Short Communication

Effects of human immunodeficiency virus type 1 transframe protein p6* mutations on viral protease-mediated Gag processing

Hsu-Chen Chiu,[1,2] Fu-Der Wang,[1,4] Yi-Ming Arthur Chen[2,3] and Chin-Tien Wang[1,5]

Correspondence
Chin-Tien Wang
chintien@ym.edu.tw

[1,2,3]Institute of Clinical Medicine[1], Institute of Public Health[2] and AIDS Prevention and Research Center[3], National Yang-Ming University, Taipei, Taiwan

[4,5]Department of Internal Medicine, Division of Infectious Disease[4] and Department of Medical Research and Education[5], Taipei Veterans General Hospital, 201 Section 2 Shih-Pai Road, Taipei 11217, Taiwan

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The proteolytic processing of human immunodeficiency virus (HIV) particles mediated by the viral *pol*-encoded protease (PR) is essential for viral infectivity. The *pol* coding sequence partially overlaps with the *gag* coding sequence and is translated as a Gag–Pol polyprotein precursor. Within Gag–Pol, the C-terminal p6^{gag} domain is replaced by a transframe peptide referred to as p6*, which separates the Gag nucleocapsid domain from PR. Several previous *in vitro* studies have ascribed a PR-suppression regulatory function to p6*. Here, it was demonstrated that an HIV-1 Gag–Pol lacking p6* is efficiently incorporated into virions when coexpressed with HIV-1 Gag precursor. However, the released virions are not processed appropriately and show a greatly reduced viral infectivity. This suggests that the p6* is indispensable during the process of PR-mediated virus particle maturation.

The human immunodeficiency virus (HIV) *gag* gene encodes the viral structural protein Gag, which is translated initially as a precursor Pr55^{gag} (Wills & Craven, 1991; Hunter, 1994). Pr55^{gag} is transported to the plasma membrane where several thousand Pr55^{gag} molecules assemble into virus particles that bud out from the cell membrane. During or after virus budding, Pr55^{gag} is cleaved by the viral *pol*-encoded protease (PR) into four major proteins: matrix (MA; p17), capsid (CA; p24), nucleocapsid (NC; p7) and the C-terminal p6 (Erickson-Viitanen *et al.*, 1989; Freed, 1998; Henderson *et al.*, 1992; Kaplan *et al.*, 1994; Leis *et al.*, 1988; Mervis *et al.*, 1988; Swanstrom & Wills, 1997). The *gag* and *pol* coding sequences partially overlap. A −1 ribosomal frameshift event occurs at a frequency of about 5 % during *gag* translation, resulting in Pol being translated as a Gag–Pol fusion protein (Jacks *et al.*, 1988). Within the Gag–Pol, the p6 domain is truncated and replaced by a transframe domain referred to as p6* or p6^{pol} (Partin *et al.*, 1990). Proteolytic processing of Gag–Pol by the PR yields reverse transcriptase (RT), RNase H and integrase (IN) in addition to the Gag cleavage products. The PR-mediated virus maturation process is not required for virus assembly and budding, but is essential for viral infectivity (Gottlinger *et al.*, 1989; Kohl *et al.*, 1988; Peng *et al.*, 1989).

How the PR is activated to mediate Gag particle maturation is still not completely understood. It is thought that dimerization of Gag–Pol is a prerequisite for PR activation (Navia & McKeever, 1990). The activated PR then cleaves itself out from Gag–Pol and functions as a homodimer to process Gag and Gag–Pol. An *in vitro* study has suggested that sequences flanking the PR domain may contribute to the process of PR activation (Louis *et al.*, 1999; Pettit *et al.*, 2003; Wondrak & Louis, 1996). Several other studies have also demonstrated that HIV-1 Gag–Pol molecules lacking the *gag* coding sequence or truncated in the RT domain are significantly defective in autoprocessing or *in trans* processing of Gag particles (Engelman *et al.*, 1995; Liao & Wang, 2004; Quillent *et al.*, 1996; Zybarth & Carter, 1995). Thus, sequences downstream or upstream of PR may potentially affect the PR activity, presumably via facilitating formation of a proper Gag–Pol dimer, which is thought to be required for activating the embedded PR.

The p6* domain is located directly N-terminal to the PR and separates NC from Pol. One previous study demonstrated that the removal of the p6* improves the proteolytic processing of Gag–Pol *in vitro*, suggesting an inhibitory effect of p6* on PR activation (Partin *et al.*, 1991). In support of this notion, mutations preventing cleavage of p6* from the PR have been shown to markedly impair PR-mediated Gag processing (Chen *et al.*, 2004; Tessmer & Krausslich, 1998; Zybarth *et al.*, 1994). Additionally, synthetic p6* peptides have been reported to be able to suppress PR activity *in vitro* (Louis *et al.*, 1998; Paulus *et al.*, 1999). These results strongly suggests that the presence of p6* may interfere with the

functioning of PR, and that p6* does not appear to make a positive contribution to the process of PR activation.

To investigate the role of the HIV-1 p6* domain in PR-mediated virus particle processing, deletion mutations in p6* were engineered by the two-megaprimer PCR extension method (Sambrook & Russell, 2001) using HIVgpt, which carries the SV40 *ori* and *gpt* (xanthine-guanine phosphoribosyltransferase) genes in the *env* region (Page *et al.*, 1990), or a $Pr160^{gag-pol}$-expression plasmid, GPfs (Chiu *et al.*, 2002) as template. Primer sequences and detailed procedures for creating the mutations are available on request. Wild-type GPfs or each of the GPfs mutants (Fig. 1) was coexpressed with a $Pr55^{gag}$ expression plasmid, pGAG, in 293T cells. At 48 h post-transfection, culture medium from transfected 293T cells was filtered through 0·45 μm pore-size filters, followed by centrifugation through 2 ml 20 % sucrose in TSE [10 mM Tris/HCl (pH 7·5), 100 mM NaCl, 1 mM EDTA] plus 0·1 mM PMSF at 4 °C for 40 min at 274 000 ***g*** (SW41 rotor at 40 000 r.p.m.). The cells were rinsed with ice-cold PBS, pelleted, and were resuspended in 250 μl immunoprecipitation buffer plus 0·1 mM PMSF, and then subjected to microcentrifugation at 4 °C for 15 min at 13 700 ***g*** to remove cell debris. Supernatant and cell samples were prepared and subjected to Western immunoblot analysis as described previously (Wang *et al.*, 1998). When cotransfected with pGAG plasmid at a DNA ratio of 1 : 10, both D2fs and D3fs exhibited a virus particle processing pattern similar to that of wild-type (wt) GPfs, with mature $p24^{gag}$ representing the major species of virus-associated Gag products (Fig. 2a, lanes 15 and 17). In contrast, D1fs produced a total amount of unprocessed and incompletely processed Gag slightly higher than that of wt (Fig. 2a, lanes 13 vs 11 and Fig. 2c, lanes 15 vs 13), suggesting that PR-mediated particle processing has been affected when most of the p6* codons were removed. However, virus particle production was markedly reduced or abolished when the amount of plasmid DNA of the wt GPfs or p6* deletion mutants used for cotransfection was equivalent to that of pGAG (Fig. 2a, lanes 10, 12, 14 and 16). This indicates that when both wt and mutant GPfs are overexpressed they can efficiently suppress virus budding, presumably due to premature cleavage of Gag precursors by Gag–Pol (Arrigo & Huffman, 1995; Burstein *et al.*, 1991; Krausslich, 1991; Park & Morrow, 1991; Rose *et al.*, 1995; Wang *et al.*, 2000; Xiang *et al.*, 1997).

The results shown above do not support the proposal that removal of the p6* can improve PR activity. On the contrary, it seems that the PR-mediated particle processing was somewhat impaired by the p6*-deleted mutation under a near normal physiological condition. Since previous studies have suggested that the upstream *gag* sequence may affect the PR-mediated Gag processing (Chiu *et al.*, 2002; Zybarth *et al.*, 1995) it was of interest to test the effects of a p6*-deleted mutation on PR-mediated virus processing in a

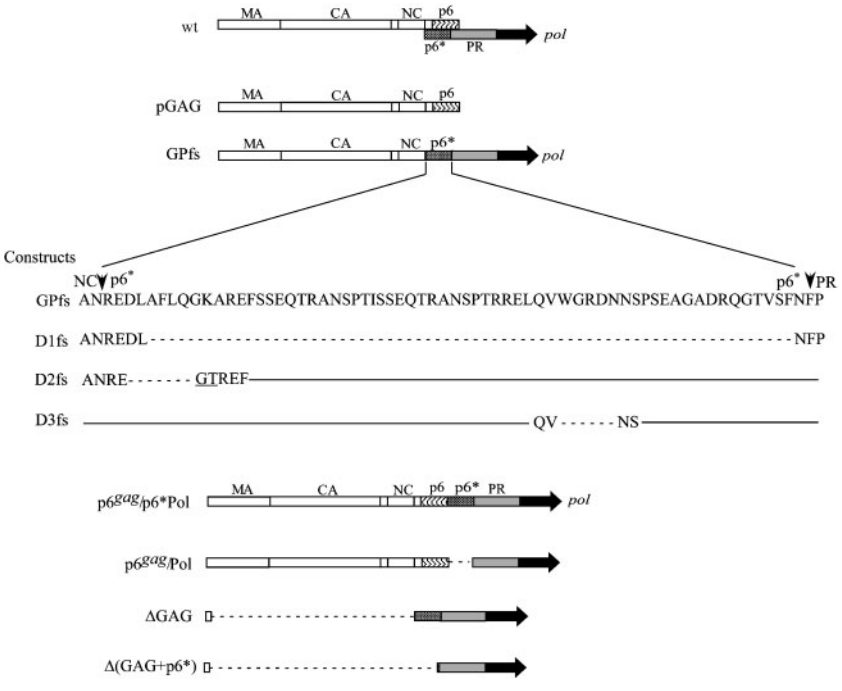

Fig. 1. Schematic representatives of HIV-1 Gag and Gag–Pol expression vectors. All of the plasmids are in the context of an HIV replication-defective expression vector, HIVgpt. The wild-type (wt) construct can express both $Pr55^{gag}$ and $Pr160^{gag-pol}$. The pGAG and GPfs can only express $Pr55^{gag}$ and $Pr160^{gag-pol}$, respectively. HIV Gag protein domains MA (matrix), CA (capsid), NC (nucleocapsid) and p6; and the *pol*-encoded transframe domains p6* (crosshatched rectangle) and PR (protease; shaded rectangle) are indicated. Arrowheads indicate the boundary of p6* that separates the PR from NC. Residues flanking and comprising p6* are shown, with dashed lines indicating deleted sequences. Substitution residues or altered amino acids in the deleted region are underlined. DNA sequences of the junctions in mutated regions are as follows, where the HIV positions of the first and then last nucleotides are indicated (deletions are not shown): D1, nt 2093-GAAGATCTGA-ACTTCCCT-2254; D2, nt 2090-AGGG-AAGGGATCAGGGAA-2128; D3, nt 2174-CTTCAGGTTAACTCCCCC-2206; ΔGAG, nt 831-CGATGGATCCAGGCTAAT-2084; Δ(GAG + p6*), nt 831-CGATGGATCCAC-TTCCCT-2254.

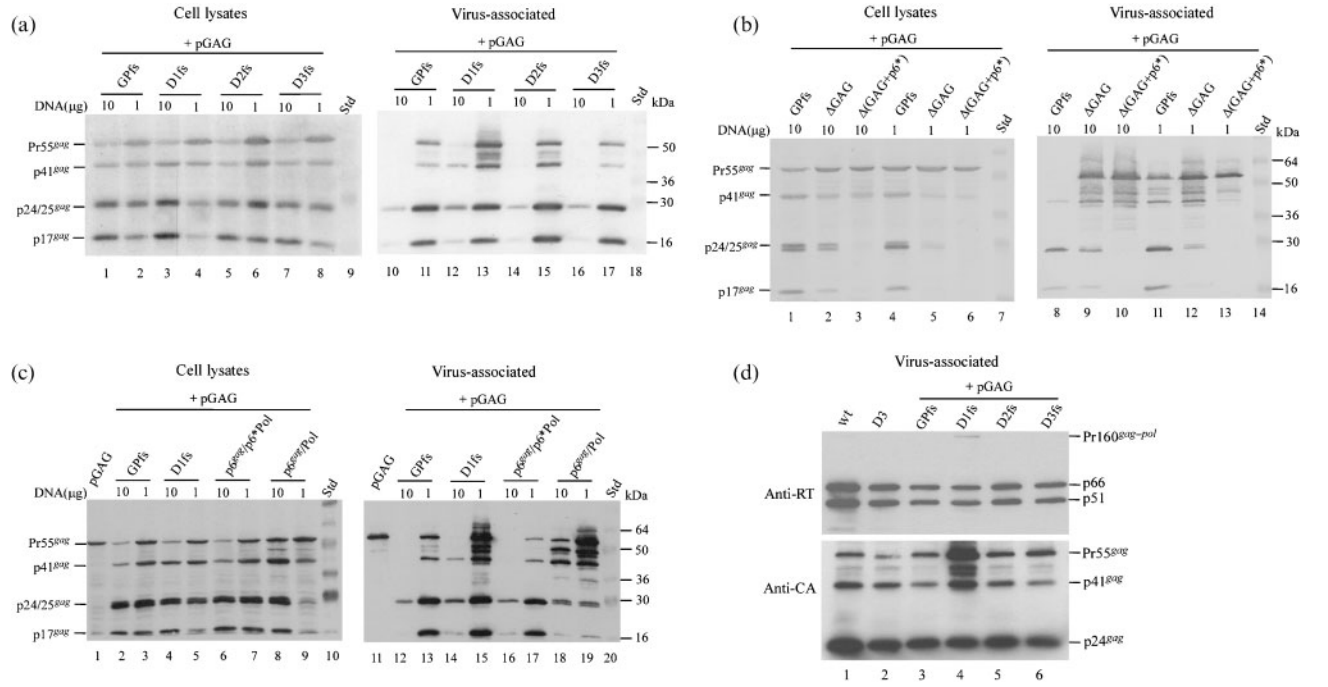

Fig. 2. Assembly and processing of HIV Gag proteins and virus-associated RT proteins. (a–c) 293T cells were cotransfected with 10 μg pGAG and 1 or 10 μg of the indicated constructs, or (d) transfected with the wild-type (wt) HIVgpt or mutant HIVgpt that contains the p6* mutation D3, or cotransfected with 10 μg pGAG plus 1 μg of the indicated plasmid, with the addition of 9 μg pBlueScript SK to keep the total DNA amount at 20 μg. Cell samples corresponding to 4 % of the total cell lysates, and supernatant samples corresponding to 50 % of the total recovered viral pellets were fractionated by 10 % SDS-PAGE. (a–c) HIV Gag proteins were probed with anti-p24^{gag} and anti-p17^{gag} monoclonal antibodies. (d) HIV Gag and Pol proteins were probed with mouse anti-CA and mouse anti-HIV-1-RT monoclonal antibodies, respectively. Positions of molecular size markers (Std) and HIV Gag proteins Pr55, p41, p24/25 and p17, and RT-associated Pol proteins Pr160$^{gag–pol}$ and p66/51 are indicated.

Gag–Pol context containing a whole intact (p6^{gag}/p6*Pol, p6^{gag}/Pol) or deleted Gag domain [ΔGAG, Δ(GAG + p6*); Fig. 1]. Unlike ΔGAG, which could still produce virus-associated p24^{gag} (Fig. 2b, lane 12), Δ(GAG + p6*) was severely defective in proteolytic processing of Pr55^{gag}, as no mature p24^{gag} was detected in either medium or cell lysates (Fig. 2b, lanes 6 and 13). Despite being cotransfected with a higher amount of plasmid DNA, Δ(GAG + p6*) still could not produce p24/25^{gag}; instead, a trace amount of p41^{gag} was observed (Fig. 2b, lanes 3 and 10). This suggests that PR-mediated Gag processing was almost abrogated by the Δ(GAG + p6*) mutation. In contrast, the p6^{gag}/p6*Pol demonstrated a Gag particle processing profile similar to that of wt GPfs when cotransfected with pGAG at a DNA ratio of either 1 : 10 or 1 : 1 (Fig. 2c, lanes 16–17 vs 12–13). However, the p6^{gag}/Pol was markedly defective in Gag particle processing, as substantial amounts of Gag proteins remained in the precursor or intermediate forms (Fig. 2c, lane 19). Overexpression of p6^{gag}/Pol does not suppress virus particle production as efficiently as the GPfs or p6^{gag}/p6*Pol (Fig. 2c, lanes 18 vs 12 and 16). These results suggests that the damage of p6*-deletion mutation to PR-mediated Gag processing is accentuated in the presence of additional mutations.

Although it is thought that the incorporation of HIV Gag–Pol into virus particles depends on interactions with the Pr55^{gag} through its N-terminal Gag domain, it is unknown whether mutations in p6* can affect the incorporation of Gag–Pol into virus particles. To test whether p6* deletion mutations have any detrimental effect on Gag–Pol incorporation, which may consequently impair viral infectivity, aliquots of the culture medium used for infection were measured for virus-associated RT by Western blot. The results shown in Fig. 2(d) suggest that the p6* deletion mutations have no significant effect on the incorporation of Gag–Pol into virions, as D1fs, D2fs and D3fs all produced a level of virus-associated p66/51 RT comparable to that of wt GPfs (Fig. 2d, lanes 3–6). However, in addition to relatively higher levels of unprocessed and incompletely processed Gag, trace amounts of Pr160$^{gag–pol}$ precursors were readily detected in virions produced from cotransfections with D1fs (Fig. 2d, lane 4). This supports the proposal that the D1 mutation has impaired PR activity.

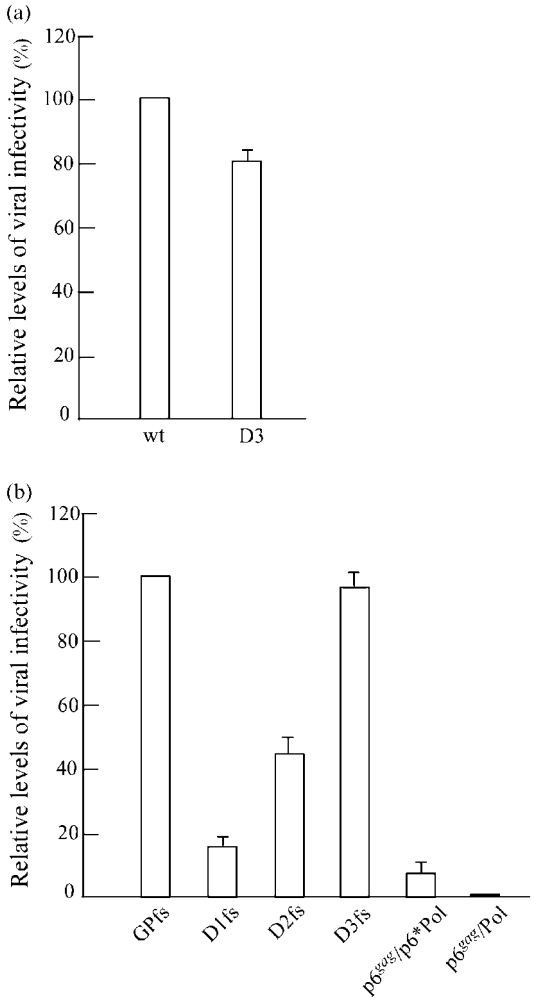

Fig. 3. Infectivity of virus particles produced by 293T cells cotransfected with $Pr160^{gag-pol}$ mutants and pGAG plus pHCMV-G. 293T cells were either (a) cotransfected with 10 μg wild-type (wt) or the mutant HIVgpt D3 and 5 μg pHCMV-G, or (b) cotransfected with 1 μg of the GPfs or the GPfs version of the designated plasmid with 10 μg pGAG plus 5 μg pHCMV-G. At 48–72 h after transfection, supernatants were used to infect HeLa cells. Infection and selection of drug-resistant colonies were as described in the text. Viral titre per Gag protein unit for each mutant was plotted relative to that of wt HIVgpt or GPfs in parallel experiments. Data were obtained from at least three independent experiments with different DNA, or at different times, or both.

The results shown above suggest that our p6* deletion mutations in Gag–Pol have no major or only a modest effect on PR-mediated particle processing. Since the PR-mediated virus maturation process is a prerequisite for viral infectivity, immature or inappropriately processed virus particles ought to lose infectivity. We performed a single-cycle-infection assay to measure how the p6* deletion mutations affected viral infectivity. To do so, the wt GPfs or each of the mutant GPfs constructs was cotransfected with the pGAG plus a vesicular stomatitis virus glycoprotein expression vector, pHCMV-G (Yee *et al.*, 1994). At 48–72 h, culture supernatants of transfected 293T cells were collected and the filtered supernatants were used to infect HeLa cells, which had been split and grown to 20 % confluence at the time of infection. Adsorption of virus was allowed to proceed at 37 °C in the presence of 4 μg Polybrene ml^{-1}. Two days after infection, the cells were trypsinized and split 1 : 10 into 10 cm dishes containing selection medium (50 μg xanthine ml^{-1}, 3 μg hypoxanthine ml^{-1}, 4 μg thymidine ml^{-1}, 10 μg glycine ml^{-1} and 150 μg glutamine ml^{-1}) plus 25 μg mycophenolic acid (Gibco) ml^{-1} (Chen *et al.*, 1997). Ten to 14 days later, colonies of drug-resistant cells were fixed and stained with 0·5 % methyl blue in 50 % methanol. Drug-resistant colonies were converted to titres (infectious units ml^{-1}) and normalized to the corresponding virus-associated Gag protein level. As shown in Fig. 3(b), the infectivity of virions produced from cotransfections with D1fs or D2fs is markedly reduced, with viral infectivity at 20–50 % relative to that of wt GPfs. In contrast, D3fs could produce virus particles with a level of infectivity comparable to that of wt GPfs. HIVgptD3 showed an infectivity level of about 80 % compared with the wt (Fig. 3a), which agrees with previous reports that suggest deletions in this region do not significantly affect HIV-1 replication (Bleiber *et al.*, 2004; Paulus *et al.*, 2004). In the case of cotransfection with $p6^{gag}$/p6*Pol, the infectivity of the released virions was only about 10 % relative to wt GPfs, although the virus particles were processed as well as those produced from the wt GPfs cotransfection (Fig. 2c, lanes 17 vs 13). These results suggest that a significant portion of the processed virions from cotransfections with Gag–Pol mutants were non-infectious. Functional interference in the post-assembly post-processing stage of virus replication by the $p6^{gag}$ embedded in the incorporated Gag–Pol may account in part for the reduced viral infectivity. The defect in viral particle processing is certainly able to impair viral infectivity. However, efficient PR-mediated virus processing is necessary but not sufficient for viral infectivity since several other factors may affect viral infectivity. For instance, the incorporated Gag–Pol mutants may have an impact on proper Gag assembly, and consequently interfere with virus replication. Additionally, tRNA incorporation (Mak *et al.*, 1994) or the stability of genomic RNA dimer (Shehu-Xhilaga *et al.*, 2001) may be influenced by the Gag–Pol mutants, resulting in reduced viral infectivity. This may possibly explain why D1fs, which is unable to process virus particles as well as the wt and contains a near wt level of RT, produced virions with infectivity reduced to only 20 % relative to that of the wt.

In conclusion, our results strongly suggest that the presence of p6* is essential during the process of PR-mediated Gag cleavage. It is clear that p6* may have a contribution to make in facilitating Gag–Pol dimerization or in stabilizing the Gag–Pol dimer, which is required for the induction of the activation of embedded PR; however, removal of the p6* may eventually be necessary to allow the freed PR to become fully functional.

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References

Arrigo, S. J. & Huffman, K. (1995). Potent inhibition of human immunodeficiency virus type 1 (HIV-1) replication by inducible expression of HIV-1 PR multimers. *J Virol* **69**, 5988–5994.

Bleiber, G., Peters, S., Martinez, R., Cmarko, D., Meylan, P. & Telenti, A. (2004). The central region of human immunodeficiency virus type 1 p6 protein (Gag residues S14–I31) is dispensable for the virus *in vitro*. *J Gen Virol* **85**, 921–927.

Burstein, H., Bizub, D. & Skalka, A. M. (1991). Assembly and processing of avian retroviral *gag* polyproteins containing linked protease dimers. *J Virol* **65**, 6165–6172.

Chen, Y.-L., Ts'ai, P.-W., Yang, C.-C. & Wang, C.-T. (1997). Generation of infectious virus particles by transient co-expression of human immunodeficiency virus type 1 *gag* mutants. *J Gen Virol* **78**, 2497–2501.

Chen, S.-W., Chiu, H.-C., Liao, W.-H., Wang, F. D., Chen, S.-S. & Wang, C.-T. (2004). The virus-associated human immunodeficiency virus type 1 Gag-Pol carrying an active protease domain in the matrix region is severely defective both in autoprocessing and in *trans* processing of gag particles. *Virology* **318**, 534–541.

Chiu, H.-C., Yao, S.-Y. & Wang, C.-T. (2002). Coding sequences upstream of the human immunodeficiency virus type 1 reverse transcriptase domain in Gag-Pol are not essential for incorporation of the $Pr160^{gag\text{-}pol}$ into virus particles. *J Virol* **76**, 3221–3231.

Engelman, A., Englund, G., Orenstein, J. M., Martin, M. A. & Craigie, R. (1995). Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. *J Virol* **69**, 2729–2736.

Erickson-Viitanen, S., Manfredi, J., Viitanen, P., Tribe, D. E., Tritch, R., Hutchison, C. A., III, Loeb, D. D. & Swanstrom, R. (1989). Cleavage of HIV-1 gag polyprotein synthesized in vitro: sequential cleavage by the viral protease. *AIDS Res Hum Retroviruses* **5**, 577–591.

Freed, E. O. (1998). HIV Gag proteins: diverse functions in the virus life cycle. *Virology* **251**, 1–15.

Gottlinger, H. G., Sodroski, J. G. & Haseltine, W. A. (1989). Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus 1. *Proc Natl Acad Sci U S A* **86**, 5781–5785.

Henderson, L. E., Bowers, M. A., Sowder, R. C., II Serabyn, S. A., Johnson, D. G., Bess, J. W., Jr, Arthur, L. O., Bryant, D. K. & Fenselau, C. (1992). Gag proteins of the highly replicative MN strain of human immunodeficiency virus type 1: posttranslational modifications, proteolytic processing, and complete amino acid sequences. *J Virol* **66**, 1856–1865.

Hunter, E. (1994). Macromolecular interactions in the assembly of HIV and other retroviruses. *Semin Virol* **5**, 71–83.

Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J. & Varmus, H. E. (1988). Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. *Nature* **331**, 280–283.

Kaplan, A. H., Manchester, M. & Swanstorm, M. (1994). The activity of the protease of human immunodeficiency virus type 1 is initiated at the membrane of infected cells before the release of viral proteins and is required for release to occur with maximum efficiency. *J Virol* **68**, 6782–6786.

Kohl, N. E., Emini, E. A., Schleif, W. E., Davis, L. J., Heimbach, J. C., Dixon, R. A. F., Scolnick, E. M. & Sigal, I. S. (1988). Active human immunodeficiency virus protease is required for viral infectivity. *Proc Natl Acad Sci U S A* **85**, 4686–4890.

Krausslich, H.-G. (1991). Human immunodeficiency virus proteinase dimer as component of the viral polyprotein prevents particle assembly and viral infectivity. *Proc Natl Acad Sci U S A* **88**, 3213–3217.

Leis, J., Baltimore, D., Bishop, J. B. & 8 other authors (1988). Standardized and simplified nomenclature for proteins common to all retroviruses. *J Virol* **62**, 1808–1809.

Liao, W.-H. & Wang, C.-T. (2004). Characterization of human immunodeficiency virus type 1 $Pr160^{gag\text{-}pol}$ mutants with truncations downstream of the protease domain. *Virology* **329**, 180–188.

Louis, J. M., Dyda, F., Nashed, N. T., Kimmel, A. R. & Davies, D. R. (1998). Hydrophilic peptides derived from the transframe region of Gag-Pol inhibit the HIV-1 protease. *Biochemistry* **37**, 2105–2110.

Louis, J. M., Clore, G. M. & Gronenborn, A. M. (1999). Autoprocessing of HIV-1 protease is tightly coupled to protein folding. *Nat Struct Biol* **6**, 868–875.

Mak, J., Jiang, M., Wainberg, M. A., Hammarskjöld, M. L., Rekosh, D. & Kleiman, L. (1994). Role of Pr160gag-pol in mediating the selective incorporation of tRNA(Lys) into human immunodeficiency virus type 1 particles. *J Virol* **68**, 2065–2072.

Mervis, R. J., Ahmad, N., Lillehoj, E. P., Raum, M. G., Salazar, F. H. R., Chan, H. W. & Venkatesan, V. (1988). The *gag* gene products of human immunodeficiency virus type 1: alignment within the *gag* open reading frame, identification of posttranslational modifications, and evidence for alternative *gag* precursors. *J Virol* **62**, 3993–4002.

Navia, M. A. & McKeever, B. M. (1990). A role for the aspartyl protease from the human immunodeficiency type 1 (HIV-1) in the orchestration of virus assembly. *Ann N Y Acad Sci* **616**, 73–85.

Page, K. A., Landau, N. R. & Littman, D. R. (1990). Construction and use of a human immunodeficiency virus vector for analysis of virus infectivity. *J Virol* **64**, 5270–5276.

Park, J. & Morrow, C. D. (1991). 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. *J Virol* **65**, 5111–5117.

Partin, K., Krausslich, H. G., Ehrlich, L., Wimmer, E. & Carter, C. (1990). Mutational analysis of a native substrate of the human immunodeficiency virus type 1 proteinase. *J Virol* **64**, 3938–3947.

Partin, K., Zybarth, G., Ehrlich, L., DeCrombrugghe, M., Wimmer, E. & Carter, C. (1991). Deletion of sequences upstream of the proteinase improves the proteolytic processing of human immunodeficiency virus type 1. *Proc Natl Acad Sci U S A* **88**, 4776–4780.

Paulus, C., Hellebrand, S., Tessmer, U., Wolf, H., Krausslich, H.-G. & Wagner, R. (1999). Competitive inhibition of immunodeficiency virus type 1 protease by the Gag-Pol transframe protein. *J Biol Chem* **274**, 21539–21543.

Paulus, C., Ludwig, C. & Wagner, R. (2004). Contribution of the Gag-Pol transframe domain p6* and its coding sequence to morphogenesis and replication of human immunodeficiency virus type 1. *Virology* **330**, 271–283.

Peng, C., Ho, B. K., Chang, T. W. & Chang, N. T. (1989). Role of human immunodeficiency virus type 1-specific protease in core protein maturation and viral infectivity. *J Virol* **63**, 2550–2556.

Pettit, S. C., Gulnik, S., Everitt, L. & Kaplan, A. H. (2003). The dimer interfaces of protease and extra-protease domains influence the activation of protease and the stability of Gag-Pol cleavage. *J Virol* **77**, 366–374.

Quillent, C., Borman, A. M., Paulous, S., Dauguet, C. & Clavel, F. (1996). Extensive regions of *pol* are required for efficient human immunodeficiency virus polyprotein processing and particle maturation. *Virology* **219**, 29–36.

Rose, J. R., Babe, L. M. & Craik, C. S. (1995). Defining the level of human immunodeficiency virus type 1 (HIV-1) protease activity required for HIV-1 particle maturation and infectivity. *J Virol* **69**, 2751–2758.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Shehu-Xhilaga, M., Crowe, S. M. & Mak, J. (2001). Maintenance of the Gag/Gag-Pol ratio is important for human immunodeficiency virus type 1 RNA dimerization and viral infectivity. *J Virol* **75**, 1834–1841.

Swanstrom, R. & Wills, J. W. (1997). Synthesis, assembly, and processing of viral proteins. In *Retroviruses*. Edited by J. M. Coffin, S. H. Hughes & H. E. Varmus. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Tessmer, U. & Krausslich, H.-G. (1998). Cleavage of human immunodeficiency virus type 1 proteinase from the N-terminally adjacent p6* protein is essential for efficient Gag polyprotein processing and viral infectivity. *J Virol* **72**, 3459–3463.

Wang, C.-T., Lai, H.-Y. & Li, J.-J. (1998). Analysis of minimal human immunodeficiency virus type 1 *gag* coding sequences capable of virus-like particle assembly and release. *J Virol* **72**, 7950–7959.

Wang, C.-T., Chou, Y.-C. & Chiang, C.-C. (2000). Assembly and processing of human immunodeficiency virus gag mutants containing a partial replacement of the matrix domain by the viral protease domain. *J Virol* **74**, 3418–3422.

Wills, J. W. & Craven, R. C. (1991). Form, function, and use of retroviral *gag* proteins. *AIDS* **5**, 639–654.

Wondrak, E. M. & Louis, J. M. (1996). Influence of flanking sequences on the dimer stability of human immunodeficiency virus type 1 protease. *Biochemistry* **35**, 12957–12962.

Xiang, Y., Ridky, T. W., Krishna, N. K. & Leis, J. (1997). Altered Rous sarcoma virus Gag polyprotein processing and its effects on particle formation. *J Virol* **71**, 2083–2091.

Yee, J. K., Friedmann, T. & Burns, J. C. (1994). Generation of high-titer pseudotyped retroviral vectors with very broad host range. *Methods Cell Biol* **43**, 99–112.

Zybarth, G. & Carter, C. (1995). Domains upstream of the protease (PR) in human immunodeficiency virus type 1 Gag-Pol influence PR autoprocessing. *J Virol* **69**, 3878–3884.

Zybarth, G., Krausslich, H. G., Partin, K. & Carter, C. (1994). Proteolytic activity of novel human immunodeficiency virus type 1 proteinase proteins from a precursor with a blocking mutation at the N terminus of the PR domain. *J Virol* **68**, 240–250.