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Overexpression of the *gag-pol* Precursor from Human Immunodeficiency Virus Type 1 Proviral Genomes Results in Efficient Proteolytic Processing in the Absence of Virion Production

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The expression of the *gag-pol* polyprotein of human immunodeficiency virus type 1 (HIV-1) occurs via ribosomal frameshifting between the *gag* and *pol* genes. Because low levels of the *gag-pol* precursor are naturally produced in HIV-1-infected cells, a limited amount of information is available on the biology of this molecule. To further study this polyprotein, two mutant HIV-1 proviral genomes were created to position the *gag* and *pol* genes in the same translational reading frame. The mutations inserted a single thymidine nucleotide at the site of ribosomal frameshifting (nucleotide 1635), which results in the addition of a phenylalanine residue (frameshift 1 [FS1]), or a single adenine nucleotide, which results in the addition of a leucine residue (frameshift 2 [FS2]). Transfection of the mutant proviral genomes into COS-1 cells resulted in the expression of the $p160^{gag\text{-}pol}$ polyprotein precursor as well as the proteolytically processed *gag* and *pol* gene products. Metabolic labeling of the transfected cells with [^{3}H]myristic acid revealed that the $p160^{gag\text{-}pol}$ and $p17^{gag}$ proteins expressed from the mutant genomes were myristylated. While the supernatants from COS-1 cells transfected with wild-type or mutant proviral genomes contained similar amounts of p24 antigen, the levels of reverse transcriptase were, on the average, 10 times greater in the supernatants from cells transfected with the FS1 and FS2 proviral genomes. The cells transfected with the wild-type proviral genome released infectious viral particles, while the mutant proviral genomes released p24 and reverse transcriptase in the absence of detectable particle formation. The mutant proviral genomes were completely noninfectious as determined by coculture of the transfected COS-1 cells with SupT1 cells. These results demonstrate that the *gag-pol* polyprotein of HIV-1 contains the appropriate signals for proteolytic processing and association with intracytoplasmic membranes in the absence of virion formation.

The genome of human immunodeficiency virus type 1 (HIV-1) contains the same *gag-pol-env* organization characteristic of all retroviruses (3, 21, 25, 28, 32). The expression of the *gag* gene results in the synthesis of a precursor protein, p55, which is subsequently processed by the viral protease to release the mature *gag* proteins p17 (matrix protein), p24 (core antigen), and p15 (structural protein) (11, 20). The translational reading frame of the *pol* gene is in a minus one position from the *gag* gene, and the expression occurs via a ribosomal frameshift to produce a *gag-pol* polyprotein (4, 12, 34). The percentage of ribosomal frameshifting has been estimated to be between 5 and 10%, which results in a *gag* to *gag-pol* polyprotein ratio of approximately 9:1 (4, 12, 34).

The *gag-pol* polyprotein plays a central role in the viral life cycle. Even though relatively low amounts of the *gag-pol* polyprotein are produced in infected cells, the *gag-pol* polyprotein is incorporated into mature virus particles and is essential for infectivity. It is assumed, on the basis of analogy with the HIV-1 *gag* protein (2, 10), that *gag-pol* polyprotein is posttranslationally modified by an amino-terminal myristylation. This feature probably facilitates the selective transport and association of the polyprotein with intracytoplasmic membranes, which is required for virus maturation and release (2, 10, 26, 30).

The *gag-pol* polyprotein contains the viral protease, p10, which subsequently processes this protein to produce mature *gag* and *pol* gene products (reverse transcriptase [p66/51] and integrase [p31]) (13, 15, 19, 22, 35) (Fig. 1A). It is not clear what regulates the HIV-1 processing events during virus assembly and release. From ultrastructure studies on HIV-1, it is evident that proteolytic processing occurs after the release of the assembled virus (8). However, processed *gag* precursors (e.g., p24) can also be found in infected cells, indicating the presence of an active HIV-1 protease. Previous studies on the *gag-pol* polyprotein of Moloney murine leukemia virus (MLV), Rous sarcoma virus (RSV), and spleen necrosis virus (SNV) have yielded different results with respect to the processing requirements of *gag* and *gag-pol* precursors (1, 6, 33). In MLV and RSV, the *gag-pol* polyprotein is not processed by the viral protease into the mature protein products, giving rise to the suggestion that the protease is not active in the absence of the *gag* gene product (6). In contrast, expression of *gag-pol* polyprotein of SNV resulted in high levels of intracellular protease and efficient processing of the polyprotein (33). In all cases, though, no virion assembly or release was observed from the expression of the *gag-pol* polyprotein.

To further investigate the biological features of the HIV-1 *gag-pol* polyprotein, we have constructed mutant proviral genomes in which the *gag* and *pol* genes are positioned in the same translational reading frame. These mutations were constructed at the proposed site of ribosomal frameshifting at nucleotide 1635 (25). Previous studies have suggested that two different amino acids could be incorporated into the *gag-pol* polyprotein as a result of the ribosomal frameshifting (12). To ascertain whether these different amino acid insertions would have any effect on the *gag-pol* polyprotein, we constructed proviral genomes with each mutation. The

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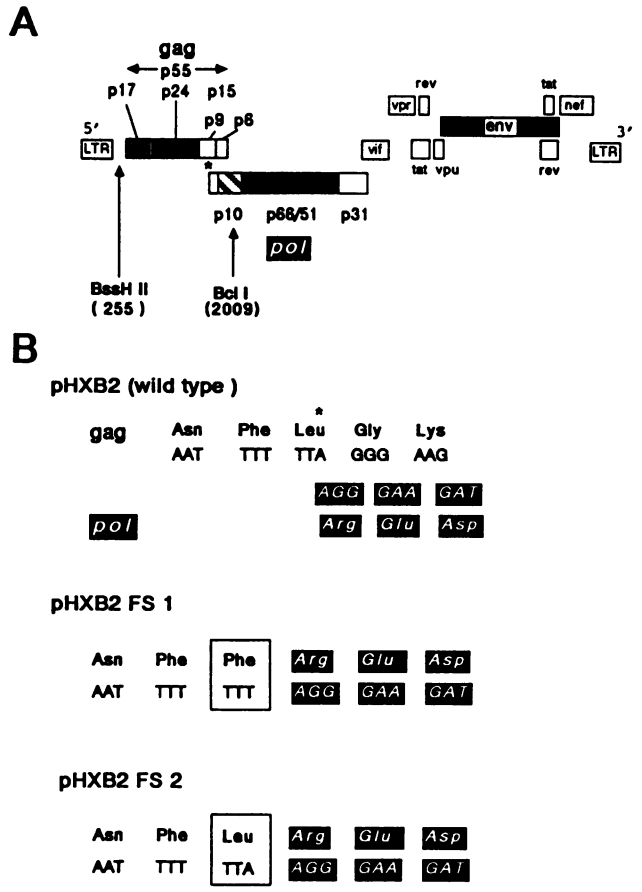

FIG. 1. Construction of HIV-1 proviral genomes containing the frameshift mutations. (A) HIV-1 proviral genome. The HIV-1 proviral genome is depicted with the relevant restriction sites, *Bss*HII at nucleotide 255 and *Bcl*I at 2009. The *gag* gene product is initially synthesized as a precursor protein, p55, and subsequently processed by the viral protease, p10, to yield p17, p24, p9, and p6. A processing intermediate of the *gag* gene, p41, consists of p15 and p24. The asterisk refers to a position at which translational frameshifting occurs to produce the *gag-pol* polyprotein. The positions of the p9 and p6 proteins in the *gag* gene are noted; the *gag-pol* polyprotein contains a truncated p9 and no p6 protein because of the translational frameshifting. (B) Expanded view of the translational frameshifting site. The region at which the *gag* and *pol* genes are positioned into the same translational reading frame is depicted. In the wild-type proviral genome (pHXB2), frameshifting results in the positioning of the amino acids leucine and arginine in the same translational reading frame, thus resulting in the production of the *gag-pol* polyprotein. In pHXB2FS1, an additional thymidine nucleotide was added by oligonucleotide site-directed mutagenesis, resulting in a duplication of the phenylalanine residue (in box). In pHXB2FS2, an additional adenine nucleotide was inserted, which results in a *gag-pol* polyprotein identical to that encoded by the wild-type genome (in box). LTR, long terminal repeat.

first, designated frameshift 1 (FS1), inserted a single thymidine nucleotide at position 1635, which results in the addition of a phenylalanine residue (Fig. 1B). A previous study suggested that, approximately 30% of the time, this amino acid is incorporated into the *gag-pol* polyprotein (12). A second mutation, frameshift 2 (FS2), inserted an adenine residue at nucleotide 1635 (Fig. 1B). This translational fusion results in the addition of a leucine residue, which has been demonstrated to be present 70% of the time in the *gag-pol* protein (12). DNA manipulation and oligonucleotide site-directed mutagenesis was performed by using standard protocols (18, 36). Briefly, the *Eco*RI-*Bal*I DNA fragment, which contains nucleotides 1 to 2201 of the HIV-1 proviral genome, was isolated from the plasmid pHXB2 (wild type) (24), ligated into the *Eco*RI-*Sma*I site of the replicative form of M13mp19, and transformed into *Escherichia coli* DH5αF′. The replicative forms from the resultant phage were screened for the presence of the desired HIV-1 DNA fragments by restriction digests. Single-strand DNA was prepared from the recombinant phage and used as a template for oligonucleotide site-directed mutagenesis (36). The two DNA oligomers were designed complementary to the following nucleotides of the HIV-1 proviral genome: (i) 5′CAG GCT AAT TTT TTT AG3′ (FS1) (1621 to 1636) and (ii) 5′G GCT AAT TTT TTA AGG GAA GAT CTG G3′ (FS2) (1623 to 1647). The nucleotides inserted into the HIV-1 proviral genome are underlined and result in the placement of the *gag* and *pol* genes in the same translational reading frame (Fig. 1B). Following mutagenesis and transformation into *E. coli* DH5αF′, the desired recombinant phages were identified by using plaque hybridization with the mutagenic DNA oligomer phosphorylated with [γ-^{32}P]ATP. Positive plaques were grown and sequenced by using the DNA primer 5′GCA CA CA GCC AG AAA TT 3′ (17-mer), which hybridizes to nucleotides 1530 to 1546, to confirm the desired mutation (29).

Polymerase chain reaction (PCR) (27) was used to amplify the region of DNA flanking the mutation. The following two primers, which are specific for nucleotides in the 5′ long terminal repeat region and for nucleotides 2090 to 2119, respectively, of the HIV-1 proviral genome were used: (i) 5′CAA GGC TAC TTC CCT GAT TAG CAG AAC TAC3′ (30-mer) and (ii) 5′GTG CAA CCA ATC TGA GTC AAC AGA TTT CTT3′ (30-mer). The PCRs were carried out with 100 pmol of each primer (approximately 600 ng) and with 50 ng of the single-strand template, 5 U of *Thermus aquaticus* (*Taq*) polymerase, 0.5 mM dNTPs, and buffer supplied by the manufacturer (Perkin Elmer/Cetus). A 30-cycle repetition of 1 min at 94°C, 3 min at 37°C, and 3 min at 60°C was used for amplification. The PCR-amplified DNA consisted of 2,509 bp with unique restriction enzyme sites *Bss*HII and *Bcl*I flanking the desired mutations.

For these studies, pHXB2 (wild type) was prepared from *E. coli* GM119, a methylase-deficient strain. The PCR-amplified DNA, containing either the FS1 or FS2 mutation, was digested with *Bss*HII and *Bcl*I, ligated with the similarly digested pHXB2, and transformed into *E. coli* HB101. The DNA from the resultant colonies was screened by restriction enzyme digestion, with the final confirmation of the desired mutant (pHXB2FS1 or pHXB2FS2) obtained by direct DNA sequencing of the plasmid DNA by the Sanger dideoxy-chain termination method (29).

To analyze the expression of HIV-1 proteins, pHXB2 (wild type), pHXB2FS1, or pHXB2FS2 was transfected into COS-1 cells, and the cell extracts were analyzed for the presence of HIV-1-specific proteins 48 h later by immunoprecipitation by using an antibody specific for the HIV-1 p24/25 (31) (Fig. 2A). The cells were lysed by the addition of 1.0 ml of RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 150 mM NaCl, 25 mM Tris-HCl [pH 7.34]) followed by the addition of the anti-p24/25 antibody for 12 h. The immunoprecipitates were collected by the addition of protein A-Sepharose for 1 h at room temperature, followed by three washes with RIPA buffer. To release the immunoprecipitates, the beads were

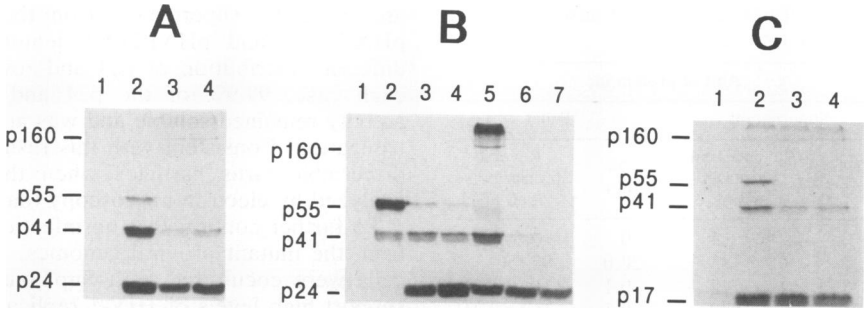

FIG. 2. Analysis of HIV-1 proteins expressed in the COS-1 cells transfected with wild-type and mutant proviral genomes. (A) COS-1 cells were transfected with 5 μg of CsCl-purified pHXB2 (wild type), pHXB2FS1, or pHXB2FS2 by using previously described conditions (17). The transfected cells were metabolically labeled with [^{35}S]methionine (Translabel) (50 μCi/ml), and the intracellular proteins were immunoprecipitated with rabbit anti-p24/25 antibody (31). The relevant viral proteins are noted. Lanes: 1, mock; 2, pHXB2; 3, pHXB2FS1; 4, pHXB2FS2. (B) Pulse-chase analysis of HIV-1 viral protein expression in COS-1 cells transfected with wild-type and mutant proviral genomes. COS-1 cells were transfected with pHXB2 (wild type) or pHXB2FS2, and at 36 h posttransfection the cells were labeled with [^{35}S]methionine (Translabel) for 20 min (pulse), followed by the addition of unlabeled media for 3 or 6 h (chase). The cell extracts were immunoprecipitated with rabbit anti-p24/25 antibody (31) and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Lanes: 1, control; 2, pHXB2 (pulse); 3, pHXB2 3-h chase; 4, pHXB2 6-h chase; 5, pHXB2FS2 (pulse); 6, pHXB2FS2 3-h chase; 7, pHXB2FS2 6-h chase. The relevant viral proteins are noted. (C) Myristylated HIV-1 proteins expressed in COS-1 cells transfected with wild-type and mutant proviral genomes. COS-1 cells were transfected with plasmids containing the wild-type and mutant proviral genomes and metabolically labeled with [^{3}H]myristic acid by using previously described conditions (26). The cell extracts were immunoprecipitated with sera from AIDS patients to detect HIV-1-specific proteins and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Lanes: 1, control; 2, pHXB2 (wild type); 3, pHXB2FS1; 4, pHXB2FS2. The relevant viral proteins are noted.

boiled in gel sample loading buffer (50 mM Tris-HCl [pH 6.8], 5% SDS, 5% β-mercaptoethanol, 0.1% bromphenol blue), and the proteins were analyzed by using SDS-polyacrylamide gel electrophoresis followed by fluorography. The gels were autoradiographed by using Kodak X-Omat AR film with an intensifier at −70°C.

The HIV-1 *gag* proteins immunoprecipitated from the COS-1 cells transfected with pHXB2 (wild type) consisted of p55, p41, and p24. The expression of HIV-1 proteins from COS-1 cells transfected with pHXB2FS1 or pHXB2FS2 was different from that observed for the wild-type genome in several respects. First, as would be expected (Fig. 1A), the *gag* precursor, p55, was absent in these cells, although *gag* proteins were synthesized, as demonstrated by the immunoprecipitation of proteins with molecular masses consistent with those of the p41 and p24 proteins. Second, a protein migrating at a molecular mass consistent with that of the HIV-1 *gag-pol* polyprotein (160 kDa) was immunoprecipitated. Third, no significant differences in the expression of viral proteins from proviral genomes with the FS1 or FS2 mutation were observed. Therefore, the addition of the phenylalanine or leucine residue at the site of ribosomal frameshifting does not appear to affect the expression of the *gag-pol* polyprotein. The levels of *env* gene product (gp160 and gp120), as determined by immunoprecipitation with sera from AIDS patients, were similar in cells transfected with pHXB2 (wild type), pHXB2FS1, and pHXB2FS2 (data not shown). To further analyze *pol* gene products, antibodies were generated to a peptide, NH_2-PISPIETVPVKLKPGM DGPG-COOH, which corresponds to the amino terminus of reverse transcriptase (5, 16). The anti-peptide antibody was used to immunoprecipitate proteins from the COS-1 cells transfected with plasmids containing wild-type and mutant genomes. Proteins of 66 kDa, corresponding to the predicted molecular mass of HIV-1 reverse transcriptase, and 160 kDa (*gag-pol* polyprotein) were evident from extracts of the COS-1 cells transfected with pHXB2FS1 or pHXB2FS2 (data not shown).

To further investigate the kinetics of the proteolytic processing of the *gag-pol* polyproteins, pulse-chase experiments were performed (Fig. 2B). The COS-1 cells were transfected with pHXB2 (wild type) or pHXB2FS2 and metabolically labeled for 20 min (pulse), followed by incubation in complete media for 3 or 6 h (chase). The viral proteins were then immunoprecipitated with anti-p24/p25 antibody. During the pulse period, the *gag* precursor, p55, was evident in the cells transfected with pHXB2 (wild type). Processing to the mature *gag* protein, p24, was apparent in cells transfected with pHXB2 at the 3- and 6-h chase periods. In contrast, in the cells transfected with pHXB2FS2, the unprocessed *gag-pol* polyprotein (p160), an intermediate product (p41), and the mature p24 antigen were all immunoprecipitated during the pulse period. Following the chase period, only proteolytically processed *gag-pol* polyprotein was detected, as evidenced by the immunoprecipitation of the p24 protein.

To further characterize the viral proteins from the mutant proviral genomes, we analyzed expression by using [^{3}H]myristate as the label. Previous studies have established that the *gag* protein of HIV-1 is modified by the amino-terminal addition of myristate to the *gag* precursor, p55 (2, 10). The expression of the myristylated viral proteins in the COS-1 cells transfected with pHXB2 (wild type), pHXB2FS1, and pHXB2FS2 were analyzed by using AIDS patient sera for immunoprecipitation (Fig. 2C). From the cells transfected with pHXB2, we immunoprecipitated the *gag* precursor, p55, as well as the proteolytically processed p41 and p17 products. In the cells transfected with the plasmids containing the proviral genomes with the frameshift mutations, immunoprecipitation of the proteolytically processed p17 protein was evident. Only small amounts of myristylated *gag-pol* polyprotein were detected, possibly because of the rapid proteolytic processing of the *gag-pol* polyprotein.

To determine whether *gag-pol* polyproteins were released from the transfected cells, the supernatants were analyzed for p24 antigen and reverse transcriptase during the 3-day period posttransfection. The levels of p24 detected in the supernatants from the cells transfected with pHXB2 (wild

TABLE 1. Fractionation of p24 and reverse transcriptase released from transfected cells[a]

DNA	Amt of protein in:			
	Supernatant		Pellet	
	p24[b] (ng)	Reverse transcriptase[c] (cpm)	p24 (ng)	Reverse transcriptase (cpm) (%)[d]
None	0	5	0	0.5
pHXB2 (wild type)	132	185	20.0	92 (50)
pXHB2FS1	475	16,140	0.1	16 (<0.1)
pXHB2FS2	295	18,620	0.1	16 (<0.1)

[a] COS-1 cells were transfected with the designated plasmids and the culture supernatants were fractionated as described in the text. Supernatant and pellet refer to samples after ultracentrifugation. The values are representative of three experiments.

[b] The amounts of p24 antigen were measured by immunoassay and are given as total nanograms of p24 per sample.

[c] The reverse transcriptase activity was measured as described in the text. The values presented are for the total sample (counts per minute of [^{35}S]TTP incorporated divided by 1,000).

[d] The percentages are obtained by dividing the levels of reverse transcriptase in the pellet by the levels in the supernatant and multiplying by 100.

type), pHXB2FS1, and pHXB2FS2 were similar over the 3-day period. In contrast, a significant difference between the wild-type and frameshift mutant proviral genomes with respect to reverse transcriptase activity was noted. Over the 3-day period, the levels detected in the supernatant from the cells transfected with pHXB2FS1 and pHXB2FS2 were up to 10 times higher than with pHXB2 (data not shown). The release of p24 antigen and reverse transcriptase from the COS-1 cells transfected with the mutant HIV-1 proviral genomes suggested that viruslike structures might be released from these cells. To test for this possibility, the supernatants from transfected COS-1 cells were fractionated by using ultracentrifugation to pellet any viruslike structures. The media was clarified by centrifugation at 10,000 × *g* for 5 min, followed by filtration through a 0.2-μm-pore-size Acrodisc (Gelman Sciences). The supernatant was layered on 2 ml of a 15% sucrose cushion and ultracentrifuged in an SW 41 rotor at 120,000 × *g* for 2 h. The levels of p24 antigen and reverse transcriptase were quantitated in the supernatant and pellet after ultracentrifugation for each sample (Table 1). For the analysis of reverse transcriptase activity, 25 μl of cell supernatant was placed in a well of a 96-well plate and 75 μl of a reaction cocktail was added which contained the following: 50 mM Tris-HCl (pH 8.0), 5 mM dithiothreitol, 5 mM $MgCl_2$, 150 mM KCl, 0.1% Triton X-100, 0.5 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1.25 μg of poly(A)-oligo(dT), and 5 μCi of [α-^{35}S]TTP. The reaction was allowed to proceed for 90 min before the mixture was directly spotted onto NA-45 paper. The samples were washed once with 0.2 M $NaPO_4$ (pH 7.5) and air dried, and the radioactivity was quantitated with an AMBIS densitometer. The p24 antigen capture assays were performed by using a kit provided by Abbott Laboratories according to the manufacturer's instructions. An assay with known amounts of p24 was used to generate a standard curve, and the samples were diluted until readings were obtained in the linear region. Both p24 antigen and reverse transcriptase activity were recovered in the pellet fractions from the cells transfected with pHXB2 (wild type). Furthermore, the analysis of the pellet fractions by electron microscopy revealed the presence of virions consistent with HIV-1 (data not shown). The analysis of the supernatants from the cells transfected with pHXB2FS1 and pHXB2FS2 demonstrated a completely different distribution of p24 and reverse transcriptase. In each case, 99.9% of the p24 and reverse transcriptase activity remained soluble and was not pelleted by ultracentrifugation. Consistent with this result was the lack of any discernible virus particles when the pellet fraction was analyzed by electron microscopy (data not shown).

To further confirm that no infectious viruses were made from the mutant proviral genomes, the transfected COS-1 cells were cocultured with SupT1 cells. The SupT1 cells support high levels of HIV-1 replication, and this assay is used to measure the spread of infectious virus. After 2 days of coculture, the SupT1 cells were isolated and the production of virus from these cultures was analyzed over a 2-week period by p24 antigen production (Fig. 3A). As expected, an increase in p24 antigen production was evident from the cultures derived from the cells transfected with pHXB2 (wild type), indicating the presence of infectious HIV-1. No increase was observed in SupT1 cultures cocultivated with the COS-1 cells previously transfected with pHXB2FS1 or pHXB2FS2. To confirm that no infectious virus was present, we metabolically labeled the SupT1 cultures and analyzed the extracts by immunoprecipitation with sera from AIDS patients (Fig. 3B). Consistent with the previous data, we detected HIV-1-specific proteins from SupT1 cultures cocultured with the COS-1 cells transfected with pHXB2 (wild type) but not with pHXB2FS1 or pHXB2FS2. Thus, under our experimental conditions, we conclude that the mutations in the proviral genome to position the *gag* and *pol* genes in the same translational reading frame result in the inability of these genomes to produce virions.

In conclusion, we have described the construction and characterization of HIV-1 proviral genomes which contain the *gag* and *pol* genes in the same translational reading frame. The transfection into COS-1 cells of plasmids containing these mutant proviral genomes resulted in the expression and proteolytic processing of the *gag-pol* polyprotein. The release of *gag* and *pol* gene products in the forms of p24 and p66, respectively, was detected in the supernatants of cells transfected with HIV-1 proviral genomes containing the frameshift mutations; however, no infectious viruses or viruslike particles were detected.

Our results are significant because, for the first time, the *gag-pol* polyprotein of HIV-1 has been exclusively expressed from the context of the entire HIV-1 proviral genome. Previous reports have described the proteolytic processing of a truncated *gag-pol* polyprotein by using an in vitro translation system (14, 23). We wanted to determine whether the overexpression of the HIV-1 *gag-pol* in the context of the entire proviral genome would provide insights into the regulation of the expression and proteolytic processing of this polyprotein. Previous reports have described proviral genomes of RSV (1), MLV (6), and SNV (33) with *gag-pol* fusions. The proteolytic processing of the expressed *gag-pol* was not detected for the RSV and MLV polyproteins. In the case of MLV, it was suggested that the cleavage of the *gag-pol* polyprotein was dependent upon virus assembly or contact with free *gag* protein (6). In contrast, expression of the SNV *gag-pol* polyprotein resulted in high levels of intracellular proteolytic activity and efficient processing. The results of our studies clearly demonstrate that expression of the *gag-pol* polyprotein of HIV-1 also results in the activation of the protease, as evidenced by the presence of processed *gag* and *pol* gene products. The initial step in the activation of the HIV-1 protease is postulated to be the

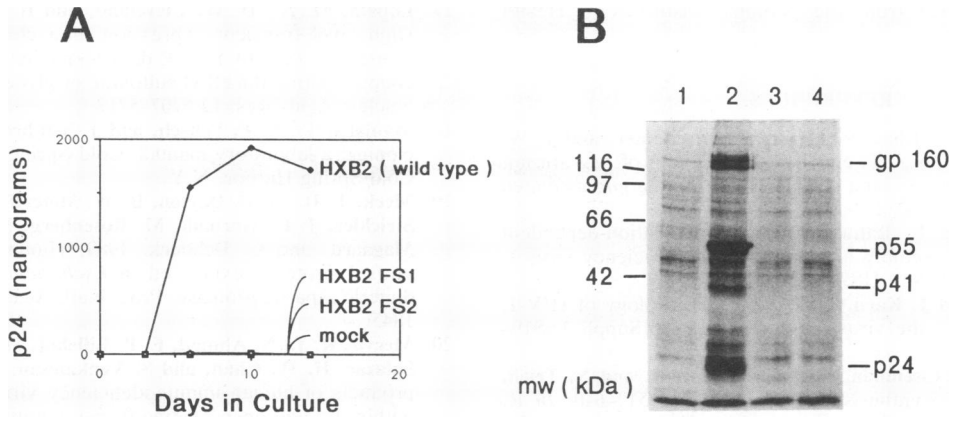

FIG. 3. Infectivity of wild-type and mutant proviral genomes. (A) The wild-type plasmid (pHXB2) and mutant plasmids (pHXB2FS1 and pHXB2FS2) were transfected into COS-1 cells on day 0. The CD4+ human lymphoid cells (SupT1) were added on day 2 and removed from the adherent cells on day 4 (designated day 0 in the figure). The SupT1 cultures were maintained in culture for an additional 2 weeks and assayed for p24 antigen by enzyme immunoassay. The values represent total nanograms of p24 antigen per culture. The individual cultures are as marked in the figure. (B) The SupT1 cells were metabolically labeled with [^{35}S]methionine (Translabel) at day 14, and viral proteins were immunoprecipitated with AIDS patient sera and then analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Lanes: 1, control; 2, pHXB2 (wild type); 3, pHXB2FS1; 4, pHXB2FS2. The molecular mass markers and relevant viral proteins are marked.

formation of a molecular dimer between the *gag-pol* polyproteins (15, 22, 35). In the wild-type virus-infected cell, the high ratio of *gag* to *gag-pol* might act as a buffer to prevent the necessary protein-protein interactions required for activation of the protease. As suggested by others, the concentration of the *gag-pol* polyprotein during virus assembly might then facilitate the necessary protein-protein interactions required to activate the protease (22). In our system, the high-level expression of *gag-pol* in the absence of the *gag* protein probably results in the efficient dimerization and subsequent intracellular activation of the HIV-1 viral protease.

It is interesting that, even with the increased proteolytic processing, both p24 and reverse transcriptase are detected in the medium of the cells transfected with the plasmids pHXB2FS1 and pHXB2FS2 (Table 1). At present, it is not clear how the p24 and reverse transcriptase are released from transfected cells containing the HIV-1 proviral genomes with the frameshift mutations. It is possible that the overexpression of the viral protease in some way disrupts the cellular integrity, leading to the release of viral proteins. To further explore this question, we are preparing *gag-pol* frameshift mutants with secondary mutations in the protease gene to determine whether the proteolytic processing is a requirement for the release of the *gag-pol* polyprotein (22a).

We did not detect any virion formation or assembly of viruslike structures in the cells transfected with plasmids containing the *gag-pol* frameshift mutants. Similar results were obtained with the expression of *gag-pol* polyprotein from other retroviruses (1, 6, 33). The expression of the p55^{gag} precursor protein of HIV-1 in the absence of the viral protease, though, results in the assembly and release of viruslike particles (7). The *gag-pol* polyprotein, like the p55^{gag} protein, contains a myristylated NH_2 terminus which might promote intracytoplasmic transport and association with membranes necessary for the assembly and release of virus. In support of this notion, previous studies have demonstrated that HIV-1 proviral genomes with mutations that abolish the myristylation of the capsid proteins do not contain viral structures within the cell or produce infectious virus (2, 10). One significant difference between the *gag* and *gag-pol* polyproteins is the truncation of the p15^{gag} gene products, which results in the deletion of the p6^{gag} gene from the *gag-pol* polyprotein (Fig. 1). Recent studies have demonstrated that deletion of the p6 region of *gag* prevents the assembly and subsequent generation of infectious viruses (7, 9). These results are consistent with the inability of the *gag-pol* polyprotein to assemble and produce infectious virions. An alternative explanation for these results is the possibility that the increased proteolytic processing, resulting from the overexpression of the *gag-pol* polyprotein, prevents necessary protein interactions required for the release of viruslike particles (33). As evidenced by the pulse-chase analysis, the expression of the *gag-pol* polyprotein results in sufficient levels of intracellular protease to completely process the precursor protein. Experiments are under way to resolve this question.

Finally, a previous study with SNV demonstrated the rescue of proviruses with *gag-pol* polyprotein by transfection into cell lines producing the *gag* protein (33). It might be possible, then, to genetically complement the HIV-1 proviral genomes containing the frameshift mutations by coexpression with p55^{gag} if the proper ratio of *gag* to *gag-pol* polyproteins is found. These experiments should provide important insights into the mechanism of virus assembly and the nature of *gag* and *gag-pol* polyprotein interactions required for the production of infectious HIV-1 virus.

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