently suppressed. It was possible to observe expression in chicken cells as well as the mammalian cells since the endogenous β-gal activity of that cell type apparently does not harbor the epitope recognized by the monoclonal antibody to the *E. coli* enzyme. The results with the avian cells corresponded with those obtained for the mammalian cells. It is important to note that in this physical assay direct quantitation of the amount of β-gal expressed is difficult. However, several separate assays all showed copious synthesis of β-gal in pRSV c-*lac* transfectants, a lesser but detectable amount in pRSV c-*gp-lac* transfectants, and levels similar to those of untransfected cells for pRSV c-*am-lac*.

Disparity in the relative RNA pools generated during the transient assay might have led to variant β-gal levels which could be misinterpreted as translational amber codon suppression. For example, mRNA from pRSV c-*am-lac* might be degraded at a higher rate than that of pRSV c-*gp-lac*. To determine whether this might be a problem, I performed an S1 nuclease analysis of the RNA. RNA was isolated from

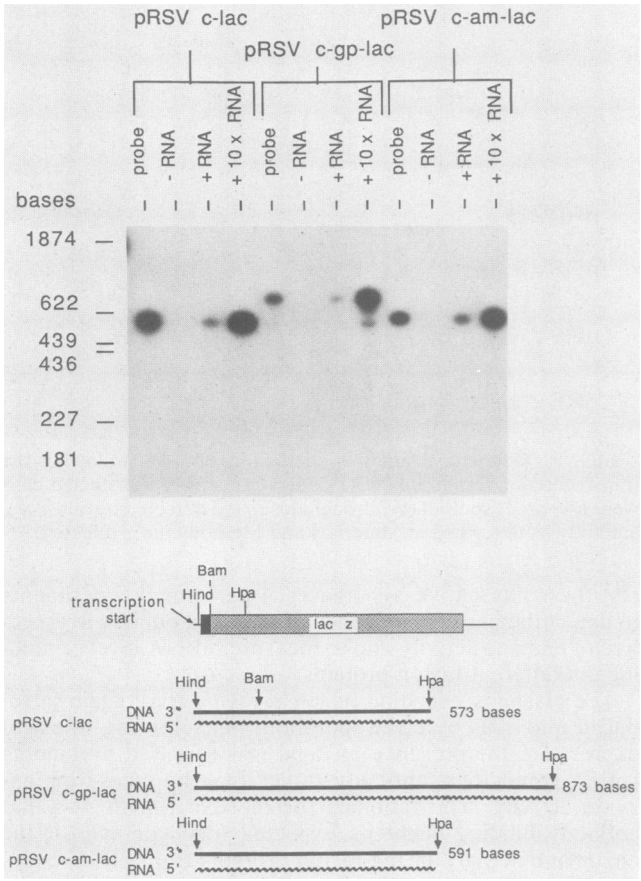

FIG. 4. S1 nuclease analysis of RNA from transfected cells. (Bottom) Location of the *Hind* and *Hpa* sites used to generate the probe for pRSV c-*lac*, pRSV c-*gp-lac*, and pRSV c-*am-lac* relative to the *lacZ* coding region and transcriptional start site. (Top) Hybridization and S1 analysis were performed as described in the text, and the resulting mixtures were electrophoresed on a denaturing DNA gel. −RNA, No RNA; +RNA, 1.5 μg of cellular RNA; 10× RNA, 15 μg of cellular RNA in the hybridization reaction. Lanes labeled probe contain untreated DNA. Size markers were labeled *Hin*fI fragments of pBR322.

dog cells following electroporation with each of the plasmids and hybridized with a denatured 3′-end-labeled *Hin*d-*Hpa* DNA fragment corresponding to the region near the 5′ end of the RNA including the amber codons of pRSV c-*gp-lac* and pRSV c-*am-lac* (Fig. 4, bottom). The RNA-DNA hybrids were then examined by protection against S1 nuclease and subjected to electrophoresis on a denaturing DNA gel (Fig. 4, top). The analysis was performed in DNA excess, so protection against S1 nuclease was a function of the RNA concentration in the hybridization reaction. This can be seen by the increase in protected labeled DNA following a corresponding increase in the amount of RNA added to the hybridization. Additionally, RNA levels were measured by dot-blot analysis with a DNA probe from the 3′ end of the *lacZ* gene (data not shown). The results of both experiments indicated that there are similar amounts of RNA present in cells transfected with the different plasmids. It is therefore unlikely that the difference in the amount of β-gal protein in the transfected cells was due to large differences in the sizes of steady-state RNA pools from the different templates.

DISCUSSION

Direct examination of tRNAs from mouse cells indicates that there is a relatively small amount (1 to 2%) of the total Gln-tRNA species containing an anticodon that can act as an amber codon suppressor in an in vitro translation system with tobacco mosaic virus RNA (19). This suppressor tRNA species is therefore potentially capable of nonspecifically suppressing amber codons in vivo and is a candidate for suppression of the *gag* amber codon. However, the data presented in this report are consistent with a suppression mechanism dependent on a unique *cis*-acting region of the viral RNA rather than general low-level suppression of all amber codons. Additionally, studies with an amber codon in the *cat* gene expressed in mammalian cells indicate that this stop codon is not leaky (2). So it would appear that there is a *cis*-acting component restricted to the adjacent 300 nucleotides of the viral mRNA that results in relatively efficient readthrough.

Examination of the flanking sequences surrounding nonsense codons in many eucaryotic genes and viruses reveals that there are preferred nucleotides at specific positions adjacent to the stop codon (17). This might reflect a functional role for the adjacent nucleotides in recognition of stop codons by translational release factors (21). For the AKV amber codon, the sequence in the vicinity matches the consensus sequence expected for an efficiently used stop codon. Thus, it does not appear that suppression is due merely to the localization of the UAG codon next to nucleotides in the mRNA that render termination inefficient.

Previous experiments indicate that the amount of the minor suppressor tRNA increased in response to virus infection (19). This observation led to a model in which suppression is effected by this suppressor tRNA population and in which efficient suppression requires an increase in the suppressor tRNA pool. However, suppression of the *gag* amber codon does not seem to be markedly affected by virus infection or by expression of viral gene products. Therefore, if this suppressor tRNA is responsible for translational readthrough, the amount present in uninfected cells is sufficient for efficient suppression. An alternative is that the normal Gln-tRNA and not the minor suppressor is responsible for suppression of the *gag* amber codon.

If suppression is due to a minor population of suppressor Gln-tRNA in the several cell types used in the analysis, then there must be some specific feature of the viral mRNA that facilitates relatively efficient suppression. Sequence analysis of the potential amber suppressor Gln-tRNA (19) indicates that anticodon-codon recognition must necessarily involve both first and third position wobble. This is somewhat surprising in light of the fact that base pairing of such an RNA-RNA interaction would be expected to be quite weak. Paradoxically, a better nucleotide match can be made for the abundant normal Gln-tRNA since first, but not third, position wobble would have to be invoked for anticodon-codon pairing (Fig. 5A).

In stop codon suppression of the IIb gene of Qβ and in *E. coli* tryptophan operon attenuation, readthrough is thought to involve specific interaction of the base immediately 3′ to the stop codon in the mRNA with the base 5′ to the anticodon in the tRNA (4, 8, 12, 25, 36). In these two instances, it has been suggested that this base pairing, in conjunction with base pairing between two of the three nucleotides in the codon and anticodon, results in a more stable interaction between the mRNA and aminoacylated tRNA and permits low-level translational elongation at a site

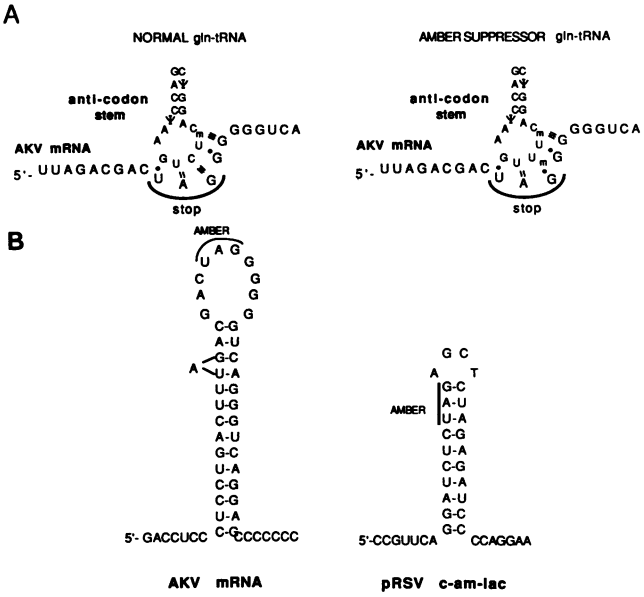

FIG. 5. Potential mRNA-tRNA interaction at the *gag* amber codon and potential intramolecular interaction at the AKV *gag-pol* junction. (A) Possible intermolecular interaction between the normal Gln-tRNA (15, 37) or the suppressor tRNA (19) with AKV mRNA at the *gag* amber codon. Codon-anticodon hydrogen bonding between the mRNA and the normal Gln-tRNA is likely to be superior to that with the amber suppressor Gln-tRNA. (B) Probable intramolecular secondary mRNA structure in the vicinity of the suppressed amber codon of AKV (pRSV c-*gp-lac*) (11) and the unsuppressed amber codon of pRSV c-*am-lac*. Although the structure is likely to form in naked AKV mRNA, it is unlikely to account for suppression (see Discussion).

where termination usually occurs. Examination of the nucleotide sequence in the anticodon loop of the normal bovine and rat liver Gln-tRNA (16, 38) and of the amber suppressor tRNA (19) indicates that there is the possibility for interaction between two of the nucleotides of the mRNA with two of the nucleotides of the anticodon loop. In particular, the two bases immediately 3′ to the amber codon in the message could potentially participate in hydrogen bonding with the two nucleotides 5′ to the anticodon; Fig. 5A delineates this hypothetical interaction. However, two considerations argue against the contention that this Gln-tRNA-mRNA interaction is solely responsible for suppression. First, the pairing of U in the tRNA with G in the mRNA may be relatively week outside of the codon and anticodon and can therefore be envisioned to provide only marginal stabilization. Second, examination of the nucleotide sequence 3′ to the amber codon of pRSV c-*am-lac* mRNA reveals the potential for pairing with the Gln-tRNA anticodon loop in a manner similar to the pairing of pRSV c-*gp-lac* mRNA with Gln-tRNA. Thus, while it may be that the two nucleotides 3′ to the AKV amber codon are necessary it is unlikely that they are sufficient for suppression.

Comparison of the mRNA sequences among those viruses that utilize the frame-shifting mechanism for *pol* gene expression (14) rather than suppression reveals that there are short regions of sequence conservation in the region of the overlap between the *gag* and *pol* open reading frames (5′-AAAAAAC and 5′-UUUA) (13). In addition, there is a potential hairpin loop structure located immediately downstream of the overlap. It has been postulated that this distally located stem and loop results in translational pausing which facilitates frame shifting (13). Examination of the AKV sequence around the amber codon reveals that the conserved heptanucleotide and tetranucleotide sequences are not present but that there may be a stable (34) hairpin loop structure with the UAG codon located in the hairpin (11) (Fig. 5B). However, there are at least three reasons that make it unlikely that this structure functions in suppression. First, since the AKV amber codon falls within the hairpin loop, it seems improbable that the role of this potential structure is to allow ribosomal pausing at the site of the amber codon; to read the amber codon, the secondary structure would have to first be disrupted. Second, the unsuppressed amber codon of pRSV c-*am-lac* is also likely to be situated in a stem-and-loop structure of stability similar to that of the AKV amber codon (Fig. 5B). Finally, in the case of at least one virus (spleen necrosis virus), such a secondary structure would not be expected to form. Isolation of mutants that stabilize or destabilize the stem surrounding the *gag* amber codon should help define the role of this or alternative downstream secondary structure in retroviral amber codon suppression. Such point mutants should also indicate the importance of specific nucleotides in hydrogen bonding with Gln-tRNA.

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