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Human immunodeficiency virus type 1 Gag proteins are processed in two cellular compartments

(human immunodeficiency virus type 1 protease/cytoplasmic processing/AIDS/capsid assembly)

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ABSTRACT **The structural proteins of the retroviral capsid are translated as a polyprotein (the Gag precursor) that is cleaved by a virally encoded protease. Processing of the human immunodeficiency virus type 1 Gag precursor Pr55 was analyzed through a combination of pulse–chase labeling, cell fractionation, and immunoprecipitation. We observed a membrane-associated processing pathway for the Gag precursor that gives rise to virions. In addition, we found that a significant amount of processing occurs in the cytoplasm of infected cells resulting in the intracellular accumulation of appropriately processed viral proteins. This observation suggests the viral protease is active in the cytoplasmic compartment of the cell. Processing of the Gag protein was blocked in both compartments by the addition of a viral protease inhibitor. A comparison of the amount of cytoplasmic processing seen in lytically infected cells with that seen in chronically infected cells showed that cytoplasmic processing was associated with the lytic infection. These observations raise the possibility that activation of the human immunodeficiency virus type 1 protease in the cytoplasm of lytically infected cells might result in the cleavage of cellular proteins and thus contribute to cytotoxicity.**

The last steps of replication of a virus include assembly of new virions and release of particles from the infected cell. For most retroviruses, these two processes are inextricably linked; virion assembly occurs at the inner cell membrane as the virus buds out of the infected cell (1–3). Virion particles are formed from precursor proteins that are proteolytically cleaved during the assembly process. However, the relationship between virion assembly, protein processing, and virion release is poorly understood.

The virion structural proteins of the human immunodeficiency virus type 1 (HIV-1) are translated as a polyprotein, the product of the *gag* gene, which is processed by a virally encoded protease (4–7). The processing of the Gag precursor protein Pr55 gives rise to the structural proteins of the viral capsid: the matrix protein p17 (MA), the capsid protein p24 (CA), and the nucleocapsid protein p7 (NC) (Fig. 1). For most retroviruses, the viral Gag precursor protein is synthesized in the cytoplasm and directed to the cell membrane by myristoylation of the precursor (3, 8–12). With murine leukemia virus, proteolytic processing of the Gag precursor requires association of the precursor with the membrane and virion assembly, such that mutations that block either of these events also block cleavage of the Gag precursor (3, 9, 10, 13).

A Gag/Pol fusion protein is expressed at a level ≈5% that of the Gag protein. In this precursor, protein sequences encoded downstream of the *gag* gene in the *pol* gene are linked to the Gag protein during translation (14). The *pol* gene encodes viral enzymes used in replication, including, in the case of HIV-1, the viral protease responsible for processing

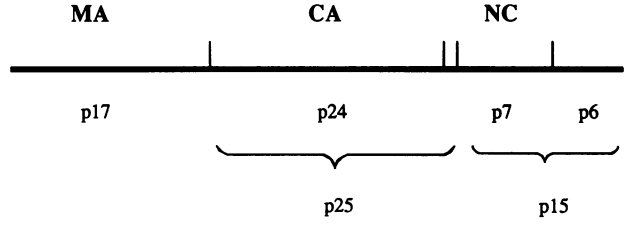

FIG. 1. Gag precursor of HIV-1 and cleavage products. MA, matrix protein; CA, capsid protein; NC, nucleocapsid protein. Vertical lines indicate processing sites (4). The incompletely processed proteins p25 and p15 are indicated.

the Gag and Gag/Pol precursors (7, 15, 16). Structural studies of the protease reveal that it is an aspartic proteinase consisting of two identical subunits that must dimerize to form the active site (17–20). Dimerization of the protease subunits to make an active protease, and thus initiate processing, must occur between two Gag/Pol precursor molecules, an event that by its concentration dependence would be favored during virion assembly.

We have examined human cells infected with HIV-1 in an effort to define the relationship between protein processing and virion assembly. We identified two compartments in the infected cell in which processing of viral capsid proteins occurs: membrane-associated processing resulting in virion formation and cytoplasmic processing leading to the accumulation of soluble processed viral proteins in the cell cytoplasm. The detection of processing in the cytoplasm of infected cells suggests that activation of the viral protease occurs in an inappropriate cellular compartment and this may contribute to cell killing by this virus. Portions of this work have been presented elsewhere.§

MATERIALS AND METHODS

Cells and Virus. CEM cells, a human T-cell lymphoma cell line (a gift of M. Cloyd, University of Texas, Galveston), were maintained in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum. Four independent HIV-1 isolates (205, 214, SK1, and G, a gift of M. Cloyd and R. Buckheit) were used in these experiments (21). All experiments were done with HIV-1_G unless otherwise indicated. To infect cells, virus was added to 2×10^6 CEM cells in a total volume of 400 μl in the well of a 48-well plate for 2 hr, at which time the cells were transferred to a T25 flask and fresh medium was added to a total volume of 3 ml. The cells were pelleted and suspended in fresh medium daily. The cultures were monitored daily for cytopathicity and cell killing. Experiments with acutely infected cells were performed at the point of

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Abbreviations: HIV-1, human immunodeficiency virus type 1; MA, matrix protein; CA, capsid protein; NC, nucleocapsid protein.
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maximal (i.e., 50%) cell killing. Chronic lines were established by maintaining the acutely infected cells until the cells surviving the acute infection grew out. Experiments with the chronically infected cells were performed at about 14 days after the initial infection when the viability of these cultures was between 85% and 90%.

Analysis of Viral Proteins: Fractionation and Immunoprecipitation. Infected cells were pulse-labeled with either 150 μCi of [^{35}S]methionine per ml (1000 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) or 500 μCi of [^{3}H]leucine per ml (50 mCi/mmol; New England Nuclear) for 20 min. The cells were washed with cold phosphate-buffered saline (PBS) at the end of the labeling period and then suspended in complete medium, which includes 0.1 mM methionine and 0.4 mM leucine. Aliquots containing 2×10^6 cells were removed at the end of the pulse period and at various intervals during the chase period. The cells were pelleted from the aliquot and the remaining medium was frozen and designated supernatant virus. All cell fractionation manipulations were performed at 4°C following published procedures (9). The cells were washed in PBS and suspended in swelling buffer (10 mM KCl/20 mM Tris·HCl, pH 7.8/1 mM EDTA/0.1% 2-mercaptoethanol/1% Trasylol). After 20 min at 4°C, the cells were lysed using a tight-fitting "B" glass Dounce homogenizer. Nuclei were pelleted by centrifugation at $1000 \times g$ for 5 min. The supernatant from this spin was centrifuged at $100,000 \times g$ for 30 min to pellet the membranes. The resulting supernatant (S100 supernatant) was frozen and the pellet (S100 pellet) was suspended in a RIPA buffer (0.15 M NaCl/20 mM Tris·HCl, pH 7.4/2 mM EDTA/1% Triton X-100/1% sodium deoxycholate). The fractionation was validated using uninfected CEM cells and subjecting each of the fractions to SDS/polyacrylamide gel electrophoresis and Western analysis using a monoclonal antibody to CD4. All of the stainable protein appeared in the membrane fraction (data not shown). Infected cells were also labeled with 500 μCi of [^{3}H]myristic acid per ml (50 mCi/ml; New England Nuclear) for 12 hr. These cells were then fractionated as described above.

To isolate labeled viral proteins, the samples were incubated for 1 hr at 4°C with serum from a HIV-1 seropositive person. The immune complexes were collected by binding to protein A-Sepharose (Pharmacia) followed by centrifugation. The complexes were washed three times in RIPA buffer, suspended in a SDS loading buffer, and boiled for 2–3 min. The samples were then loaded on a SDS/12% polyacrylamide gel (22). After electrophoresis the gel was dried under vacuum and then exposed to x-ray film. Gels containing proteins labeled with ^{3}H were treated with EN^{3}HANCE according to the instructions of the manufacturer (New England Nuclear). In some cases, radioactivity in the dried gel was quantitated using a radioanalytical analyzing system (AMBIS Systems, San Diego).

Inhibition of the Viral Protease. Infected cells were maintained in the presence of one of two inhibitors of the viral protease (kindly provided by Thomas Meek and Geoffrey Dryer, SmithKline Beecham). The cells were grown for 1 hr in methionine-deficient medium that contained one of the inhibitors at a concentration of 20 μM. Labeling was then carried out for 6 hr with 150 μCi/ml in the presence of the inhibitor. Aliquots containing 2×10^6 cells were removed and the cells were fractionated into cytoplasmic, membrane, and supernatant components as described above.

RESULTS

Pulse–Chase Labeling and Cell Fractionation of Acutely Infected Cells Reveal Processing in Two Cellular Compartments. In an effort to define the temporal order of events in HIV-1 virion formation we examined the intracellular site of viral protein processing. Viral proteins were analyzed by combining pulse–chase analysis with subcellular fractionation at the peak of cell killing, which occurred ≈5 days after infection. Cellular and viral proteins were pulse-labeled with [^{35}S]methionine for 20 min followed by a chase period in the presence of unlabeled methionine. At various times during the chase, cells were removed and fractionated into cell supernatant (containing released virus particles), a nuclear fraction, a cytoplasmic fraction, and a membrane fraction. Viral proteins in each fraction were isolated by immunoprecipitation using human serum obtained from a HIV-1 seropositive person. The proteins were then analyzed by SDS/polyacrylamide gel electrophoresis followed by autoradiography. The results are shown in Fig. 2. In the cytoplasmic and membrane fractions the Gag precursor Pr55 was present at the end of the pulse-labeling period. At 30 min into the chase period the membrane fraction had small and equivalent amounts of two proteolytically processed forms of CA, p25 and p24, which differ by a short C-terminal extension in the longer protein (23, 24). The mature p24 species predominated by 60 min in the membrane fraction and was the only species apparent at 3 hr. This relationship between p25 and p24 was also seen in extracellular virus. At 15 min equivalent amounts of p25 and p24 were present in virus. During the rest of the chase period p24 predominated. The similarity in these two patterns is consistent with the interpretation that proteolytic processing involved in virion formation occurs either at the membrane during virion formation or rapidly after virus budding.

Surprisingly, the processing of viral proteins was not restricted to the membrane fraction. A portion of the Pr55 Gag precursor present in the cytoplasm at the end of the chase period persisted with some of it being replaced by the p25 and p24 forms of CA (Fig. 2). The p25 intermediate appeared initially, and by 60 min there were equivalent amounts of the two forms of CA (p25 and p24). This situation persisted through 3 hr and even to 14 hr (not shown). Other processing intermediates are seen below Pr55 at the end of

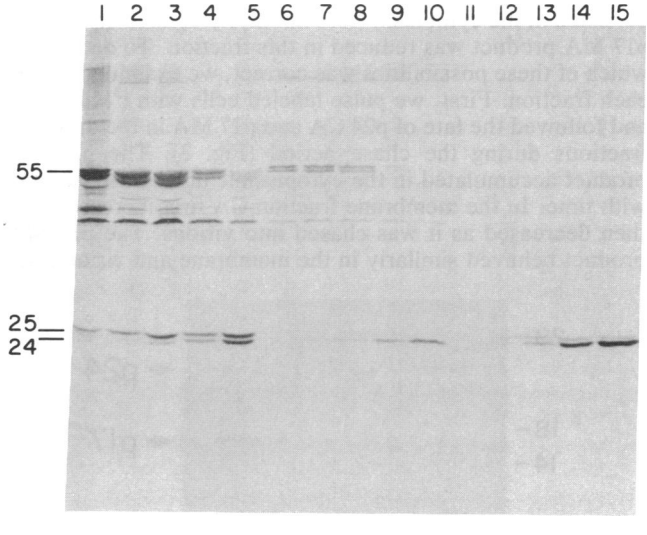

FIG. 2. Pulse–chase analysis of viral proteins from different fractions of infected cells. CEM cells were infected with stock virus (HIV-1$_G$) and the labeling was performed at the peak of cell killing (5 days after infection). Lanes 1–5, cytoplasmic fraction (S100 supernatant); lanes 6–10, membrane fraction (S100 pellet); and lanes 11–15, extracellular fraction (supernatant virus). Samples were taken at the end of the chase period (lanes 1, 6, and 11), after 15 min of chase (lanes 2, 7, and 12), after 30 min of chase (lanes 3, 8, and 13), after 1 hr of chase (lanes 4, 9, and 14), and after 3 hr of chase (lanes 5, 10, and 15). Each sample was immunoprecipitated and analyzed by polyacrylamide gel electrophoresis. The positions of p55 Gag and the two forms of CA p25 and p24 are indicated on the left.

the pulse (Fig. 2), and steady-state levels of the p41 intermediate (MA/CA) are seen in the cytoplasm using Western analysis (data not shown) (25). Processing in the cytoplasmic fraction was similar to processing that occurs in the membrane fraction but was clearly distinct: the two forms of CA remained in equivalent amounts in the cytoplasmic fraction, whereas the p24 form predominated in the membrane fraction and in virions. Similar results were obtained with three additional independent HIV-1 isolates, although the persistence of p25 in the cytoplasmic fraction was not seen with all isolates or resolved in all experiments (data not shown).

The fact that the processed products found in the cytoplasm resembled the products found in the membrane and in supernatant virus fractions suggested the viral protease was responsible for all of the observed processing. To provide a test of this hypothesis we analyzed the extent of processing in each compartment in the presence of a viral protease inhibitor. Pulse–chase labeling experiments were carried out in the presence of two synthetic analogues of one of the protease cleavage sites (26). We found that processing was inhibited in the membrane and cytoplasmic fractions in the presence of both viral protease inhibitors (data not shown). These results support the conclusion that the processed proteins that appear in these two compartments are processed by the viral protease and not by a cellular protease.

MA Accumulates to a Lesser Extent than CA in the Cytoplasm of Acutely Infected Cells. The Gag precursor is normally directed to the membrane through the addition of an N-terminal myristic acid moiety (11, 12). To determine what role myristoylation might play in the accumulation of cytoplasmic protein, we labeled cells for 12 hr with [^{3}H]myristic acid and looked for the distribution of labeled viral proteins in the different fractions. Labeled Pr55 Gag and p17 MA were present in the membrane fraction and labeled p17 accumulated in the virus fraction, but no labeled viral proteins were detected in the cytoplasmic fraction (data not shown). Labeling of Gag in the cytoplasmic fraction was not expected since Gag turned over during this long labeling period, but the absence of labeled p17 suggested that either the proteins that persisted in the cytoplasm were not myristoylated or that the p17 MA product was reduced in this fraction. To determine which of these possibilities was correct, we examined p17 in each fraction. First, we pulse labeled cells with [^{3}H]leucine and followed the fate of p24 CA and p17 MA in the different fractions during the chase period (Fig. 3). The p24 CA product accumulated in the cytoplasmic and virus fractions with time. In the membrane fraction CA first increased and then decreased as it was chased into virions. The p17 MA product behaved similarly in the membrane and virion fractions, but this protein was significantly underrepresented in the cytoplasmic fraction. This analysis was complicated by what appears to be a general underrepresentation of p17 in each fraction (p17 and p24 have a similar number of leucines that can be labeled and are made in equimolar amounts). This difference may be due to differing affinities by the antibodies in the immunoprecipitating serum. However, the specific underrepresentation of p17 in the cytoplasmic fraction was confirmed by analysis of steady-state levels of protein. Using Western analysis to measure the steady-state level of protein, we estimate that only 25% of the expected MA is present in the cytoplasm based on the amount of CA in this fraction (data not shown). We do not know if the unaccounted for p17 MA is myristoylated and directed to the membrane fraction or turned over and lost in the cytoplasmic fraction. The remaining portion of Gag encodes p7 NC, which first appears as an intermediate with the C-terminal p6 protein as a 15-kDa protein, p15 (ref. 27; Fig. 1). Western analysis of each fraction using a monoclonal antibody to p6 (27) revealed that although the mature product, p6, was present in the membrane and the virus fractions only the p15 precursor was seen in the cytoplasm (data not shown).

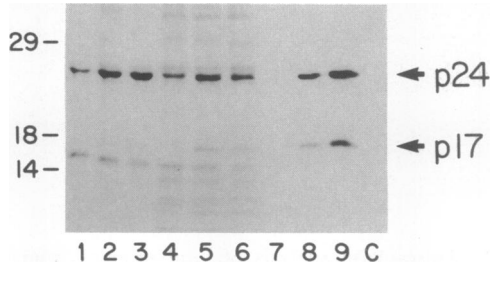

FIG. 3. Pulse–chase analysis of p17 MA. Cells were pulse labeled for 20 min with [^{3}H]leucine (lanes 1, 4, and 7) and then chased for either 1 hr (lanes 2, 5, and 8) or 3 hr (lanes 3, 6, and 9). For each time point, the cells were fractionated into a cytoplasmic fraction (lanes 1–3), a membrane fraction (lanes 4–6), and a supernatant virus fraction (lanes 7–9). Each fraction was immunoprecipitated and analyzed by polyacrylamide gel electrophoresis. Molecular mass markers (in kDa) are shown on the left. The positions of p24 CA and p17 MA are shown on the right. Lane C is an immunoprecipitate of a lysate of labeled uninfected cells.

Cytoplasmic Processing Does Not Occur in Chronically Infected Cells. The occurrence of accurate cytoplasmic processing of the viral precursor proteins suggested that the viral protease was active in this fraction of the cell. An inappropriately active protease in the cytoplasm of the cell is a potential source of toxicity. One test of this possibility is to examine the extent of cytoplasmic processing in cells chronically infected with HIV-1. Infection of CEM cells with HIV-1 *in vitro* results in extensive cell death followed, at about 14 days, by the appearance of cells that are chronically infected. These survivor cells produce viral proteins but are not killed. To evaluate further the role of processing in this noncytopathic infected state, chronic lines were established from eight independent infections of CEM cells with the same virus isolate. The cells were labeled with [^{35}S]methionine for 20 min and the labeled proteins were chased with complete medium for 1 and 3 hr. The cells were fractionated and viral proteins were isolated by immunoprecipitation and analyzed by polyacrylamide gel electrophoresis. Processing in all eight lines occurred primarily at the membrane, with only a small amount of p25/24 accumulating in the cytoplasm after a 3-hr chase (data not shown). The extent of processing was quantitated for three of the cell lines; a graphic comparison of the loss of Pr55 and the accumulation of cytoplasmic p25/24 in the cytoplasmic fraction of cells from two acute and three chronic infections is shown in Fig. 4. These results indicate virtually no cytoplasmic processing in the chronically infected cells while the Gag precursor is chased from this compartment. Thus, increased cell viability is correlated with a reduced amount of cytoplasmic processing of the viral Gag protein.

DISCUSSION

HIV encodes the Gag and Gag/Pol precursor proteins, Pr55 and Pr160, respectively, which, when processed into the mature proteins, subserve the structural and enzymatic requirements of the virus. Accurate processing of these precursors is an obligatory step in the formation of an infectious virion and is accompanied by a readily observable structural rearrangement in which the immature particles attain the more condensed core characteristic of an infectious particle (5, 7). The viral Gag precursor is directed to the cell membrane by myristoylation of the precursor. Although processing of the Gag precursor is thought to be a late event in the maturation of the murine and avian oncoviruses (3, 4), the location of processing has not been determined for HIV or the other lentiviruses. We have identified two compartments in

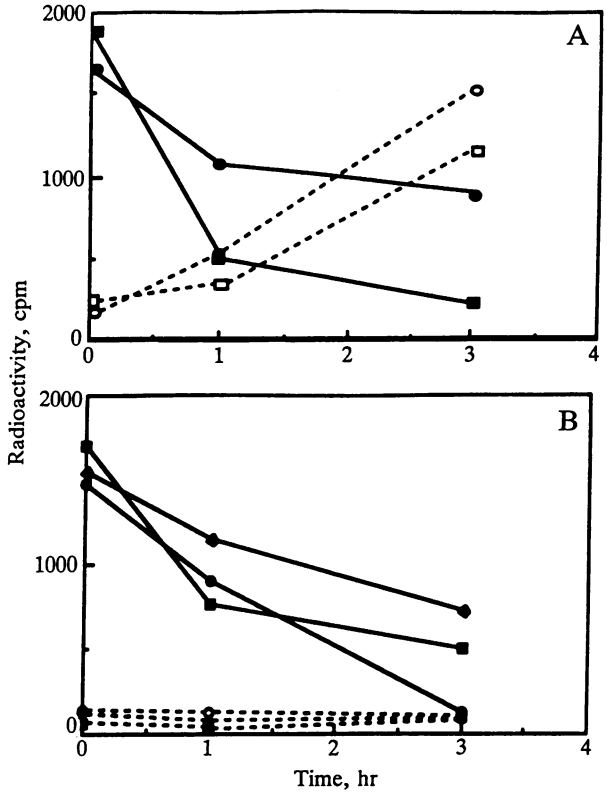

FIG. 4. Comparison of cytoplasmic processing in chronically and acutely infected cells. Acutely and chronically infected cells were pulse-labeled with [^{35}S]methionine and then chased with complete medium. Aliquots of cells were removed at intervals during the chase period, the cells were fractionated, and the viral proteins were immunoprecipitated. Radioactivity in the dried gels was quantitated by a radioanalytical analyzing system (AMBIS Systems). The total radioactivity present in the cytoplasm as p55 and as p24/p25 is plotted versus time of chase. (*A*) Acute infection for two different experiments. The lines with open squares represent data from Fig. 2. The other experiment was similar to that shown in Fig. 2 in that at the long chase times there were equal amounts of p25 and p24. (*B*) Chronic infection from three experiments. Closed symbols and solid lines represent p55; open symbols and dashed lines represent p24/25, respectively, for the individual experiments.

cells acutely infected with HIV-1 where processed viral proteins are found: in the membrane, where processing gives rise to virions, and in the cytoplasm, where processing results in the accumulation of processed viral capsid protein.

Processing of viral Gag and Gag/Pol proteins could be occurring either at the membrane or at both the membrane and in the cytoplasm. In the former case, the appearance of processed viral proteins in the cytoplasm could be the result of a breakdown of virion assembly at the membrane with release of viral proteins into the cytoplasm. Alternatively, viral proteins may accumulate in the cytoplasm at high enough levels to permit activation of the viral protease. A high level of viral protein is one of the features of acute HIV-1 infection (28), and the potential for cytoplasmic processing has been demonstrated using a mutant viral genome that encodes a Gag protein that cannot be myristoylated (11). In this case, high level expression was obtained after transfection and amplification of viral DNA in COS cells, and cell-associated processing of Gag proteins was observed. We have observed in the cytoplasm of acutely infected cells (*i*) the presence of mature products of processed Gag protein (p17 MA and p24 CA), (*ii*) processing intermediates (p41, p25, and p15), and (*iii*) a dynamic change in the ratio of the p25 intermediate and the p24 product over time that was slower than the processing associated with virion formation. These features of viral protein processing were not seen in the cytoplasm of chronically infected cells. Taken together, the evidence suggests that the viral protease is active in the cytoplasmic fraction of acutely infected cells and capable of processing viral Gag protein that persists in the cytoplasm.

The mechanisms by which viruses kill cells during lytic infection are poorly understood. One mechanism for HIV-1-induced cell killing is through cell fusion, or syncytium formation, mediated by the viral *env* gene product (29). However, in many circumstances cell killing by HIV-1 occurs in the absence of significant syncytium formation and therefore alternative mechanisms of cell killing must be considered (30). Lytic infections by retroviruses have been correlated with the appearance of large amounts of unintegrated viral DNA (31), utilization of a specific cellular receptor (32), and the production of large amounts of viral protein (28). These observations suggest that one feature of cell killing is the ability to carry out efficient initial infection, perhaps augmented by a period of efficient reinfection. Perturbations in phospholipid synthesis (33) and calcium metabolism (34) have also been identified in infected cells and may play a role in viral pathogenesis.

In several picornavirus systems, where processing of viral precursor proteins and virion assembly occur in the cytoplasm, a viral protease has been implicated in cell killing. These proteases may inhibit the function of several cellular proteins, including histone 3 (35) and a subunit of the cap-binding protein complex (36). Thus, the action of a viral protease on cellular proteins can contribute to the cell toxicity associated with virus replication.

Dimerization is required to form the active site of the HIV-1 protease and thus presumably required for activity. Therefore, proteolytic cleavage (of either viral or possibly cellular proteins) can occur only in those situations in which the concentration of the protease is sufficiently high to permit dimerization. The high intracellular viral protein concentration noted for certain HIV-1-infected cells (28) may provide the opportunity for premature dimerization and activation of the viral protease. Although specific cellular targets of the HIV-1 protease have not been identified, it is clear that the viral protease is toxic when expressed at high levels in bacteria (16, 37, 38), and it is able to cleave heterologous proteins (39, 40). Furthermore, injection of purified protease directly into mammalian cells resulted in altered cell morphology (40). Our observation of processing of viral proteins in the cytoplasm of infected cells indicates that the HIV-1 protease is active in an inappropriate cellular compartment during lytic infection, which suggests another mechanism for HIV-1-induced cell toxicity.

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1. Bolognesi, D., Montelaro, R., Frank, H. & Schafer, W. (1978) *Science* **199**, 183–186.
2. Dickson, C., Eisenman, R., Fan, H., Hunter, E. & Teich, N. (1984) in *Molecular Biology of Tumor Viruses: RNA Tumor Viruses*, eds. Weiss, R. A., Teich, N. M., Varmus, H. E. & Coffin, J. M. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), p. 513–649.
3. Witte, O. N. & Baltimore, D. (1978) *J. Virol* **26**, 750–761.
4. Oroszlan, S. & Luftig, R. B. (1990) *Curr. Top. Microbiol. Immunol.* **157**, 153–187.

5. Katoh, I., Yoshinaka, Y., Rein, A., Shibuya, M., Odaka, T. & Oroszlan, S. (1985) *Virology* **145,** 280–292.
6. Crawford, S. & Goff, S. P. (1985) *J. Virol.* **53,** 899–907.
7. Kohl, N. E., Emini, E. A., Schlief, W. A., Davis, L. J., Heimbach, J. C., Dixon, R. A., Scolnik, E. M. & Sigal, I. S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4686–4690.
8. Rhee, S. S. & Hunter, E. (1987) *J. Virol.* **61,** 1045–1053.
9. Rein, A., McClure, M. R., Rice, N. R., Luftig, R. B. & Schultz, A. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7246–7250.
10. Schultz, A. & Rein, A. (1989) *J. Virol.* **63,** 2370–2376.
11. Gottlinger, H. G., Sodroski, J. G. & Haseltine, W. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5781–5785.
12. Bryant, M. & Ratner, L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 523–527.
13. Schwartzberg, P., Colicelli, J., Gordon, M. L. & Goff, S. P. (1984) *J. Virol.* **49,** 918–923.
14. Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J. & Varmus, H. E. (1988) *Nature (London)* **331,** 280–283.
15. Farmerie, W. G., Loeb, D. D., Casavant, N. C., Hutchinson, C. A., Edgell, M. H. & Swanstrom, R. (1987) *Science* **236,** 305–308.
16. Debouck, C., Gorniak, J. G., Strickler, J. E., Meek, T. D., Metcalf, B. W. & Rosenberg, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8903–8906.
17. Miller, M., Jaskolski, M., Rao, J. K. M., Leis, J. & Wlodowar, A. (1989) *Nature (London)* **337,** 576–579.
18. Navia, M. A., Fitzgerald, P. M., McKeever, B. M., Leu, C. T., Heimbach, J. C., Herber, W. K., Sigal, I. S., Darke, P. L. & Springer, J. P. (1989) *Nature (London)* **337,** 615–620.
19. Wlodowar, A., Miller, M., Jaskolski, M., Sathyanarayana, B. K., Baldwin, E., Weber, I. T., Selk, L. M., Clawson, L., Schneider, J. & Kent, S. B. (1989) *Science* **245,** 616–621.
20. Lapatto, R., Blundell, T., Hemmings, A., Overington, J., Wilderspin, A., Wood, S., Merson, J. R., Whittle, P. J., Danley, D. E., Geoghegan, K. F., Hawrylik, S. J., Lee, S. E., Scheld, K. G. & Hobart, P. M. (1989) *Nature (London)* **342,** 299–302.
21. Cloyd, M. W., Lynn, W. S., Ramsey, K. & Baron, S. (1989) *Virology* **173,** 581.
22. Laemmli, U. (1970) *Nature (London)* **277,** 680–685.
23. Mervis, R. J., Ahmad, N., Lillehoj, E. P., Raum, M. G., Salazar, F. H. R., Chan, H. W. & Venkatesan (1988) *J. Virol.* **62,** 3993–4002.
24. Veronese, F., Copeland, T. D., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1988) *J. Virol.* **62,** 795–801.
25. Gowda, S., Stein, B. & Engleman, E. (1989) *J. Biol. Chem.* **264,** 8459–8462.
26. Meek, T. D., Lambert, D. M., Dreyer, G. B., Carr, T. J., Tomaszek, T. A., Jr., Moore, M. L., Strickler, J. E., Debouck, C., Hyland, L. J., Mathews, T. J., Metcalf, B. W. & Petteway, S. R. (1990) *Nature (London)* **343,** 90–92.
27. Veronese, F. D., Rahman, R., Copeland, T. D., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1987) *AIDS Res. Hum. Retroviruses* **3,** 253–264.
28. Somosundaran, M. & Robinson, H. L. (1988) *Science* **242,** 1554–1556.
29. Sodroski, J. G., Goh, W. C., Rosen, C. A., Cambell, K. & Haseltine, W. A. (1986) *Nature (London)* **322,** 470–474.
30. Somasundaran, M. & Robinson, H. L. (1987) *J. Virol.* **61,** 3114–3119.
31. Weller, S. K., Joy, A. E. & Temin, H. M. (1980) *J. Virol.* **33,** 494–506.
32. Dorner, A. J. & Coffin, J. M. (1986) *Cell* **45,** 365–374.
33. Lynn, W. S., Tweedale, A. & Cloyd, M. W. (1988) *Virology* **163,** 43–51.
34. Rasheed, S., Gottlieb, A. A. & Garry, R. F. (1986) *Virology* **154,** 395–400.
35. Falk, M. M., Grigera, P. R., Bergmann, I. E., Zibert, A., Multhaup, G. & Bech, E. (1990) *J. Virol.* **64,** 748–756.
36. Rose, J. K., Trachsel, H., Leong, K. & Baltimore, D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2732–2736.
37. Erikson-Viitanan, S., Manfredi, J., Viitanen, P., Tribe, D. E., Tritch, R., Hutchinson, C. A., Loeb, D. D. & Swanstrom, R. (1989) *AIDS Res. Hum. Retroviruses* **5,** 577–591.
38. Baum, E. Z., Berbernitz, G. A. & Gluzman, Y. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5573–5577.
39. Tomasselli, A. G., Olsen, M. K., Hui, J. O., Staples, D. J., Sayer, T. K., Heinrickson, R. L. & Tomich, C. C. (1990) *J. Biol. Chem.* **265,** 408–413.
40. Shoeman, R. L., Honer, B., Stoller, T. J., Kesselmeier, C., Miedel, M. C., Traub, P. & Graves, M. C. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6336–6340.