Retroviral vectors elevate coexpressed protein levels in *trans* through cap-dependent translation

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Edited by Peter K. Vogt, The Scripps Research Institute, La Jolla, CA, and approved February 3, 2015 (received for review October 24, 2014)

Retroviruses cause immunodeficiency and cancer but also are used as vectors for the expression of heterologous genes. Nevertheless, optimal translation of introduced genes often is not achieved. Here we show that transfection into mammalian cells of lentiviral or gammaretroviral vectors, including those with specific shRNAs, increased expression of a cotransfected gene relative to standard plasmid vectors. Levels of most endogenous cellular proteins were unchanged. Transfer of lentiviral vector sequences into a standard plasmid conferred the ability to give increased expression of cotransfected genes (superinduction). Superinduction by the retroviral vector was not dependent on the cell type or species, the type of reporter gene, or the method of transfection. No differences were detected in the IFN, unfolded protein, or stress responses in the presence of retroviral vectors. RT-PCRs revealed that RNA levels of cotransfected genes were unchanged during superinduction, yet Western blotting, pulse labeling, and the use of bicistronic vectors showed increased cap-dependent translation of cotransduced genes. Expression of the mammalian target of rapamycin (mTOR) kinase target 4E-BP1, but not the mTOR inhibitor Torin 1, was increased in trans by mTOR-independent signaling mechanism. Our experiments have broad applications for the design of retroviral vectors for transfections, DNA vaccines, and gene therapy.

Translational control is critical for mammalian cells and the viruses that infect them. For example, picornaviruses and some flaviviruses have an internal ribosomal entry site (IRES) that allows cap-independent translation of viral RNAs and provides preferential translation over most host cell mRNAs (2). Structural modifications at the 5′ ends of viral RNAs, such as differences in cap methylation or absence of a cap, often distinguish viral mRNAs from their cellular counterparts (3). Multiple cellular proteins recognize foreign RNAs or DNAs, which trigger specific signaling pathways that lead to general translational arrest and/or selective viral RNA degradation (4). Many viral RNAs trigger the IFN signaling pathway and translational inhibition, but viruses encode various proteins or RNAs that mute this response (5). Recognition of foreign nucleic acids by cellular surface or cytosolic receptors also leads to signaling events that provide an innate antiviral response (6).

Retroviruses are positive-sense RNA viruses that have been used extensively for introduction of genes or small hairpin RNAs (shRNAs) into cells, both in culture and for gene therapy. Unlike most RNA viruses, retroviruses replicate through a DNA intermediate and use RNA polymerase II to produce mRNAs with structures that are very similar to those of host mRNAs, thus avoiding some crucial RNA sensors (7). Nevertheless, the unspliced and partially spliced RNAs are required for the synthesis of viral structural proteins. These RNAs require a highly structured cis-acting element to facilitate export from the nucleus to the cytoplasm. These cis-acting sequences include a constitutive transport element (CTE) or a Rev-like response element (RRE) that requires binding of a protein adapter (8). Furthermore, retroviruses specify several other highly structured RNA elements, including the packaging sequence Psi, plus-strand priming sites, and splice acceptor sites (9).

We have made the unique observation that transfection of retrovirus-based vectors leads to increased levels (superinduction) of proteins encoded by cotransfected plasmids. Both lentiviral and gammaretroviral vectors lacking viral protein-coding potential, but not plasmid-based vectors, elevated translation of proteins expressed from cotransfected plasmids, such as GFP, but not most endogenous proteins. Increased translation of exogenous proteins was cap-dependent and did not lead to additional mammalian target of rapamycin complex 1 (mTORC1) signaling. Retroviral sequences did not require transcription to facilitate cap-dependent translation of cotransfected genes. These results indicate that retrovirus-based vectors can be used for improved gene expression during transfection and DNA vaccination in multiple cell types without additional cloning.

Results

Lentiviral Vector Cotransfection Causes Superinduction in *Trans*. We used calcium-phosphate transfection of an shRNA-expressing lentiviral vector (pLKO.1, Sigma) (Fig. 1A) to measure the effect

Significance

Translation is a key process that is regulated by cellular health and responses to the environment, including virus infection. We show here that introduction of lentivirus and gammaretroviral vectors into cells by transfection increased translation (superinduction) of cotransfected genes but not most endogenous proteins. Superinduction was independent of the unfolded protein, stress, and interferon responses and did not require retroviral vector transcription. Retroviral vectors elevated cap-dependent translation initiation without increased mammalian target of rapamycin (mTOR) kinase activity. Thus, DNA sequences from HIV-1 and other retroviruses increase translation of cotransfected genes in *trans* by mTOR complex 1-independent signaling. Our results suggest that retroviral DNA manipulates translation, which has practical implications for protein expression and design of vectors for transfection assays, DNA vaccines, and shRNA knockdown experiments.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/doi/10.1073/pnas.1420477112.

www.pnas.org/cgi/doi/10.1073/pnas.1420477112
of targeted knockdowns of specific cellular genes on the activity of mouse mammary tumor virus (MMTV) Rem, a Rev-like RNA-binding protein (10, 11). Rem activity was measured by cotransfection of a reporter plasmid (pHMRLuc) expressing Renilla luciferase in 293T cells. Increased luciferase levels require binding of Rem signal peptide (Rem-SP) to the Rem-responsive element (RnRE) in the reporter transcript (12). As anticipated, Rem expression elevated Renilla expression by ~12-fold (Fig. 1B, Top). Unexpectedly, cotransfection of pLKO.1 expressing a control shRNA (pLKO.1c) elevated both basal and Rem-induced luciferase expression in 293T cells (an additional 6.6-fold and 8.9-fold, respectively). Further, Western blotting indicated that Rem levels were increased in the presence of the lentiviral vector (Fig. 1C). Effects of the lentivirus vector were not limited to Rem-responsive plasmids. A firefly luciferase reporter vector that lacked Rem responsiveness, pGL3-C, showed ~threefold higher levels in the presence of the lentiviral vector (Fig. 1B, Bottom). These results indicated that the lentivirus increased expression of two different reporter genes (firefly or Renilla luciferase genes) from either the SV40 (pGL3-C) or CMV (pHMRLuc) promoters.

We then determined whether different shRNA inserts downstream of the U6 promoter affected superinduction. Cotransfection of lentivirus vectors expressing a control shRNA (pLKO.1c) or an shRNA designed to knock down the AAA ATPase, p97 (ΔU6) or the retroviral packaging region (pLKΔ4250 or LK-4252), each elevated reporter expression (Fig. 1D). The highest superinduction occurred with ΔU6, which gave the highest Rem levels by Western blotting (Fig. 1C), but no effect on p97 levels was observed. Because the same total amount of DNA (6 μg) was used, different DNA levels were not responsible for increased reporter activity. Thus, vectors expressing various shRNAs, including those with no known target, could enhance superinduction. Similar results were obtained in transfections of XC rat fibroblast cells using a lipid-based method or Jurkat human T cells using electroporation (Figs. S1 and S2), indicating that lentivirus superinduction was not cell-type-, species-, or method-specific. The effect was independent of the presence of the RnRE and Rem (Fig. S3).

To test the effect of pLKO.1c on other transgenes, we cotransfected this lentivirus vector with either EGFP or ER-mCherry expression vectors (13) and monitored it by fluorescence microscopy (Fig. 1D). The data showed that both fluorescent proteins were induced by the presence of pLKO.1c in trans, consistent with previous findings (Fig. 1B and C). Similar transfections were quantitated by FACS analysis and revealed 8- to 10-fold induction for EGFP, which diffuses throughout cells, or ER-mCherry, which is localized to the endoplasmic reticulum (13) (Fig. 1E and F). Thus, lentivirus cotransfection increased expression of different exogenous proteins that were localized to distinct cellular compartments.

Determinants of Lentivirus-Mediated Superinduction. To investigate the lentivirus sequences needed for superinduction, we deleted the shRNA hairpin (pLKΔsh) (Fig. 2A) and repeated cotransfection into 293 cells with both Rem-expression and Rem-responsive vectors. Removal of the shRNA decreased Rem expression, but the deleted vector still caused Rem induction as determined by reporter assays and Western blots (Fig. 2B and C and Fig. S4A). We also deleted sequences through the U6 promoter (pLKΔU6) or the retrorival packaging region (pLKΔPs) also in pLKO.1c (Fig. 2A). Cotransfections of the lentivirus vector lacking the shRNA, U6 promoter, RRE, and Psi (pLKΔPs) retained superinduction capability of 2.6-fold. Additional transfections of reporter and expression vectors with a different lentivirus vector lacking an shRNA (pLL3.7) revealed two-to-threefold induction of both Renilla and firefly reporter vectors and ~5- to 10-fold Rem induction (Fig. S5). Thus, although the shRNA was a major determinant of increased expression, lentiviral sequences also caused superinduction of exogenous proteins from cotransfected vectors.

![Fig. 1. Lentivirus vectors stimulate cotransfected gene expression. (A) Diagram of pLKO.1-based lentivirus vectors. (B) The pLKO.1c vector stimulates activity from both Renilla and firefly reporter vectors. Cells (293) were cotransfected with the empty vector pCDA3 or 5 μg of pLKO.1c with a control shRNA or pLKO.1 with either one of two different shRNAs against the AAA ATPase p97 (pLK-4250 or 4252) in the presence or absence of GFP-tagged Rem (12.5 ng) as indicated. The numbers in parentheses give fold induction by pLKO.1 vectors of the Renilla reporter vector in the presence of Rem. Significant differences were observed for Renilla luciferase comparing lane 1 to all other lanes and in comparing lane 5 to lanes 6–8. (C) Exogenous expression increases in the presence of the pLKO.1c vector. Extracts from the transfections in Fig. 1B were subjected to Western blotting. (D) Lentivirus vector cotransfection stimulates EGFP and mCherry expression. Fluorescence microscopy of cells transfected with an EGFP or mCherry expression vector in the absence or presence of pLKO.1c. A representative field is shown. (E) Quantitation of EGFP expression by FACS. The GFP fluorescence intensities and SDs of triplicate transfections were determined in the presence or absence of 2.5 ng pEGFP vector and reported relative to the pCDA3 control. (F) Quantitation of mCherry expression by FACS. Values are reported as in Fig. 1E.](image-url)
RNA region (IRES), we deleted these sequences. Cotransfection of the deleted derivative MigΔGFP induced Renilla and firefly reporter activity similar to MigR1 (Fig. 2G and Fig. S4C) as well as either GFP or GFP-Rem-SP levels (Fig. 2F and H). Thus, different types of retroviral vectors enhanced expression of cotransfected genes.

**Lentivirus Vector Does Not Induce IFN or Stress Responses.** Double-stranded RNAs activate RNA-dependent protein kinase (PKR), which phosphorylates the translation initiation factor, eIF2α, resulting in reduced translation initiation (15). To determine if pLKO.1c altered eIF2α phosphorylation, we repeated cotransfections with MMTV Rem expression vectors and extracts were tested by Western blotting (Fig. S6A). No difference in phosphorylated eIF2α was observed in the presence and absence of pLKO.1c. Further, BiP levels increase during the unfocused protein response and/or viral infections (16). Western blotting of transfected cell extracts revealed no differences in BiP, B23, or GAPDH (Fig. S6B). Cellular stress was also assessed by cotransfection of pLKO.1c with the NF-κB reporter plasmid, NF-κB-GL2, expressing firefly luciferase. No evidence of NF-κB induction by lentivirus cotransfection was observed (Fig. S7). Stress granules and levels of the stress granule protein G3BP (17) were unchanged by the lentivirus vector (Figs. S6C and S8). Furthermore, levels of the IFN-induced protein RIG-G (18) were not affected by introduction of pLKO.1c (Fig. S6D). Therefore, lentivirus cotransfection does not influence levels of exogenous proteins through IFN signaling, stress, or the unfolded protein response.

**Lentivirus Vector Affects eIF4E-Dependent Translation.** To determine whether superinduction increased transcription, we performed cotransfection experiments with Rem expression and reporter vectors in the presence and absence of pLKO.1c. Semiquantitative RT-PCR results indicated that pLKO.1c did not affect the steady state level of exogenous rem or Rluc or endogenous gapdh mRNAs (Fig. S9A) when exogenous protein levels increased (Fig. S9B). Thus, the lentivirus sequences did not superinduce transcription.

To test whether lentivirus sequences increased expression of exogenous genes, cells were cotransfected with EGFP expression vector in the presence of the lentivirus or pcDNA3 only and used for pulse labeling with [35S]methionine. Radioactive incorporation into endogenous proteins was observed (Fig. S9A). Additional aliquots were used for GFP affinity purification and analysis on denaturing polyacrylamide gels. Few differences in radioactive background incorporation into endogenous proteins were observed (Fig. S9D), but GFP levels greatly increased in the presence of pLKO.1c (Fig. S9E). These experiments indicated increased translation of exogenous genes cointroduced with the lentivirus vector. To assess whether vector-induced translational effects were cap-dependent, we tested different bicistronic vectors that expressed Renilla luciferase and firefly luciferase separated by an IRES from the same transcript (Fig. 3A). Experiments using vectors with either the hepatitis C virus (HCV) or cricket paralysis virus (CrPV) IRES sequence showed significant differences in transfection with and without the lentivirus vector. However, no difference in reporter activity was observed with the bicistronic Rluc/GFP vector (Fig. 3B).

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To test whether lentivirus sequences increased expression of exogenous genes, cells were cotransfected with EGFP expression vector in the presence of the lentivirus or pcDNA3 only and used for pulse labeling with [35S]methionine. Radioactive incorporation with and without the lentivirus vector was not statistically different (Fig. S9C). Additional aliquots were used for GFP affinity purification and analysis on denaturing polyacrylamide gels. Few differences in radioactive background incorporation into endogenous proteins were observed (Fig. S9D), but GFP levels greatly increased in the presence of pLKO.1c (Fig. S9E). These experiments indicated increased translation of exogenous genes cointroduced with the lentivirus vector. To assess whether vector-induced translational effects were cap-dependent, we tested different bicistronic vectors that expressed Renilla luciferase and firefly luciferase separated by an IRES from the same transcript (Fig. 3A). Experiments using vectors with either the hepatitis C virus (HCV) or cricket paralysis virus (CrPV) IRES sequence showed significant differences in transfection with and without the lentivirus vector. However, no difference in reporter activity was observed with the bicistronic Rluc/GFP vector (Fig. 3B).
showed that lentiviral induction was cap-dependent (Fig. 3B). Transfection of plasmids carrying retroviral sequences confirmed cap dependence of both lentiviral (pLKO.1c and Psi-sh) and gammaretroviral (MigAGFP) vectors, yet cap dependence did not completely correspond to induction of other reporter proteins, suggesting different signaling mechanisms (20) (Fig. 3C).

To determine signaling pathways affected by lentivirus vectors, we cotransfected 293 cells with an EGFP expression vector in the presence and absence of pLKO.1c. No change in total or phosphorylated p38 MAPK (Fig. 3D) or in total or phosphorylated ERK1/2 (p44/p42) was detected (Fig. 3E). Differences in phosphorylation of AKT or p70 S6K also were not observed (Fig. 3F). Chemical inhibitors for MNK1 and p38 MAPK had no specific effect on pLKO.1c induction of reporter genes (Fig. S10). These data suggested that signaling pathways through AKT, ERK1/2, MAPK, MNK1, and mTORC1 were not responsible for superinduction.

Cap-dependent translation is controlled through eIF4E (21). Unexpectedly, we observed a ~twofold increase in total, but not phosphorylated, eIF4E in multiple transfection experiments with pLKO.1c (Fig. 4 A and B). Unphosphorylated 4E-BP1 binds eIF4E to prevent assembly of the cap-binding complex (22, 23), and 4E-BP1 phosphorylation by mTORC1 increases cap-dependent translation. To test whether phosphorylation of 4E-BP1 was affected by the lentiviral vector, we performed additional transfections in the presence and absence of pLKO.1c. As expected, GFP was superinduced by pLKO.1c, yet no difference in total or phospho-4E-BP1 levels was detected (Fig. 4C), consistent with no lentivirus-induced change in mTOR kinase activity (Fig. 3).

To determine whether vector-mediated superinduction is controlled by 4E-BP1, we cotransfected 293 cells with 4E-BP1 and EGFP expression vectors in the presence or absence of pLKO.1c. Superinduction was greatly reduced by excess 4E-BP1, but protein overexpression had little effect on GFP levels in the absence of pLKO.1c (Fig. 4D). Similarly, excess 4E-BP1 partially inhibited cap-dependent translation using a bicistronic vector in the presence of pLKO.1c (Fig. S11). The effect of exogenous 4E-BP1 expression on superinduction was demonstrable by pulse labeling, consistent with a reduced level of cap-dependent GFP fluorescence intensities and SDs of triplicate transfections in the absence of pLKO.1c (Fig. 4F), which has been normalized to 1.0 for each condition (pcDNA3 only or in the presence of wild-type or mutant 4E-BP1). Expression of 4E-BP1 significantly reduced pLKO.1c-mediated superinduction (compare lanes 4 and 6; P < 0.005), whereas F114A expression had no effect (compare lanes 4 and 5; P = 0.75). The mTOR inhibitor Torin 1 similarly reduced both basal and pLKO.1c-induced GFP expression. Two exposures of the Western blot with GFP-specific antibody are shown. (F) FACS quantitation of Torin 1 effects on pLKO.1c-mediated superinduction of GFP. Although 4E-BP1 expression significantly inhibited the effect of pLKO.1c (compare lanes 4 and 5; P < 0.001), lentivirus-induced GFP expression was not significantly different when Torin 1 was added (compare lanes 4 and 6; P = 0.14).

Fig. 3. Superinduction affects cap-dependent translation of exogenous proteins but not mTOR activity. (A) Structure of bicistronic vectors used to measure cap-dependent translation. Cells (293) were cotransfected in triplicate with either pcDNA3 or pLKO.1c in the presence of a bicistronic vector. The ratio of the two luciferase values ± SD is shown. The differences in the presence and absence of pLKO.1c were highly significant. (C) Lentiviral and gammaretroviral vector cotransfection induces cap-dependent translation of reporter genes. Cells (293) were cotransfected with the CrPV bicistronic vector as in B. (D) Total and phosphorylated p38 MAPK were unchanged after pLKO.1c transfection. Cells (293) were transfected as in Fig. 18 in all panels, and lysates were subjected to Western blotting. (E) Total and phosphorylated p44/p42 ERK are unaltered after pLKO.1c transfection. Western blots were performed on transfected cell lysates. (F) Phosphorylated AKT and p70 S6K are not changed by pLKO.1c transfection. Western blots of transfected cell lysates are shown.

Fig. 4. Lentivirus vectors selectively affect eIF4E-dependent translation in cotransfection experiments. (A) Total levels of eIF4E increase after pLKO.1c transfection. Western blots of transfected cell lysates are shown. (B) Quantitation of eIF4E levels after pLKO.1c transfection. (C) Wild-type 4E-BP1 selectively inhibits pLKO.1c superinduction but not basal GFP levels. Cells were transfected with the indicated plasmids, except that equivalent levels of pcDNA3 were substituted for pLKO.1c. Transfected cell extracts were subjected to Western blotting. (D) FACS quantitation of wild-type and mutant 4E-BP1 effects on pLKO.1c-mediated superinduction of GFP expression. The fluorescence intensities and SDs of triplicate transfections in the absence of pLKO.1c have been shown normalized to 1.0 for each condition (pcDNA3 only or in the presence of wild-type or mutant 4E-BP1). Expression of 4E-BP1 significantly reduced pLKO.1c-mediated superinduction (compare lanes 4 and 5; P < 0.005), whereas F114A expression had no effect (compare lanes 4 and 6; P = 0.75). The mTOR inhibitor Torin 1 similarly reduced both basal and pLKO.1c-induced GFP expression. Two exposures of the Western blot with GFP-specific antibody are shown. (F) FACS quantitation of Torin 1 effects on pLKO.1c-mediated superinduction of GFP. Although 4E-BP1 expression significantly inhibited the effect of pLKO.1c (compare lanes 4 and 5; P < 0.001), lentivirus-induced GFP expression was not significantly different when Torin 1 was added (compare lanes 4 and 6; P = 0.14).
unphosphorylated forms, had a selective effect on superinduction (Fig. 4D). Cotransfection of pLKO.1c with the EGFP expression plasmid in the presence of the mTORC1 inhibitor, Torin 1, similarly decreased levels of both basal and lentivirus-induced GFP expression (Fig. 4E and F). Thus, although mTORC1 activity affects superinduction, cap-dependent translation of GFP is more sensitive to 4E-BP1 inhibition in the presence of pLKO.1c than in its absence. Our experiments suggest that retroviral sequences induce an mTORC1-independent signaling event that increases cap-dependent translation of exogenous genes.

**DNA Causes Superinduction.** To assess whether the transfected DNAs or their resulting transcripts increase expression of cotransfected genes, we cloned the Psi to shRNA sequence from pLKO.1c into the vector pTRE3G-BI-mCherry, which contains a bidirectional doxycycline (DOX)-inducible promoter (Fig. 5A). Thus, although mTORC1 activity affects superinduction, cap-dependent translation of GFP is more sensitive to 4E-BP1 inhibition in the presence of pLKO.1c than in its absence. Our experiments suggest that retroviral sequences induce an mTORC1-independent signaling event that increases cap-dependent translation of exogenous genes.

**Discussion**

Using lentiviral vectors for RNA interference experiments, we observed vector-induced expression of cotransfected genes (superinduction). Although vectors lacking retroviral sequences did not increase expression from cotransfected plasmids, transfer of the 5′ end of the lentivirus vector, including the shRNA sequences, caused superinduction of at least five different exogenous genes (mCherry, EGFP, Renilla luciferase, and Rennilla luciferase). Cotransfected plasmids showed no difference in the steady state level of RNA expressed, whereas Western blotting or fluorescence microscopy of transfected cells expressing retroviral sequences revealed 5- to 10-fold increases in protein expression. Deletions of pLKO.1c suggested that retroviral and shRNA sequence effects are additive. An unrelated gammaretroviral vector, MigR1, also gave superinduction (Fig. 2). Superinduction was observed with different transfection methods (electroporation, calcium phosphate, and lipid reagents) and independent cell lines. Thus, the retroviral effect occurs in trans, is not dependent on specific reporters or transfection protocol, and is not limited to specific cell types or species.

Experiments with a vector containing the pLKO.1c Psi-shRNA region downstream of an inducible promoter indicated that vector transcription inhibited superinduction of cotransfected...
genes and cap-dependent translation (Fig. 5). Furthermore, elimination of the 5′ LTR within plKO.1c had little or no effect on cotransfected gene expression. Linearization of plKO.1c within the 5′ enhancer (SpI) or downstream of the shRNA (EcoRI), which would reduce transcript levels by different means, did not affect superinduction, but multiple cleavages by HinfI did. Together, these data strongly suggest that DNA is responsible for superinduction. Because transfection of multiple sequences that have little sequence identity, such as HIV-1, shRNA, and MuLV, increased cotransfected gene expression, our results are consistent with superinduction by a distinct DNA structure.

Several pieces of evidence argue that retroviral sequences alter the translation of coexpressed genes. First, no difference in RNA levels of cotransfected genes was detected. Second, pulse-labeling experiments showed increased incorporation of radioactive amino acids into GFP expressed from a plasmid cotransfected with plKO.1c. Third, transfection of bicistronic vectors together with plKO.1c increased cap-dependent expression preferentially compared with IRES-dependent reporter expression from the same transcript. Therefore, introduction of retroviral vectors has the opposite effect of stress or IFN induction.

Increased translation of cotransfected genes indicates that retroviral sequences trigger cell signaling. Many signals affecting translation alter the mTORC1 kinase activity (26). Nonetheless, no differences were detected in the phosphorylation of targets upstream or downstream of mTORC1, such as AKT, p70 S6K, and 4E-BP1, in the presence or absence of plKO.1c. The mTORC1 kinase inhibitor Torin 1 (27) showed no selective effect on lentivirus-mediated superinduction versus basal expression. The 4E-BP1 mutant, F114A, which cannot interact with Raptor in the mTORC1 complex and cannot be phosphorylated and released from eIF4E (24), inhibited basal and lentivirus-induced translation to similar extents (Fig. 4D). In contrast, expression of wild-type 4E-BP1 selectively inhibited plKO.1c-mediated GFP, but not basal GFP levels (Fig. 4 C and D). Increased expression of 4E-BP1 dramatically increased phosphorylated forms (Fig. 4C), but how this would inhibit translation initiation of capped mRNAs is unclear. We detected twofold increased levels of eIF4E in the presence of plKO.1c, suggesting signaling that elevates eIF4E and its ability to stimulate eIF-4A helicase activity (28). Many cancer cells have elevated eIF4E levels, resulting in increased translational efficiency (29). Nevertheless, eIF4E overexpression did not reproduce retroviral superinduction.

DNA elements in both lentiviral and gammaretroviral vectors increased cap-dependent translation of cotransfected genes in trans and in cis (Fig. 5). Because modified vectors would eliminate the need for recombining, addition of these vectors during transfection will save both time and expense while improving protein production. Our results reveal unforeseen effects of shRNA expression vectors. Expression of an shRNA, even those with no known targets, by transient transfection may dramatically increase levels of protein products from cotransfected expression plasmids. In addition, we found that cap-dependent translation can be controlled by mechanisms that are independent of phosphorylation by the mTOR kinase. These experiments suggest that retroviral DNA controls the translation of coexpressed genes, thus affecting subgenomic mRNA expression. Understanding the activity of these critical retroviral sequences will improve our knowledge of translational control as well as guide the design of vectors for gene therapy, DNA vaccines, and molecular biology.

Materials and Methods

Conditions for growth of cell lines and transfections have been previously reported (30). Detailed methods and reagents are given in SI Materials and Methods.

ACKNOWLEDGMENTS. We acknowledge helpful discussions with members of the J.P.D. laboratory and Drs. Jon Huibregtse, Arlen Johnson, and Chris Sullivan. This work was supported by Grant R01CA167053 from the National Institutes of Health.

10. Byun H, et al. (2010) Retroviral Rem protein requires processing by signal peptidase complexes or downstream of mTORC1, suggesting signaling that elevates eIF4E and its ability to stimulate eIF-4A helicase activity (28). Many cancer cells have elevated eIF4E levels, resulting in increased translational efficiency (29). Nevertheless, eIF4E overexpression did not reproduce retroviral superinduction.