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**The Cellular Protein La Functions in Enhancement of Virus Release through Lipid Rafts Facilitated by Murine Leukemia Virus Glycosylated Gag**

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**ABSTRACT** Murine leukemia viruses (MuLVs) encode two forms of Gag polyprotein: the precursor for the viral core proteins (Pr65\(^{\text{gag}}\) for Moloney MuLV [M-MuLV]) and a longer glycosylated form (glyco-gag, or gPr80\(^{\text{gag}}\)). gPr80\(^{\text{gag}}\) is translated from the same unspliced viral RNA as Pr65\(^{\text{gag}}\), from an upstream in-frame CUG initiation codon. As a result, gPr80\(^{\text{gag}}\) contains 88 unique N-terminal amino acids that include a signal peptide that conducts gPr80\(^{\text{gag}}\) into the rough endoplasmic reticulum, where it is glycosylated, exported to the cell surface, and cleaved into two proteins of 55 and 40 kDa. The amino-terminal 55-kDa protein remains cell associated with the 88 unique amino acids exposed to the cytosol. We previously showed that gPr80\(^{\text{gag}}\) glycosylated, exported to the cell surface, and cleaved into two proteins of 55 and 40 kDa. The amino-terminal 55-kDa protein contains 88 unique amino acids, including a signal peptide that targets gPr80\(^{\text{gag}}\) for transport into the rough endoplasmic reticulum, leading to its glycosylation and export to the cell surface. At the cell surface, mature gPr80\(^{\text{gag}}\) is cleaved into two proteins of ca. 55 and 40 kDa (1, 5), and the 55-kDa amino-terminal portion is maintained in a type II integral membrane configuration, with the 88 unique amino acids in the cytosol (4).

Glycosylated Gag proteins are conserved among gammaretroviruses, but the molecular functions of these proteins have been unclear until recently. In mice, gPr80\(^{\text{gag}}\) is a major pathogenic determinant for neuropathic MuLV (6–9). MuLV mutants of gPr80\(^{\text{gag}}\) show substantial replication defects in mice, and there is strong selection for recovery of gPr80\(^{\text{gag}}\) expression (10–12). We previously showed that gPr80\(^{\text{gag}}\) plays a role in a late step in viral assembly or release. gPr80\(^{\text{gag}}\)-negative MuLVs bud from cells from aberrant tube-like structures (12, 13). Expression of gPr80\(^{\text{gag}}\) in mutant-infected cells increases virus particle release, and the tube-like structures are replaced by typical spherical particles (12). Recently, we found that there are two pathways for MuLV release...
from cells: interferon (IFN)-sensitive release through lipid rafts and interferon-resistant release through areas other than lipid rafts (14). gp80<sup>0.08</sup> facilitates viral release through lipid rafts, and this is apparently the more efficient pathway for release. We also found that Moloney MuLV (M-MuLV) gp80<sup>0.08</sup> can facilitate release of HIV-1 particles (14). It has also recently been reported that gp80<sup>0.08</sup> can complement a replication defect in human lymphocyte lines for Nef-deficient HIV-1, although in this study the results, gg88 does not appear to directly bind NA14 in mammalian cells, interferon-activated binding activity, a yeast two-hybrid screen was conducted as described in Materials and Methods. The bait plasmid encoded the 88 amino-terminal unique amino acids fused to the LexA DNA binding domain. This plasmid was used to screen a mouse cDNA expression library fused to the B42 activation domain in <i>Saccharomyces cerevisiae</i>. Four cDNA fusion clones that showed strong interaction with the bait plasmid were identified, and sequencing of the inserts indicated that they all were cDNAs for Sjogren’s syndrome nuclear autoantigen 1 (SSNA1; also known as nuclear autoantigen 14 [NA14] [16, 17]). While the initial report described SSNA1/NA14 as a nuclear antigen, subsequent studies indicated that it predominantly binds to centrosomes in the cytoplasm (16–18). To test for potential <i>in vivo</i> interactions between NA14 and gp80<sup>0.06</sup>, we performed two-color immunofluorescence microscopy on 293T cells transiently transfected with HA-gg88 (Fig. 3). Endogenous NA14 showed a cytoplasmic perinuclear location consistent with centrosomes reminiscent of previous studies (16, 18), regardless of whether the cells were expressing HA-gg88 or not. HA-gg88 was found in the cytoplasm and localized mainly in the perinuclear region surrounding the endogenous NA14 protein. However, these two proteins did not show actual colocalization. NIH 3T3 cells transiently transfected with an epitope-tagged Flag-NA14 protein showed sporadic localized signals in the cytoplasm as well as the condensed signals at centrin regions, but in cotransfections with HA-gg88, colocalization with Flag-NA14 was not observed (not shown). Glutathione S-transferase (GST) pulldown experiments with 293T cells cotransfected with a GST-tagged gg88 protein and Flag-NA14 also did not show evidence of direct binding between these two proteins (not shown). Thus, despite the two-hybrid results, gg88 does not appear to directly bind NA14 in mammalian cells.

Because the major Sjogren’s syndrome autoantigens are associated with immune responses and viral replication, we also tested
whether glyco-gag might show any interactions (direct or indirect) with other Sjogren’s syndrome autoantigens (SSA [also known as Ro] and SSB [also known as La, or the lupus antigen]). Ro is a member of the tripartite motif (TRIM) protein family; this protein is an interferon-inducible E3 ligase that ubiquitinates interferon regulatory factor 3 (IRF-3) and IRF-8 (19–21). La is a predominantly nuclear RNA binding protein that binds certain RNA polymerase III (Pol III) transcripts, facilitating their processing and trafficking (22, 23); it also facilitates replication of some viruses (24–30). Two-color immunofluorescence microscopy for Ro and HA-gg88 was carried out with 293T cells. For these experiments, it was necessary to transfect cells with a Ro expression plasmid since endogenous Ro protein could not be detected by the anti-Ro antibody. 293T cells were cotransfected with the Ro expression plasmid and either pcDNA3.1 or HA-gg88. Ro protein was found in both the cytoplasm and the nucleus (more in the cytoplasm) (data not shown), and the distribution pattern was consistent with previously published studies (31, 32). However, coexpression of HA-gg88 did not change the cellular distribution of Ro, and colocalization of HA-gg88 and Ro was not observed (data not shown).

In contrast, expression of HA-gg88 affected the intracellular localization of La (Fig. 4). In 293T or NIH 3T3 cultures transfected with HA-gg88 (Fig. 4, second row and bottom row), La showed nuclear fluorescence in the cells that did not express HA-gg88, while the cells that were positive for HA-gg88 showed substantial cytoplasmic fluorescence for La. One possible explanation for the results is that transfection of any expression plasmid could lead to realocalization of La in those cells that took up the DNA. However, in cells transfected with the JSRV, jaagsiekte sheep retrovirus, Env
expression vector, with ΔGP-HA (Fig. 4, top and third rows), or with the backbone pcDNA3 expression plasmids (not shown), La localization was largely nuclear. When the results were quantified, 43.5% of 293T cells from HA-gg88-transfected cultures showed cytoplasmic La staining, versus 18.6% showing cytoplasmic staining, after ΔGP-HA transfection. Likewise, NIH 3T3 cultures transfected with HA-gg88 showed cytoplasmic staining in 27.5% of cells, compared to 6.2% for ΔGP-HA-transfected cells. Taken together, these results indicated that HA-gg88 relocalizes La to the cytoplasm, which suggested that cytoplasmic La might play a role in the mechanism of glyco-gag action.

Overexpression of La phenocopies glycosylated gag. If M-MuLV glyco-gag facilitates virus release via La protein, one mechanism could be that when La is directed to the cytoplasm by glyco-gag, La plays a positive role in facilitating virus release through lipid rafts. We reasoned that overexpression of La might result in enhanced cytoplasmic levels of the protein, which might facilitate virus release in the absence of glyco-gag expression. As shown in Fig. 5A, cotransfection of 293T cells with expression plasmids for mouse or human La along with AKAQ188 resulted in significantly enhanced virus release, with human La more effective than mouse La (ca. 3-fold and 6-fold increases, respectively). Overexpression of mouse La also enhanced the appearance of Pr65gag in cellular DRMs (Fig. 5B and C), and virus released from cells overexpressing mouse La showed the characteristic shift to lighter buoyant density, indicative of higher cholesterol content. Thus, in the absence of glyco-gag, overexpression of La appears to result in the same enhancement of virus release through lipid rafts as that seen when glyco-gag is expressed.

Knockdown of La antagonizes the effects of HA-gg88. While the effects of overexpressing La were consistent with the idea that glyco-gag functions through La, we sought additional support for this model. We tested whether knockdown of human La would reduce HA-gg88-enhanced virus release from transfected 293T cells. As shown...
in Fig. 6, 293T cells were transiently transfected with AKAQ188, with or without HA-gg88, as well as two small interfering RNAs (siRNAs) against human La. The siRNAs were effective in reducing the amount of La present in the cells (Fig. 6, third panel from the top). As shown in the second panel, in the absence of the siRNAs, HA-gg88 substantially enhanced virus release; both siRNAs significantly reduced this enhancement. On the other hand, the lower levels of virus released in the absence of HA-gg88 were not affected by knockdown of La. Therefore, the effects of La knockdown were specific to glyco-gag-enhanced virus release. As shown in the top panel of Fig. 6, neither HA-gg88 nor anti-La siRNAs affected the amount of intracellular Pr65\(^{gag}\).

**Overexpression of La results in interferon-sensitive virus release.** In our previous study, one of the first indications that gPr80\(^{gag}\) directs M-MuLV release through lipid rafts was that wild-type M-MuLV release was sensitive to mouse IFN-α (IFN-α) but that gPr80\(^{gag}\)-negative-virus release was largely resistant to it (14). Therefore, we tested whether the enhanced virus release resulting from overexpression of La is sensitive to IFN-α. As shown in Fig. 7, 293T cells were transiently transfected with AKAQ188 along with either HA-gg88 or the mouse La expression plasmid. The transfected cells were treated with different doses of IFN-α for 24 h, and viruses were gathered after 8 h of further incubation. As shown in the legend to Fig. 2.
expected, the enhanced virus release resulting from cotransfection with HA-gg88 was inhibited by IFN-α treatment (Fig. 7, middle panels), while the smaller amounts of virus released from the control cells (left panels) were not affected by IFN-α. (Longer phosphorimager exposures were required for the left panels due to the lower levels of virus released.) As shown in the right panels, the enhanced virus release resulting from overexpression of mouse La was also inhibited by IFN-α treatment. Thus, the enhanced virus release mediated by La overexpression is IFN sensitive.

HA-gg88 and La overexpression both enhance release of HIV. We previously showed that gPr80 \text{gag} can also enhance release of another retrovirus, HIV-1, from transfected 293T cells (14). As shown in Fig. 8A, HA-gg88 also enhanced release of HIV-1 particles from 293T cells transiently transfected with an HIV-1 gag-pol expression vector, with HA-gg88 showing slightly higher efficiency than p8065-2 (ca. 3-fold and 4.5-fold increases, respectively). Overexpression of either mouse or human La also enhanced HIV-1 particle release (Fig. 8B), with human La again showing a stronger effect than mouse La in 293T cells (ca. 3.5-fold and 7-fold increases, respectively). Thus, the amino-terminal unique region of gPr80 \text{gag} is also sufficient for enhancement of HIV-1 particle release, and glyco-gag enhancement of HIV-1 release is likely mediated through La.

DISCUSSION

In this report, we have obtained insights into the mechanism of action for MuLV-glycosylated Gag protein. We found that the N-terminal unique 88 amino acids of gPr80 \text{gag} are necessary and sufficient for the ability of gPr80 \text{gag} to target release through lipid rafts, as evidenced by the enhanced association of the Gag polyprotein precursor Pr65 \text{gag} with detergent-resistant membranes, the lower buoyant density of released virions (reflective of a higher cholesterol content), and the sensitivity of virus release to interferon. A search for cellular proteins that interact with glyco-gag led indirectly to the cellular La protein, and HA-gg88 was found to relocalize La into the cytoplasm from the nucleus. Overexpression of La in transfected 293T cells phenocopied glyco-gag in all measures tested. Moreover, knockdown of La with siRNAs abrogated the ability of HA-gg88 to enhance MuLV virus particle release. These results strongly support a role for La in the mechanism of glyco-gag action, with La being a downstream effector of glyco-gag.
La protein is a highly abundant cellular protein, predominantly localized in the nucleus. The best known function of this protein is to bind the 3' ends of RNA transcripts containing U residues, notably tRNA precursors and other RNA Pol III transcripts (22, 23). This binding protects pre-tRNAs from premature 3' end processing, allowing for proper maturation. Substantial structural and functional studies have been conducted on La (22, 23). Other functions also have been reported for La. It binds to the genomic RNAs for several cytoplasmic RNA viruses, including poliovirus (33), coxsackievirus B3 (28) (picornaviruses), and hepatitis C virus (a flavivirus) (25), and the binding is important for the function of the viral internal ribosome entry sites (IRES). The latter observations make several points. First, La protein can function in the cytoplasm as well as in the nucleus. Second, it seems likely that La may interact with other cellular proteins in carrying out its various functions. Third, other viruses have also employed La protein to carry out key parts of their replication cycles. Thus, while at first surprising, the involvement of La in glyco-gag's enhancement of MuLV release is reasonable.

While these studies have established a role for La in glyco-gag function, the mechanism of action remains to be determined. We have not detected direct interaction between HA-gg88 and La protein in pulldown assays from transfected cells (T. Nitta and R. Tam, unpublished), which might suggest that glyco-gag and La are interacting indirectly through another cellular protein(s) or particle. This notion is also consistent with the findings that neither gPr80⁰⁰⁰⁰ nor La is found in DRMs from transfected cells, even though the DRMs from such cells contain enhanced levels of Pr65⁰⁰⁰⁰ when either glyco-gag or La is expressed along with a gag-pol expression vector. Thus, we hypothesize that glyco-gag and La facilitate release through lipid rafts by interacting with other proteins or macromolecules that conduct Pr65⁰⁰⁰⁰ to the DRMs/lipid rafts. It will be of substantial interest to identify such proteins or macromolecules. The results also indicated that human La was more efficient than mouse La in enhancing virus release from 293T cells. These cells are of human origin, which might suggest that there is species preference between La proteins and the cellular factors that they interact with for enhancing virus release.

Since La is an RNA binding protein, it will be interesting in the future to test whether the RNA binding activity is important for enhancement of virus release. When retroviruses are assembled, this involves interaction at membranes of the gag and gag-pol polyproteins, the genomic RNA, and the envelope protein. One possibility is that La is interacting with genomic RNA and facilitating incorporation into virus particles. However, the experiments presented here were with transiently transfected 293T cells expressing only Gag and Gag-pol polyproteins, with no Env protein or packageable viral RNA. Others have previously shown that MuLV particles consisting of Gag and Gag-pol proteins can be assembled and released from cells in the absence of viral genomic RNA or Env protein (34, 35). Thus, interaction of La with viral RNA is not involved in the release through lipid rafts shown here.

Previous reports by chemical analyses (4, 36) and computer predictions (not shown) indicated that gPr80⁰⁰⁰⁰ is a type II integral membrane protein with a short amino-terminal cytoplasmic sequence. Here, we made HA-gg88, containing the initial 88 amino acids of gPr80⁰⁰⁰⁰, and this truncated protein showed activity similar to that of the entire gPr80⁰⁰⁰⁰ protein in enhancing M-MuLV release through lipid rafts (Fig. 2), suggesting that neither the glycosylations on the p15⁰⁰⁰⁰⁰⁰ and p30⁰⁰⁰⁰⁰⁰ regions (36) nor any of the extracellular Gag residues are required for viral release enhancement. HA-gg88 distributes in the cytoplasm, mainly at perinuclear regions. Similar to the results reported here, a truncated gPr80⁰⁰⁰⁰ containing the amino-terminal 189 amino acids showed strong localization at perinuclear regions (15). Thus, extracellular Gag residues, including those from position 89 to position 189, do not determine the perinuclear localization.

We previously showed that MuLV gPr80⁰⁰⁰⁰ also enhances release of HIV particles through lipid rafts (14). Pizzato (15) has recently shown that MuLV glyco-gag complements a replication detect for Nef-negative HIV in lymphocytes, although in the latter study the major glyco-gag effect was on Nef-negative viral infectivity as opposed to release. The glyco-gag effects of Nef-negative HIV infectivity were found to be most pronounced for infected lymphocyte cell lines, while less pronounced for other cell lines, such as 293T, and even less for some fibroblast lines. The relationships of glyco-gag effects on viral infectivity from lymphocytes and particle release from 293T cells as described here and from NIH 3T3 cells in our previous study (14) remain to be elucidated. The results shown here also demonstrate that overexpression of La can phenocopy the enhanced virus release for HIV particles. Thus, La appears to be involved in facilitating HIV release through lipid rafts as well. It will interesting to test whether more-distantly related viruses that exit cells through lipid rafts employ a similar mechanism.

Extensive research has been conducted on HIV and MuLV assembly in infected cells. The interaction of Gag L domains (through motifs such as PS/TAP or YPDL) with components of the cellular vesicular trafficking machinery (e.g., ESCRT proteins) results in transport of Gag polyprotein to the plasma membrane or maybe internal sites of assembly (e.g., multivesicular bodies) (37–40). It does not seem likely that L domains of Gag polyprotein and the resulting association with the vesicular trafficking machinery are sufficient for targeting virus release through lipid rafts. In fact, the Gag polyprotein produced in a gPr80⁰⁰⁰⁰ mutant MuLV is identical to that of wild-type virus, with the same L domains (41, 42). Thus, transport of Gag polyprotein (Pr65⁰⁰⁰⁰⁰) to the plasma membrane should not be affected by gPr80⁰⁰⁰⁰ status. Rather, once Gag polyprotein arrives at the plasma membrane MuLV, glyco-gag, working through La, may then conduct it to DRMs and lipid rafts, where efficient virus release occurs.

MATERIALS AND METHODS

Cells. Human 293T and mouse NIH 3T3 fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), and 10% fetal bovine serum (293T cells) or 10% calf serum (NIH 3T3 cells).

DNA constructs. The plasmid p8065-2, expressing MuLV gPr80⁰⁰⁰⁰, was described previously (Fig. 1) (12). To make the plasmid HA-gg88, which expresses an HA epitope tag and the 88 amino acids at the N terminus of gPr80⁰⁰⁰⁰, the tetherin-coding sequence was removed by digesting HaeIII (43) with EcoRI and NotI. The region encoding the N terminus of gPr80⁰⁰⁰⁰ was amplified from p8065-2 by PCR with primers 5'-TCCC GGAAATTCTTCGAGGGCCACCTAGGAGACGTCCAGGAGC and 5'-TCCCCGGCGCGCCGCTAATTCTTCAGAATACAGAAC. The amplified PCR product was digested with EcoRI and NotI and was ligated with the backbone of the digested HA-tetherin plasmid. For assessment of viral release from cells transiently transfected with retroviral constructs carrying gag and pol, the gPr80⁰⁰⁰⁰-negative M-MuLV Gag-Pol expression vector AKAQ188 (Fig. 1) and the HIV-1-based packaging vector PMV-DR8.74 (http://www.lentiweb.com/) were used. The plasmids pCDNA3 (Invitrogen) and pEGFP-N1 (Clontech) were used as negative controls and also added to...
equalize the total amounts of DNA transfected. cDNAs for human SSNA1/NA14, SSA/RO, and SSBA/La and mouse SSBA/La were obtained from OpenBioSystems. For the bait plasmid in yeast two-hybrid screening, the gP80\textsuperscript{Gag} unique region at the N terminus of gP80\textsuperscript{Gag} was amplified with the primers 5′-GGCGAATTCATGGGAGACGTCCAGCAGGGACCTTG and 5′-GGCGCVGCTCAATTCTCAGACAAATACAGAAACAC. The PCR product was digested with EcoRI and Sall and ligated with similarly digested pEG202-LexA to express gP80 fused to LexA DNA binding protein. The plasmid Δgp-HA, expressing JSRV Env with the HA epitope tag from the cytomegalovirus promoter, was described previously (44).

Antibodies and chemicals. Rabbit polyclonal anti-MuLV p30\textsuperscript{C-A} anti-serum was described previously (45). Mouse monoclonal anti-HIV-1 p24\textsuperscript{A-C} antibody (YDHIVp24) was purchased from MybioSource. For detection of epitope tags, mouse and rabbit anti-HA antibodies (Cell Signaling), anti-HA antibodies conjugated with horseradish peroxidase (HRP; GenScript), and anti-Flag antibodies (Cell Signaling) were used. The antibodies against Ro (D-12) and NA14 were purchased from Santa Cruz Biotechnology and ProteinTech Group. To detect La, mouse monoclonal anti-La antibodies (312B; Santa Cruz Biotechnology) and rabbit polyclonal anti-La antibodies (Abgent) were used. Beta-Tubulin was used for the loading control in Western blots and was detected by rabbit anti-beta-Tubulin (Cell Signaling). For Western blots, we used anti-mouse IgG conjugated with horseradish peroxidase (Thermo Scientific) and anti-rabbit IgG conjugated with horseradish peroxidase (GE Healthcare). For indirect fluorescence microscopy, anti-mouse and anti-rabbit IgGs conjugated with Alexa 488 or 546 (Invitrogen) were used. Mouse alpha A interferon (IFN-αA) was obtained from Calbiochem.

Indirect immunofluorescence microscopy. The 293T and NIH 3T3 cells were plated on glass coverslips 1 day before transfection. The cells were transfected with HA-gg88, a Ro-expressing vector, and pCDNA3 by Lipofectamine 2000 (Invitrogen) or BioT (Bioland) and then were incubated for 36 to 48 h. The cells were fixed with 4% paraformaldehyde for 30 min at room temperature. After the cells were washed and blocked, antibodies were added. The cells were stained with fluorescein isothiocyanate-conjugated secondary antibodies, followed by mounting with Vectashield mounting medium (Vector Laboratories). The images were analyzed with Axiovert200 and LSM510 microscopes (Carl Zeiss).

Yeast two-hybrid screening. To find cellular proteins binding to the gP80\textsuperscript{Gag} unique amino acids at the N terminus of gP80\textsuperscript{Gag}, we conducted the yeast two-hybrid screen using pLexA-gg88 and a mouse liver cDNA library. The bacterial strain X1-Blue (supE44 hsdR17 recA1 endA1) harbouring the yeast two-hybrid screen using pLexA-gg88 and a mouse liver cDNA library. The bacterial strain X1-Blue (supE44 hsdR17 recA1 endA1) harbouring pLeu-LexAop6/Psh18-34 (LexA-op-lacZ reporter) were used. The Saccharomyces cerevisiae strain EGY48 was subsequently cotransformed with pLexA-gg88 and the cDNA library and then plated on medium lacking uracil, histidine, and tryptophan (SD/-U, -H, -W) and then plated on medium lacking uracil, histidine, and tryptophan (SD/-U, -H, -W). After the resulting colonies were harvested and pooled, approximately 5 × 10\textsuperscript{4} colonies were replated in selection medium lacking uracil, histidine, tryptophan, and leucine (SD/-U, -H, -W, -L) and containing 2% galactose to induce the expression of cDNAs. The resulting colonies were streaked on the X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)-containing medium (SD/-U, -H, -W, -L) to induce and eliminate false positives.

Flotation of DRM domains. Flotation of DRMs was performed as previously described (14), with slight modifications. 293T cells transiently transfected with AKAQ188, p8065-2, HA-gg88, and mouse SSA/RO expressing vector, and control vectors (pCDNA3 or pEGFP-N1) were treated on the lysis buffer (0.8 ml/10-cm dish) containing 25 mM Tris-HCl (pH 8.0), 140 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM Na\textsubscript{3}VO\textsubscript{4}, and a protease inhibitor cocktail (Roche) for 20 min at 4°C. The lysates were harvested and then centrifuged at 10,000 × g for 5 min at 4°C. The postnuclear lysate was adjusted to 40% (wt/vol) sucrose, and then a 5 to 30% discontinuous sucrose gradient was layered on the top. Samples were centrifuged at 100,000 × g for 18 to 24 h at 4°C. The fractions were collected from the top of the density gradient. The densities of the fractionated samples were determined by use of a refractometer.

Knockdown of La expression by siRNA. Interference of La expression was conducted by siRNAs. The 293T cells on 6-well plates were transfected with AKAQ188 and HA-gg88 or control vectors by use of CalPhos mammalian transfection kit (Clontech). At 24 hours posttransfection, two different siRNAs against human La (no. 5 and no. 10; Qiagen) were transfected at 10 μM by use of Lipofectamine RNAiMAX (Invitrogen). Two days after siRNA transfection, media were replaced, and then the cells and media were harvested at 8 h of further incubation.

IFN treatment and detection of viruses. Treatment of IFN-α and detection of released viruses and viruses in cells by anti-p30\textsuperscript{C-A} antibodies were described previously (14).

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References

M-MuLV Glycosylated Gag and La


