*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 11940–11944, December 1995
Microbiology

# Production of avian leukosis virus particles in mammalian cells can be mediated by the interaction of the human immunodeficiency virus protein Rev and the Rev-responsive element

GEORGIOS NASIOULAS*†, STEPHEN H. HUGHES‡§, BARBARA K. FELBER*, AND JEANNETTE M. WHITCOMB‡

‡Gene Expression in Eukaryotes Section and *Human Retrovirus Pathogenesis Group, Advanced BioScience Laboratories–Basic Research Program, National Cancer Institute Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702-1201

*Communicated by George F. Vande Woude, Frederick Cancer Research and Development Center, Frederick, MD, July 28, 1995*

**ABSTRACT** **In human immunodeficiency virus type 1-infected cells, the efficient expression of viral proteins from unspliced and singly spliced RNAs is dependent on two factors: the presence in the cell of the viral protein Rev and the presence in the viral RNA of the Rev-responsive element (RRE). We show here that the HIV-1 Rev/RRE system can increase the expression of avian leukosis virus (ALV) structural proteins in mammalian cells (D-17 canine osteosarcoma) and promote the release of mature ALV virions from these cells. In this system, the Rev/RRE interaction appears to facilitate the export of full-length unspliced ALV RNA from the nucleus to the cytoplasm, allowing increased production of the ALV structural proteins. Gag protein is produced in the cytoplasm of the ALV-transfected cells even in the absence of a Rev/RRE interaction. However, a functional Rev/RRE interaction increases the amount of Gag present intracellularly and, more strikingly, results in the release of mature ALV particles into the supernatant. RCAS virus containing an RRE is replication-competent in chicken embryo fibroblasts; however, we have been unable to determine whether the particles produced in D-17 cells are as infectious as the particles produced in chicken embryo fibroblasts.**

Retroviruses can be classified as either simple or complex, on the basis of their genomic organization (1–3). Avian leukosis virus (ALV) is a simple retrovirus. The ALV genome contains *gag*, *pol*, and *env* genes that encode the viral structural proteins, the enzymes required for viral replication and the surface glycoproteins, respectively (4). Complex retroviruses, such as human immunodeficiency virus type 1 (HIV-1), have, in addition to the *gag*, *pol*, and *env* genes, regulatory and accessory genes that are expressed from subgenomic messages that have undergone multiple splicing reactions (5–8).

Retroviruses are (+)-strand viruses; i.e., their genomes are the same polarity as their mRNAs (9). In most retroviruses, including ALV and HIV-1, a single RNA transcript serves as the genome for the progeny virus, as one of the viral messages, and as the precursor for all of the spliced subgenomic messages. The retroviral life cycle is absolutely dependent on the appropriate regulation of splicing and the efficient transport and translation of both spliced and unspliced versions of viral RNA.

For ALV, we do not understand what controls either the ratio of spliced to unspliced RNA or the mechanisms involved in transporting the unspliced RNA from the nucleus to the cytoplasm. An element known as the negative regulator of splicing, located in the *gag* region of ALV, may be involved (10, 11) and other cis-acting sequences have been identified throughout the genome (12, 13). In the complex retroviruses, RNA splicing patterns and the transport of unspliced RNAs appear to be controlled by viral regulatory proteins (14–21). In HIV-1, the interaction of the viral protein Rev with the Rev-responsive element (RRE) allows unspliced and intermediate spliced messages to be transported and translated efficiently. It has been proposed that this interaction (22–25) affects message transport and translation at many steps, including message stability (26, 27), commitment to splicing (28, 29), nuclear/cytoplasmic transport (26, 30–32), RNA/poly(A)$^+$-binding protein interactions (33), and translatability (34–36). These different activities may, at least in part, reflect the different cell types and systems that have been used to study the Rev/RRE interactions.

In contrast to avian cells, mammalian cells do not support the replication of ALV (37–39). When a wild-type ALV is experimentally introduced into mammalian cells, low levels of Gag protein are produced (40, 41), but no infectious virus is released from the cells (37, 42). Defects in either splicing, transport, or stability of full-length ALV RNA could help to explain the low levels of ALV Gag and lack of ALV particles produced by mammalian cells. We have asked how the HIV-1 Rev/RRE system affects ALV production in a mammalian cell line.

## MATERIALS AND METHODS

**Plasmids, Cells, and Transfection.** D-17 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics. We routinely used 5 μg of RCAS-based plasmids, 2 μg of pCMV-rev (43) (when indicated), and 0.25 μg of pRSVluc (44) per dish. Transfection was by the calcium phosphate method (45). Cells and supernatants were harvested 24 hr after addition of DNA. Three plates were transfected for each data point. The supernatants from all three plates were pooled, cleared by centrifugation at 1000 × *g* for 10 min, and frozen at −70°C for later analysis. Cells from individual plates were harvested for the analysis of cell-associated proteins, total RNA, and cytoplasmic and nuclear RNA.

**Protein Preparation and Analysis.** For Western analysis, virion and cellular protein preparation was as described (46). Proteins were fractionated in an SDS/10% polyacrylamide gel, transferred to nitrocellulose, and probed with a polyclonal anti-matrix (MA) rabbit serum (generously provided by Volker Vogt, Cornell University) followed by $^{125}$I-protein A.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ALV, avian leukosis virus; CEF, chicken embryo fibroblast; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIV-1, human immunodeficiency virus type 1; RRE, Rev-responsive element; RSV, Rous sarcoma virus.

†Present address: Department of Hygiene and Epidemiology, Athens University Medical School, Athens, Greece.

§To whom reprint requests should be addressed at: National Cancer Institute Frederick Cancer Research and Development Center, P.O. Box B, Bldg. 539, Frederick, MD 21702-1201.

**RNA Preparation and Analysis.** For the preparation of total RNA and virion RNA, cells were washed twice in ice-cold phosphate-buffered saline, lysed with 1 ml of RNAzol (Tel-Test, Friendswood, TX), and transferred to a 1.5-ml microcentrifuge tube. RNA was purified according to the manufacturer's directions. Cytoplasmic and nuclear RNAs were prepared by standard procedures (47).

For Northern analysis, RNA was precipitated in the presence of vanadyl ribonucleosides, denatured in formamide, fractionated in 2.2 M formaldehyde/1% agarose gels, and transferred to nitrocellulose filters. The filters were rinsed, baked, and prehybridized at 65°C for 1 hr. RNA probes were prepared by incorporation of [$\alpha$-$^{32}$P]UTP into T7 transcripts with an RNA transcription kit (Stratagene) and subsequent fractionation on Sephadex G-25 spin columns (Boehringer Mannheim) to remove unincorporated nucleotides. The blots were hybridized at 65°C overnight and washed twice for 30 min at 70°C. The filters were dried and analyzed by autoradiography.

**Electron Microscopy.** Preparation of cells for ultrastructural studies was as described (48). Ultrathin sections (70 nm) were made and mounted on necked copper grids. The sections were double-stained with uranyl acetate and lead citrate and then stabilized with carbon in a vacuum evaporator. The sections were examined and photographed with a Hitachi H-7000 electron microscope operated at 75 kV.

## RESULTS

We introduced the HIV-1 RRE into the unique *Cla* I cloning site between *env* and the downstream long terminal repeat of two ALV-based vectors, RCAS and RCASBP (49) (Fig. 1). These two viruses differ in the source of the *pol* gene that they carry: RCAS has the *pol* gene from the Schmidt–Ruppin A strain and RCASBP contains the *pol* gene from the Bryan high-titer strain of RSV. In avian cells, RCASBP replicates to a higher titer than RCAS (51). We were curious to see whether the Bryan *pol* region would have an effect on the level of ALV protein expressed in mammalian cells.

Transfection of plasmids containing full-length infectious proviruses into chicken embryo fibroblasts (CEFs) resulted, in all cases, in the production of infectious virus (data not shown). Analysis of viral particle production by Western blot showed identical patterns of processing of the Gag polyprotein in the parental viruses and the viruses containing an RRE. However, the viruses containing an RRE grew more slowly than the parental viruses and lost their RRE sequences upon continued passage of the virus on CEFs.

The same plasmids were then transfected into the D-17 canine cell line. We chose this cell line because it can be transfected efficiently and the RSV LTR is a strong promoter in these cells (A. Zolotukhin, personal communication). Western blots of lysates from transfected D-17 cells showed that all of the viral DNAs directed the expression of cell-associated Gag polyprotein (Fig. 2*A*). However, cells that were transfected with a Rev-producing plasmid and one of the RRE-containing viruses contained significantly higher levels of Gag.

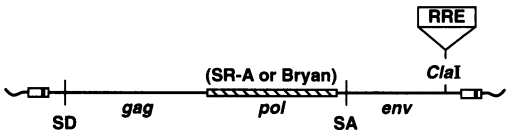


FIG. 1. Diagram of the proviral clones. RCAS is derived from the Schmidt–Ruppin A (SR-A) strain of RSV. In RCASBP, the *pol* gene of SR-A has been replaced by the *pol* gene of the Bryan high-titer strain of RSV. RRE indicates a 330-bp fragment from HIV-1 (bases 7266–7595 from the HXB2 strain of HIV-1; ref. 50). SD, splice donor; SA, splice acceptor.

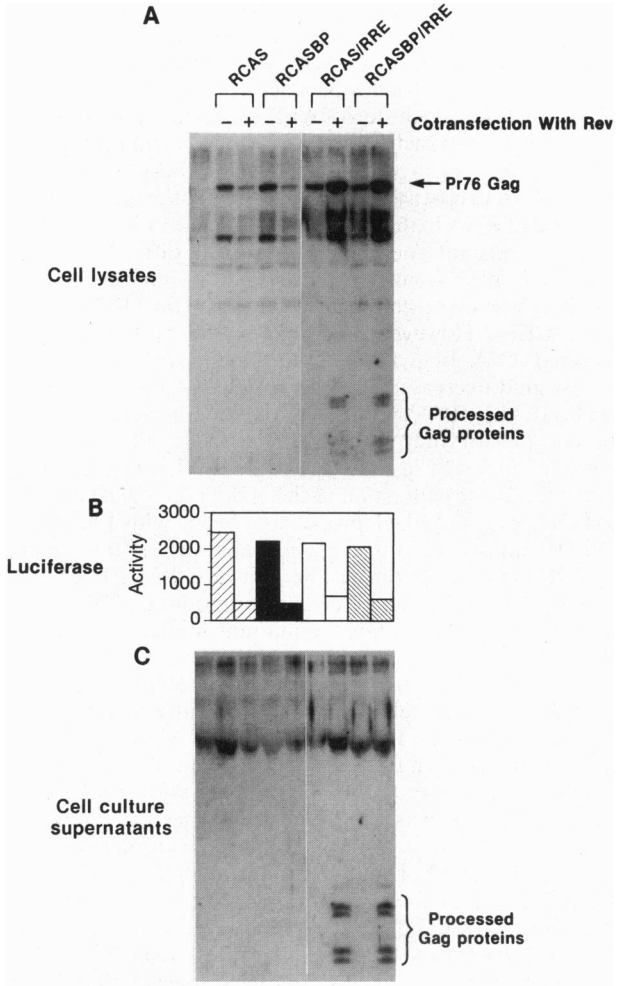


FIG. 2. Expression of viral proteins in D-17 cells transfected with the indicated proviral plasmids alone (−) or with a plasmid that expresses HIV-1 Rev (+). The Gag proteins were analyzed by Western analysis with anti-MA antibody 24 hr after transfection. Position of the Gag precursor protein (Pr76) is marked. Processed Gag proteins are also indicated. (*A*) Western blot of anti-MA-reactive proteins in D-17 cell lysates 24 hr after transfection. (*B*) Expression (in arbitrary light units standardized per $\mu$g of protein) of a luciferase reporter gene in each transfectant. (*C*) Western blot of anti-MA-reactive proteins present as particles in the culture supernatant.

The presence of Rev had a similar effect on both the RCAS/RRE and RCASBP/RRE viruses. For both of these viruses the amount of Gag in the cells was increased by the interaction of Rev and the RRE and processing of the Gag polyprotein was seen only when both Rev and the RRE were present. The RCASBP *pol* region did not appear to have any effect on the expression of ALV Gag proteins in these cells in either the presence or the absence of Rev.

When the viral genome did not contain an RRE, Rev decreased Gag production by a factor of 3–5. This inhibition appears to be a nonspecific effect of Rev on cotransfected plasmids that do not contain an RRE (50). In all experiments, the plasmid pRSVluc was cotransfected and luciferase activity was measured to determine the relative transfection efficiencies. Cotransfection with Rev consistently decreased luciferase expression (Fig. 2*B*). The decrease in luciferase activity was similar to the effect of Rev on the expression of viral protein from RCAS and RCASBP without an RRE.

Western blot analysis of particles harvested from the supernatant of D-17 cells 24 hr posttransfection showed that one effect of the Rev/RRE interaction was to permit the release of ALV particles into the supernatant (Fig. 2*C*). Particle

production occurred only when an RRE-containing virus was introduced into cells that simultaneously expressed Rev. Processing of Gag in these particles was indistinguishable from that seen in ALV particles produced in CEFs.

To investigate which step of ALV expression was affected by the Rev/RRE interaction, we performed Northern blot analysis of total, nuclear, and cytoplasmic RNAs. In total and nuclear RNA preparations (Fig. 3*A* and *B*), the ratio of spliced to unspliced RNA in the presence or absence of Rev/RRE was relatively constant and was not markedly different in transfected D-17 cells from that seen in RCAS-infected CEFs. In fact, there was slightly more spliced RNA in the D-17 cells than in the CEFs. However, all samples had both spliced and unspliced RNA. In general, cotransfection with a Rev expression plasmid decreased the overall level of both spliced and unspliced ALV RNAs produced from the transfected plasmids whether or not they contained an RRE; this effect was especially apparent in the RCAS sample. Expression of Rev did not affect the expression of the endogenous gene encoding GAPDH. The GAPDH probe does react with the chicken GAPDH message; however, in most cases the chicken GAPDH band was barely visible. Because the CEF RNA was from productively infected cells, the amount of RNA loaded was significantly less than the amount loaded for the D-17 cells.

By contrast, the distribution of viral RNA seen in the cytoplasm of D-17 cells (Fig. 3*C*) was quite different from that seen in the total RNA or nuclear RNA preparations. In cells transfected with RCAS or RCASBP viral DNAs that did not contain an RRE, the majority of the viral RNA present in the cytoplasm was the spliced *env* message. Some unspliced viral RNA was observed in the cytoplasm of cells transfected with all of the ALVs, although in the absence of an RRE and Rev the amount was quite low. A functional Rev/RRE interaction increased the level of unspliced viral RNA and decreased the level of spliced viral RNA in the cytoplasm (Fig. 3*C*). These findings suggest that in this system the Rev/RRE interaction does not stabilize the unspliced viral message but instead plays a role in transport and/or compartmentalization (and possibly translation) of the full-length viral mRNAs.

We wanted to determine whether the processed Gag protein detected in the supernatant represented authentic viral particles. Northern analysis showed that the particles contained full-length ALV RNA (data not shown), as would be expected for authentic ALV particles. Electron micrographs of the ALV particles produced in D-17 cells (Fig. 4) show that the particles have condensed cores as seen in mature ALV particles produced from CEFs (not shown). Most of the mature particles were released from the cells but particles

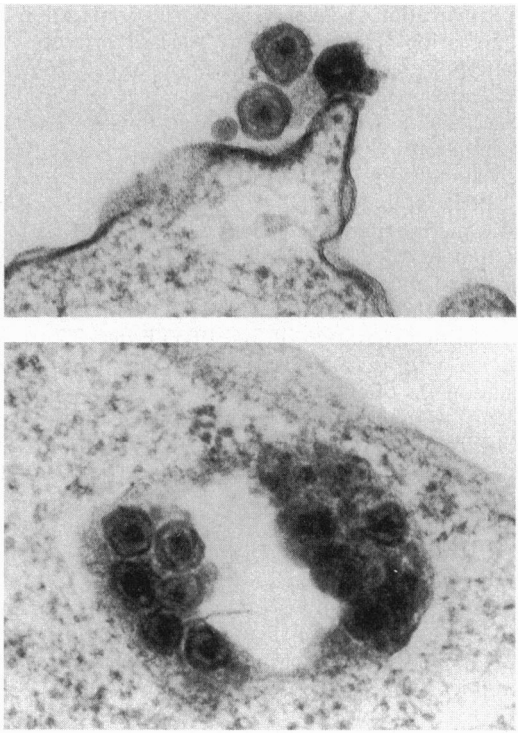

FIG. 4. Electron micrographs of particles produced by the D-17 cells. Particles were seen only in those cultures that were cotransfected with both an RRE-containing plasmid and the pCMVrev plasmid. (×135,000.)

were also present in membrane-bound vesicles that appeared to be intracellular. The data shown in Figs. 2–4 are from a single experiment.

Infectious virus was present in the supernatants from the D-17 cells, and virus could be transferred from cell-free supernatants to CEF cells and cause a productive infection. However, infectious virus was also detected by this method in supernatants from cells transfected with RCAS and RCASBP virus in the absence of a Rev/RRE interaction in which viral proteins were not readily visible in the Western blots. Very long exposures of some Western blots indicated the release of some particles even in the absence of a Rev/RRE interaction. Our assay for infectious virus, viral growth after transfer to CEFs, can detect very low levels of infectious viral particles. We are unable at this time to make any strong statements about the effects of the Rev/RRE interaction on the infectivity or titer of the virus.

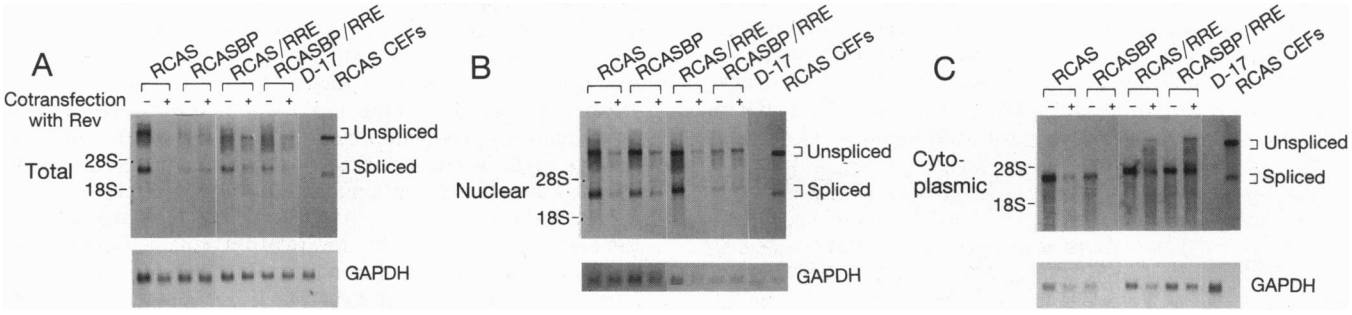


FIG. 3. Northern blot analysis of total (*A*), nuclear (*B*), and cytoplasmic (*C*) RNA from D-17 cells transfected with ALV plasmid alone (−) or with a plasmid that expresses HIV-1 Rev (+). The RNA was hybridized with an ALV-specific RNA probe and with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific RNA probe to control for the amount of RNA loaded onto each lane and for RNA integrity. Positions of unspliced and *env* spliced viral RNAs and of 28S and 18S rRNAs are indicated. RNAs from the viruses containing an RRE are slightly larger than those without an RRE. The last lane in each panel contained RNA from ALV-infected CEFs. The RNA sample in *C* lane 4 was degraded during preparation. However, in other experiments, the RNA in this sample was similar to that in lanes 1–3.

## DISCUSSION

The regulation of the splicing, transport, and stability of ALV RNAs is not well understood (13). There are sequences in *gag* that have been reported to inhibit splicing of the viral transcript (10, 52, 53), and interactions of the viral RNA with nuclear splicing factors may also play a role in the decision of whether to splice and/or transport RNAs to the cytoplasm (12, 54). However, it is clear that whatever factor(s) control these events in chicken cells, they either do not exist in mammalian cells or are sufficiently different from their chicken homologs that they cannot appropriately regulate ALV RNA expression in mammalian cells. Mammalian cells containing an integrated RSV provirus do not produce RSV particles. However, fusion of these cells with chicken fibroblasts results in the release of RSV particles (55–57), suggesting that chicken cells contain factors that are essential for proper regulation of splicing, transport, and/or translation of ALV proteins. We report here that an HIV-1 Rev/RRE interaction can promote the production of ALV particles by a mammalian cell line. These results allow us to draw several conclusions about the expression and processing of ALV RNA. (*i*) One of the main blocks to the efficient expression of ALV in mammalian cells appears to take place at the RNA level. Specifically, in the nonpermissive D-17 cells, there appears to be a block to the efficient export of full-length viral RNA from the nucleus to the cytoplasm. In the absence of Rev/RRE a moderate amount of Gag was present in the cytoplasm; however, the Gag that was produced in the absence of a Rev/RRE interaction did not assemble into viral particles, as judged by the lack of Gag proteins in the supernatant, lack of appropriate cleavage of the Gag precursor into mature Gag proteins, and our inability to find viral particles by electron microscopy. One possibility is that the Rev/RRE mediated particle release we observed was simply a result of mass action (i.e., there is a threshold level of Gag required for particle assembly to occur). We consider this explanation unlikely, as the difference in the amount of Gag produced in the presence or absence of Rev is not large. An alternative explanation is that differential splicing may be involved. For example, it is possible that, in the absence of a Rev/RRE interaction, alternative splicing results in what appears to be unspliced RNA yet codes for a defective Gag polyprotein that inhibits particle assembly. A third possibility is that the Rev/RRE interaction facilitates the transport of viral RNAs to a specific cellular compartment that somehow promotes particle assembly.

(*ii*) Although the overall levels of viral RNA were lower in the ALV-transfected D-17 cells than in ALV-infected CEFs, the ratio of spliced to unspliced RNA in the total RNA pool of the transfected D-17 cells was not significantly different from that seen in ALV-infected chicken cells. In contrast, previous studies on the expression of RSV in NIH 3T3 mouse cells transformed with RSV showed that the majority of viral RNA was present as the spliced *src* message (58, 59). However, since these cells had been selected for high levels of Src expression, it was possible that the pattern of RNA splicing seen in the RSV-transformed cells was a result of that selection. In NIH 3T3 cells transiently transfected with RSV the results were only slightly different (60). Multiple spliced forms of RNA were seen, including some spliced forms not seen in ALV-infected chicken cells. The majority of the RSV RNA was either the *src*-spliced message, or a doubly spliced *env*/*src* fusion transcript. In the ALV-transfected D-17 cells we found that the overall pattern of viral RNA was very similar to that seen in ALV-infected chicken cells. However, most of the full-length ALV RNA in D-17 cells was found in the nucleus; the ALV RNA present in the cytoplasm of D-17 cells was almost entirely *env*-spliced RNA. The lack of full-length viral RNA in the cytoplasm did not appear to be due to the instability of the unspliced viral RNA but rather to a block in its transport from the nucleus. It has been suggested that the association of unspliced RNA with nuclear splicing factors could inhibit the transport of intron-containing RNAs from the nucleus to the cytoplasm (12, 28). The presence of an additional splice acceptor site could explain, in part, the differences between RSV and ALV splicing patterns in mammalian cells. However, there are examples of intron-containing RNAs that are perfectly capable of being expressed, transported, and translated in mammalian cells (61).

(*iii*) These studies show that a functional Rev/RRE interaction can occur in non-primate cells. We have used canine cells in these experiments, but we have seen positive Rev/RRE effects on the expression of ALV proteins in mouse, chicken, and human cells as well (unpublished observations). Earlier reports had indicated that the Rev/RRE interaction requires cellular factors present only in human cells (62, 63); however, our data argue against the requirement for species-specific Rev/RRE cofactors.

(*iv*) One major difference between avian and mammalian retroviruses is that the Gag protein of mammalian retroviruses is obligately myristoylated on its N terminus (64, 65), whereas the ALV Gag protein is acetylated (66). N-terminal myristoylation of ALV Gag has been reported to promote ALV particle formation in certain mammalian cells (67–69). However, a more recent report (70) has shown that myristoylation of Gag is not required for assembly of ALV particles in mammalian cells. Since none of the vectors used in these experiments encoded signals for myristoylation of the Gag precursor protein, our studies confirm that myristoylation of the ALV Gag precursors is not an important determinant of ALV virion assembly in mammalian cells.

We were surprised to observe cell-associated processing of the Gag precursor in D-17 cells. Ordinarily, activation of the viral protease and particle maturation are tightly linked to budding of the viral particle from the cell surface. Cell-associated processing of Gag is not usually seen in ALV-infected CEFs but has been reported when ALV is expressed in other host systems, including turkey cells and several mammalian cell lines, especially when high levels of the Gag protein are produced. One possibility is that the processed proteins we observed were associated with mature viral particles that had been partially or completely extruded through the cell membrane and remained associated with the membrane in these cells. Alternatively, particles may have been extruded into deep pits in the cell membrane or into vesicles within the cell. The electron micrographs of the transfected D-17 cells suggest that such events do occur. We observed several cases in which mature membrane-bound particles were present in vesicles or pits (Fig. 5).

In most eukaryotic cells, there is a block to the export of unspliced or partially spliced RNAs from the nucleus to the cytoplasm (26, 61). Different retroviruses have found different solutions to this restriction. Lentiviruses and other complex retroviruses express a viral protein and encode a cis-acting RNA sequence to allow the efficient transport and translation of their unspliced and intermediate spliced RNAs (15–18, 19). Type D simple retroviruses (71) have been shown to contain a cis-acting RNA element (designated the constitutive transport element, or CTE) that apparently provides an RRE-like function via interaction with cellular "Rev-like" factor(s) (72, 73).

The regulation of RNA transport and translation in ALV is cell-type dependent. The mammalian counterparts of the factor(s) that provide these functions in avian cells are unable to appropriately regulate ALV RNA, either because they are not present in mammalian cells or because they do not recognize the cognate signals in the ALV RNA. We have shown that the HIV-1 Rev/RRE interaction can promote ALV particle production in mammalian cells and that the Rev/RRE interaction is sufficient to cause the efficient trans-

port and translation of unspliced RNA in mammalian cells that is essential for ALV particle production. These data demonstrate that ALV replication is dependent on appropriate posttranscriptional RNA regulation for virus production. The HIV-1 Rev/RRE system, which can act in a variety of cells and in heterologous RNAs (74), can rescue ALV production in mammalian cells. We believe that ALV and other simple retroviruses, will, like the type D retroviruses, be found to depend on cellular factors for appropriate posttranscriptional regulation of RNA. In contrast, the complex retroviruses appear to have acquired, presumably from their cellular hosts, the factors necessary to control these processes in a cell-independent manner.

We acknowledge Vicki Marshall and Kunio Nagashima of the Laboratory of Cell and Molecular Structure, Program Resources, Frederick, MD, for the electron microscopy work. We thank Volker Vogt for the anti-MA antibody, Andrei Zolotukhin for helpful discussions, G. N. Pavlakis for reagents, Jenifer Bear for technical assistance, Marjorie Strobel for critical reading of the manuscript, and Hilda Marusiodis for exquisite secretarial assistance. This research was sponsored by the National Cancer Institute under contract with Advanced BioScience Laboratories.

1. Cullen, B. R. (1991) *J. Virol.* **65,** 1053–1056.
2. Myers, G. & Pavlakis, G. N. (1992) in *The Retroviridae*, ed. Levy, J. (Plenum, New York), pp. 1–37.
3. Temin, H. M. (1992) in *The Retroviridae*, ed. Levy, J. (Plenum, New York), pp. 1–18.
4. Coffin, J. (1982) in *RNA Tumor Viruses*, eds. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 261–368.
5. Feinberg, M. B., Jarrett, R. F., Aldovini, A., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **46,** 807–817.
6. Muesing, M. A., Smith, D. H., Cabradilla, C. D., Benton, C. V., Lasky, L. A. & Capon, D. J. (1985) *Nature (London)* **313,** 450–458.
7. Schwartz, S., Felber, B. K., Fenyo, E. M. & Pavlakis, G. N. (1990) *J. Virol.* **64,** 5448–5456.
8. Viglianti, G. A., Sharma, P. L. & Mullins, J. I. (1990) *J. Virol.* **64,** 4207–4216.
9. Varmus, H. & Swanstrom, R. (1982) in *RNA Tumor Viruses*, eds. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 649–784.
10. Arrigo, S. & Beemon, K. (1988) *Mol. Cell. Biol.* **8,** 4858–4867.
11. Cullen, B. R., Kopchick, J. J. & Stacey, D. W. (1982) *Nucleic Acids Res.* **10,** 6177–6190.
12. Fu, X. D., Katz, R. A., Skalka, A. M. & Maniatis, T. (1991) *Genes Dev.* **5,** 211–220.
13. Stoltzfus, C. M. (1988) *Adv. Virus Res.* **35,** 1–38.
14. Cullen, B. R. (1992) *Microbiol. Rev.* **56,** 375–394.
15. Felber, B. K. & Pavlakis, G. N. (1993) *AIDS* **7,** Suppl., S51–S62.
16. Gitlin, S. D., Dittmer, J., Reid, R. L. & Brady, J. N. (1993) in *Human Retroviruses*, ed. Cullen, B. R. (Oxford Univ. Press, New York), pp. 159–192.
17. Green, P. L. & Chen, I. S. (1990) *FASEB J.* **4,** 169–175.
18. Greene, W. C. (1990) *Annu. Rev. Immunol.* **8,** 453–475.
19. Haseltine, W. A. (1991) *Genetic Structure and Regulation of HIV* (Raven, New York), pp. 1–537.
20. Parslow, T. C. (1993) in *Human Retroviruses*, ed. Cullen, B. R. (Oxford Univ. Press, New York), pp. 101–136.
21. Steffy, K. & Wong-Staal, F. (1991) *Microbiol. Rev.* **55,** 193–205.
22. Bartel, D., Zapp, M., Green, M. & Szostak, J. (1991) *Cell* **67,** 529–536.
23. Cook, K. S., Fisk, G. J., Hauber, J., Usman, N., Daly, T. J. & Rusche, J. R. (1991) *Nucleic Acids Res.* **19,** 1577–1583.
24. Heaphy, S., Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Karn, J., Lowe, A. D., Singh, M. & Skinner, M. A. (1990) *Cell* **60,** 685–693.
25. Holland, S. M., Chavez, M., Gerstberger, S. & Venkatesan, S. (1992) *J. Virol.* **66,** 3699–3706.
26. Felber, B. K., Hadzopoulou-Cladaras, M., Cladaras, C., Copeland, T. & Pavlakis, G. N. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1495–1499.
27. Schwartz, S., Felber, B. K. & Pavlakis, G. N. (1992) *J. Virol.* **66,** 150–159.
28. Chang, D. D. & Sharp, P. A. (1989) *Cell* **59,** 789–795.
29. Lu, X. B., Heimer, J., Rekosh, D. & Hammarskjold, M.-L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7598–7602.
30. Emerman, M., Vazeux, R. & Peden, K. (1989) *Cell* **57,** 1155–1165.
31. Hammarskjold, M. L., Heimer, J., Hammarskjold, B., Sangwan, I., Albert, L. & Rekosh, D. (1989) *J. Virol.* **63,** 1959–1966.
32. Malim, M. H., Hauber, J., Le, S. Y., Maizel, J. V. & Cullen, B. R. (1989) *Nature (London)* **338,** 254–257.
33. Campbell, L. H., Borg, K. T., Haines, J. K., Moon, R. T., Schoenberg, D. R. & Arrigo, S. J. (1994) *J. Virol.* **68,** 5433–5438.
34. Arrigo, S. J. & Chen, I. S. (1991) *Genes Dev.* **5,** 808–819.
35. D'Agostino, D. M., Felber, B. K., Harrison, J. E. & Pavlakis, G. N. (1992) *Mol. Cell. Biol.* **12,** 1375–1386.
36. Lawrence, J. B., Cochrane, A. W., Johnson, C. V., Perkins, A. & Rosen, C. A. (1991) *New Biol.* **3,** 1220–1232.
37. Altaner, C. & Temin, H. M. (1970) *Virology* **40,** 118–134.
38. Cooper, G. M., Copeland, N. G., Zelenetz, A. D. & Krontiris, T. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44,** 1169–1176.
39. Varmus, H. E., Bishop, J. M. & Vogt, P. K. (1973) *J. Mol. Biol.* **74,** 613–626.
40. Eisenman, R. N. & Vogt, V. M. (1978) *Biochim. Biophys. Acta* **473,** 187–239.
41. Vogt, V. M., Bruckenstein, D. A. & Bell, A. P. (1982) *J. Virol.* **44,** 725–730.
42. Svoboda, J., Machala, O., Donner, L. & Sovova, V. (1971) *Int. J. Cancer* **8,** 391–400.
43. Mermer, B., Felber, B. K., Campbell, M. & Pavlakis, G. N. (1990) *Nucleic Acids Res.* **18,** 2037–2044.
44. de Wet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R. & Subramani, S. (1987) *Mol. Cell. Biol.* **7,** 725–737.
45. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1373–1376.
46. Whitcomb, J. M., Ortiz-Conde, B. A., & Hughes, S. H. (1995) *J. Virol.* **69,** 6228–6238.
47. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
48. Gonda, M. A., Fine, D. L. & Gregg, M. (1978) *Arch. Virol.* **56,** 297–307.
49. Hughes, S. H., Petropoulos, C. J., Federspiel, M. J., Sutrave, P., Forry-Schaudies, S. & Bradac, J. A. (1990) *J. Reprod. Fertil. Suppl.* **41,** 39–49.
50. Solomin, L., Felber, B. K. & Pavlakis, G. N. (1990) *J. Virol.* **64,** 6010–6017.
51. Petropoulos, C. J. & Hughes, S. H. (1991) *J. Virol.* **65,** 3728–3737.
52. Arrigo, S., Yun, M. & Beemon, K. (1987) *Mol. Cell. Biol.* **7,** 388–397.
53. Stoltzfus, C. M., Chang, L. J., Cripe, T. P. & Turek, L. P. (1987) *J. Virol.* **61,** 3401–3409.
54. McNally, M. T. & Beemon, K. (1992) *J. Virol.* **66,** 6–11.
55. Machala, O., Donner, L. & Svoboda, J. (1970) *J. Gen. Virol.* **8,** 219–229.
56. Coffin, J. M. (1972) *J. Virol.* **10,** 153–156.
57. Steimer, K. A. & Boettiger, D. (1977) *J. Virol.* **23,** 133–141.
58. Quintrell, N., Hughes, S. H., Varmus, H. E. & Bishop, J. M. (1980) *J. Mol. Biol.* **143,** 363–393.
59. Deng, C. T., Stehelin, D., Bishop, J. M. & Varmus, H. E. (1977) *Virology* **76,** 313–330.
60. Berberich, S. L., Macias, M., Zhang, L., Turek, L. P. & Stoltzfus, C. M. (1990) *J. Virol.* **64,** 4313–4320.
61. McKeown, M. (1992) *Annu. Rev. Cell Biol.* **8,** 133–155.
62. Winslow, B. J. & Trono, D. (1993) *J. Virol.* **67,** 2349–2354.
63. Trono, D. & Baltimore, D. (1990) *EMBO J.* **9,** 4155–4160.
64. Henderson, L. E., Krutzsch, H. C. & Oroszlan, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 339–343.
65. Schultz, A. M. & Oroszlan, S. (1983) *J. Virol.* **46,** 355–361.
66. Palmiter, R. D., Gagnon, J., Vogt, V. M., Ripley, S. & Eisenman, R. N. (1978) *Virology* **91,** 423–433.
67. Rein, A., McClure, M. R., Rice, N. R., Luftig, R. B. & Schultz, A. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7246–7250.
68. Rhee, S. S. & Hunter, E. (1987) *J. Virol.* **61,** 1045–1053.
69. Wills, J. W., Craven, R. C. & Achacoso, J. A. (1989) *J. Virol.* **63,** 4331–4343.
70. Wills, J. W., Craven, R. C., Weldon, R. A., Jr, Nelle, T. D. & Erdie, C. R. (1991) *J. Virol.* **65,** 3804–3812.
71. Gardner, M. B., Endres, M. & Barry, P. (1994) in *The Retroviridae*, ed. Levy, J. A. (Plenum, New York), pp. 133–276.
72. Bray, M., Prasad, S., Dubay, J. W., Hunter, E., Jeang, K. T., Rekosh, D. & Hammarskjold, M. L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1256–1260.
73. Zolotukhin, A. S., Valentin, A., Pavlakis, G. N. & Felber, B. K. (1994) *J. Virol.* **68,** 7944–7952.
74. Zolotukhin, A. S., Harford, J. B. & Felber, B. F. (1994) *Nucleic Acids Res.* **22,** 4725–4732.