HIV-1 protease cleaves eukaryotic initiation factor 4G and inhibits cap-dependent translation

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Several animal viruses inhibit host protein synthesis, but only some members of the picornavirus group are known to do so by cleaving translation initiation factor eIF4G. Here we report that infection of human CD4$^+$ cells with HIV-1 also leads to proteolysis of eIF4G and profound inhibition of cellular translation. Purified HIV-1 protease directly cleaves eIF4GI at positions 678, 681, and 1086, separating the three domains of this initiation factor. Proteolysis of eIF4GI by HIV-1 protease, as with poliovirus 2A protease, inhibits protein synthesis directed by capped mRNAs but allows internal ribosome entry site-driven translation. These findings indicate that HIV-1, a member of retrovirus group, shares with picornaviruses the capacity to proteolyze eIF4G.

Protein synthesis is regulated mainly at the initiation level in eukaryotic cells. The majority of mRNAs initiates translation on recognition of the 5′ cap structure by the protein complex eIF4F (1–3). This complex comprises the cap-binding protein eIF4E, the ATP-dependent RNA helicase eIF4A, and the eIF4G subunit. The eIF4F complex acts in concert with other initiation factors to promote the joining of the small ribosomal subunit to mRNAs. eIF4G plays a key role in this process, functioning as an adaptor molecule that bridges the mRNAs to ribosomes via interaction with factors eIF4E and eIF3, respectively (4). In addition, eIF4G interacts with the poly(A)-binding protein permitting the circularization of the mRNA, an event that could facilitate the initiation of new rounds of translation (5, 6). Recently, another form of eIF4G, termed eIF4GII, has been identified. Both eIF4GI and eIF4GII are interchangeable functionally (7).

Lytic viruses usually inhibit overall host protein synthesis after infection. This effect is very marked in the case of some picornaviruses, in which cellular translation is shut down concomitantly with the proteolytic inactivation of translation initiation factor eIF4G (8). Cleavage of eIF4G is accomplished by the activity of the virus-encoded proteases 2A^{pro} (enterovirus and rhinovirus) or L^{pro} (aphtovirus). Both picornaviral proteases cleave eIF4GI into two fragments at position 641 (2A^{pro}) or 634 (L^{pro}; refs. 9 and 10). This cleavage dissociates eIF4G into two moieties, the N-terminal fragment bound to eIF4E, which mediates cap recognition, and the C-terminal domain involved in mRNA-ribosome interaction through eIF3 (11, 12). Thus, bisection of eIF4G by picornavirus proteases uncouples the activities of eIF4G domains, leading to the inhibition of cap-dependent translation (13–15). Picornavirus mRNAs escape this inhibition because of their ability to initiate protein synthesis by internal entry of ribosomes on uncapped viral mRNAs (16).

In contrast to many retroviruses, acute infection of a number of cell lines with HIV-1 also abrogates host protein synthesis by an unknown mechanism (17, 18). Notably, genomic mRNA from HIV-1 contains an internal ribosome entry site (IRES) sequence that supports translation of HIV-1 *gag* mRNAs in cells containing eIF4G cleaved by poliovirus 2A^{pro} (19). We show here that HIV-1 protease cleaves eIF4G. This finding may account for the ability of HIV-1 to inhibit host translation.

Materials and Methods

Plasmids and *in Vitro* Transcription. The pTM1-HIV-1 PR plasmid was constructed by cloning a PCR-amplified cDNA fragment encoding the protease gene of HIV-1 (BH10 strain) into the pTM1 vector (20) by using *Nco*I and *Bam*HI enzymes. The primers used were: 5′-HIV-1 PR (GCGCGCCATGGGACCTCAGATCACTCTTTGG) and 3′-HIV-1 PR (CGCGGATCCTTACTAAAAATTTAAAGTGCAACCAATCTG). pTM1–2A^{pro}, pTM1–2C, and pKS-Luc plasmids have been described previously (21, 22). The pKS5′ L GAG-PR plasmid encodes *gag* and *pr* genes under the entire 5′ leader region of HIV-1 derived from pNL4.3 plasmid. It was constructed by cloning the 3-kbp PCR-amplified DNA fragment into pBluescript KS (Stratagene) by using *Not*I and *Bam*HI enzymes. The primers used were 5′-NCRH (CGACGCGGCCGCGGTCTCTCTGGTTAGACC) and 3′-HIV-1 PR. The pGEX-4GI plasmid (a gift from M. Hentze) expressing the C-terminal half of human eIF4GI protein (amino acid 642-1560) fused to glutathione *S*-transferase (GST) protein was described previously (23). Capped luciferase and uncapped encephalomyocarditis virus-IRES-2CPolio mRNAs were synthesized *in vitro* from pKS-Luc and pTM1–2C DNA templates, respectively, by using the T7 RNA polymerase kit (Promega).

Cell Culture, Infections, and Transfections. C8166 cells were provided by the EV Programme EVA/MRC Centralised Facility for AIDS Reagents, NIBSC, U.K. and were grown in RPMI medium 1640 containing 10% fetal bovine serum. Cells were infected with pNL4.3-derived HIV-1 virus (24) by using a multiplicity of infection of ≈5 plaque-forming units per cell, and at the indicated times an aliquot of 5×10^5 cells was labeled metabolically with 50 μCi/ml (1 Ci = 37 GBq) of [^{35}S]Met/[^{35}S]Cys mixture (Promix, Amersham Pharmacia) for 1 h and lysed in sample buffer as described previously (22). Labeled products were separated on SDS-PAGE and exposed to X-film (Kodak). Coupled infection/transfection of COS-7 cells with recombinant vT7 virus and pTM1-derived plasmids was described in detail before (21).

Western Blotting. The following antibodies were used: anti-eIF4GI antisera raised against peptides derived from N- and C-terminal regions of human eIF4GI (21) at 1:1,000 dilution; rabbit antisera against N- and C-terminal regions of eIFG4GII factor (a gift from N. Sonenberg) at 1:500 dilution; hybridoma supernatant of a monoclonal antibody against eIF4A factor at 1:50 dilution (a gift from H. Trachsel); and mouse ascites of a monoclonal antibody against HIV-1 p24 antigen and sheep antiserum against HIV-1 PR (Centralised Facility for AIDS reagent) were used at 1:100 and 1:700 dilutions, respectively. Polyclonal antiserum against the 47-kDa subunit of human eIF3 factor was raised by immunizations of rabbits with the recombinant 47-kDa subunit purified from *Escherichia coli* as fusion

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Abbreviations: IF, initiation factor; IRES, internal ribosome entry site; GST, glutathione *S*-transferase; MBP, maltose-binding protein.

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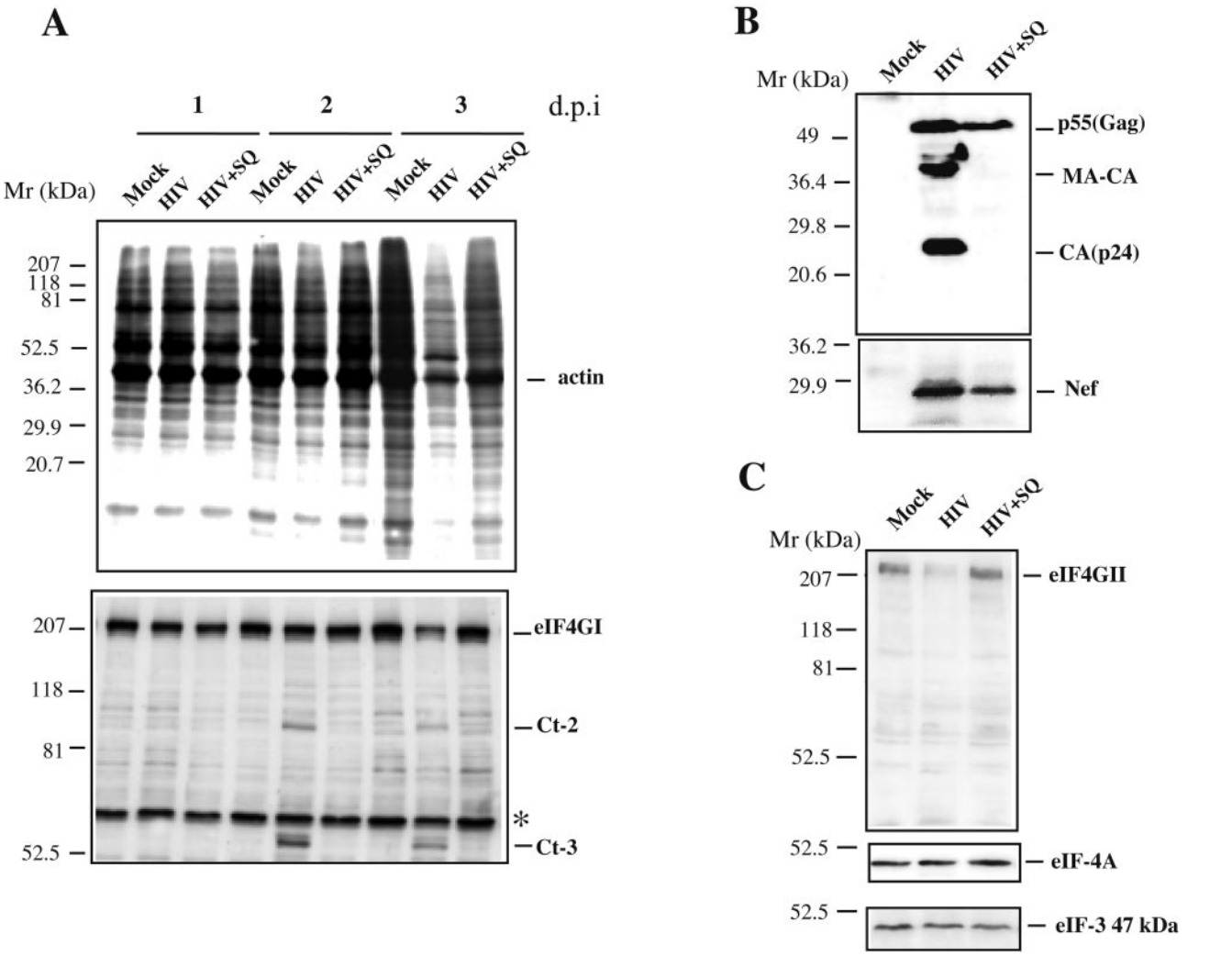

Fig. 1. Protein synthesis and eIF4G cleavage in HIV-1-infected cells. (*A*) C8166 cells were infected with HIV-1 (multiplicity of infection 5) and labeled metabolically with [^{35}S]Met/[^{35}S]Cys for 1 h at 1, 2, or 3 days post infection (d.p.i). The autoradiography of labeled proteins (*Upper*) and the corresponding Western blot against eIF4GI (*Lower*) are shown. Mock, mock-infected cells; HIV-1, cells infected with HIV-1; HIV-1+SQ, cells infected and incubated with 2 μM of saquinavir. Cleavage products derived from eIF4GI are indicated as Ct-2 and Ct-3. An unknown anti-eIF4GI-reactive band is also denoted by an asterisk. Mr, molecular mass. (*B*) Western blots against p24 and Nef proteins at 3 days post infection. (*C*) Western blots against eIF4GII factor (*Upper*) and eIF4A and 47-kDa subunit of eIF3 (*Lower*) at 3 days post infection.

protein with maltose-binding protein (MBP, New England Biolabs).

Protease Cleavage Assays. To map the cleavage sites of HIV-1 PR on eIF4GI, 50 μg of affinity-purified GST-4GI(642–1,560) protein was digested with 1 μg of HIV-1 PR for 3 h at 30°C in 50 mM Na_2HPO_4 buffer, pH 6.0, 25 mM NaCl, 5 mM EDTA, and 1 mM DTT. Cleavage products were separated by SDS-PAGE, transferred to Inmobilon polyvinylidene difluoride membrane (Bio-Rad), and subjected to automated Edman degradation with an Applied Biosystems sequenator by in-home proteomic service.

Protein Purification. GST-4GI(642–1,560) protein was purified from *E. coli* by affinity chromatography on glutathione-agarose resin (Sigma) as described (23). Recombinant eIF4E and flag-eIF4GI factors were provided kindly by Dr. N. Sonenberg. Recombinant HIV-1 PR was purchased from the Centralised Facility for AIDS reagent. MBP-2A^{pro} was purified as described previously (25).

Results

Cleavage of eIF4G in HIV-1-Infected Cells. Initiation factor eIF4G has emerged as a key target for the regulation of translation in cells infected with influenza virus and some picornaviruses (8, 26). To investigate the fate of eIF4G after HIV-1 infection, C8166 cells were infected with HIV-1 (pNL4.3-derived) at high multiplicity (5 plaque-forming units per cell). Protein synthesis and eIF4G integrity were analyzed over a time course. Cell cultures were treated in parallel with 2 μM saquinavir, a specific inhibitor of the HIV-1 protease. This compound was added 8 h after infection with HIV-1 to prevent protease activation. Such treatment did not reduce the number of infected cells at day 3 (27), as judged by fluorescence-activated cell sorting analysis, electron microscopy, and Western blotting against early viral proteins such as Nef (Fig. 1*B*), suggesting that a single round of infection suffices to infect most cells. Western-blot analysis using anti-p24 antiserum indicated that saquinavir prevented the processing of the Gag (p55) precursor (Fig. 1*B*). Protein synthesis and eIF4G integrity were determined by using equal numbers of cells. Infection of C8166 cells with HIV-1 led to a

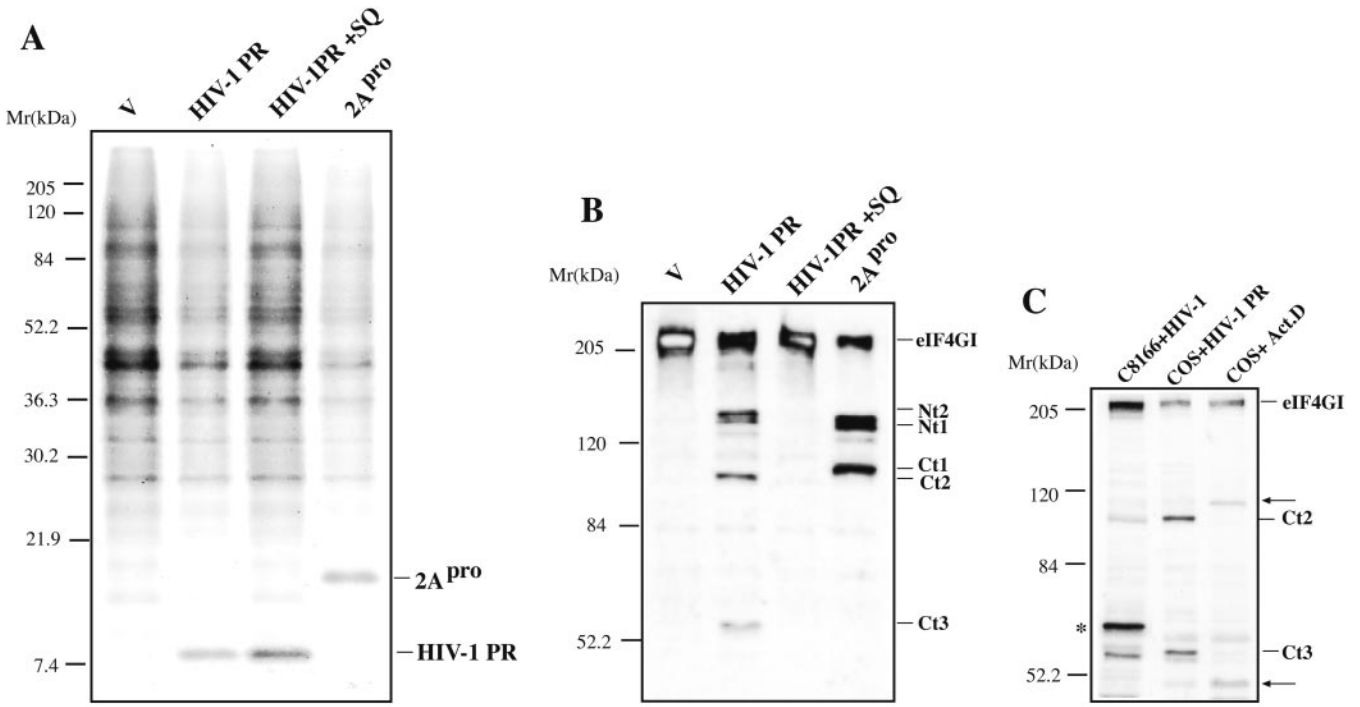

Fig. 2. Cleavage of eIF4GI in transfected cells. (*A*) COS-7 cells were transfected with pTM1 empty vector (V) or pTM1 encoding the HIV-1 *pr* gene (HIV-1 PR) or pTM1 encoding poliovirus 2A protease (2A^{pro}). Cells were labeled with [^{35}S]Met/[^{35}S]Cys from 14 to 15 h post transfection. Equal amounts of protein extract were electrophoresed and analyzed by autoradiography. +SQ, cells incubated with 2 μM saquinavir. Mr, molecular mass. (*B*) Western blot against eIF4GI using a mixture of antisera against N- and C-terminal regions of that factor. Cleavage products derived from eIF4GI are indicated. Nt-1 and Ct-1 are fragments generated by poliovirus 2A^{pro}. Nt-2, Ct-2, and Ct-3 are fragments generated by HIV-1 PR. (*C*) Comparison of eIF4GI cleavage products from HIV-1-infected cells, cells transfected with HIV-1 PR, and Actinomycin D-induced apoptotic cells. The arrows show eIF4GI cleavage products in apoptotic cells. Western blots were over-exposed to ensure detection of cleavage products.

decline of host translation at day 2; this inhibition was more apparent at day 3, coincident with active synthesis of viral proteins. Strikingly, the level of intact eIF4GI (molecular mass of 220 kDa) starts to decrease at day 2 after infection, whereas two smaller immunoreactive protein bands with molecular masses of ≈102 and 57 kDa were apparent (Fig. 1*C*). A higher decrease (80%) of intact eIF4GI occurred at day 3. The intensity of these cleavage products was lower at day 3 as compared with day 2, most probably because of their instability after long incubation times. In addition, eIF4GII (molecular mass ≈ 215 kDa) also decreased after infection by 50%, although no cleavage products were detected. The presence of saquinavir prevented the proteolysis of both forms of eIF4G as well as the inhibition of host protein synthesis. To analyze the integrity of other initiation factors in HIV-1-infected cells, the same blots were reprobed with antibodies against eIF4A and the 47-kDa subunit of eIF3. The amount of these translation factors remained constant after infection, indicating that they were not modified by HIV-1 (Fig. 1*C*). These findings suggest that cleavage of eIF4G contributes at least in part to the inhibition of host translation. However, the modification of other factors may also be involved in this blockade.

HIV-1 PR Cleaves eIF4GI. Next, we tested the ability of HIV-1 PR to cleave eIF4GI and inhibit host translation when expressed alone. The HIV-1 *pr* sequence was cloned in the pTM1 vector and transiently expressed in COS-7 cells by means of the vaccinia vT7 method (20). Cellular protein synthesis and eIF4GI integrity were analyzed at 14 h post transfection. Poliovirus 2A^{pro} was expressed also in a parallel transfection as control. The two proteases were detected easily by metabolic labeling with [^{35}S]Met/[^{35}S]Cys (Fig. 2*A*). Expression of HIV-1 PR induced a substantial (≈70%) decrease in cellular protein synthesis comparable with that obtained after transfection of poliovirus 2A^{pro}. The remaining 30% cellular translation may reflect the percentage of untransfected cells, because the efficiency of transfection in this experiment was ≈70%. The addition of saquinavir prevented the blockade of cellular protein synthesis.

The analysis of extracts of HIV-1 PR-transfected cells by Western blot revealed a cleavage of eIF4GI into three smaller fragments: an N-terminal fragment with molecular mass of ≈140 kDa (Nt-2) and two C-terminal fragments with molecular masses of ≈102 kDa (Ct-2) and ≈57 kDa (Ct-3; Fig. 2*B*; see below for details). This pattern resembles but is not identical to that obtained after expression of poliovirus 2A^{pro}, because the smaller cleavage product (Ct-3) is absent in 2A^{pro}-expressing cells. The pattern of eIF4GI cleavage products generated by HIV-1 PR was compared with that observed in HIV-1-infected cells (Fig. 2*C*). Fragments corresponding in electrophoretic mobility to both Ct-2 and Ct-3 were observed in infected cells. It should be noted that both intact eIF4GI and the Ct-3 fragment from COS-7 cells migrate slightly slower than the corresponding counterpart from C8166 cells, perhaps reflecting minor sequence differences between eIF4GI from human and monkey cells or to differential post-translational modifications. The Nt-2 fragment, faintly detected in HIV-1 PR-expressing cells, was not found in HIV-1-infected C8166 cells, perhaps because of a greater level of instability of the N-terminal fragment of eIF4GI. This pattern is clearly different from that observed in apoptotic cells (ref. 28; Fig. 2*C*).

Identification of the HIV-1 PR Cleavage Sites in eIF4GI. The activity of recombinant HIV-1 PR on eIF4GI was examined in cell-free systems. Crude HeLa S10 extracts (29) were incubated with

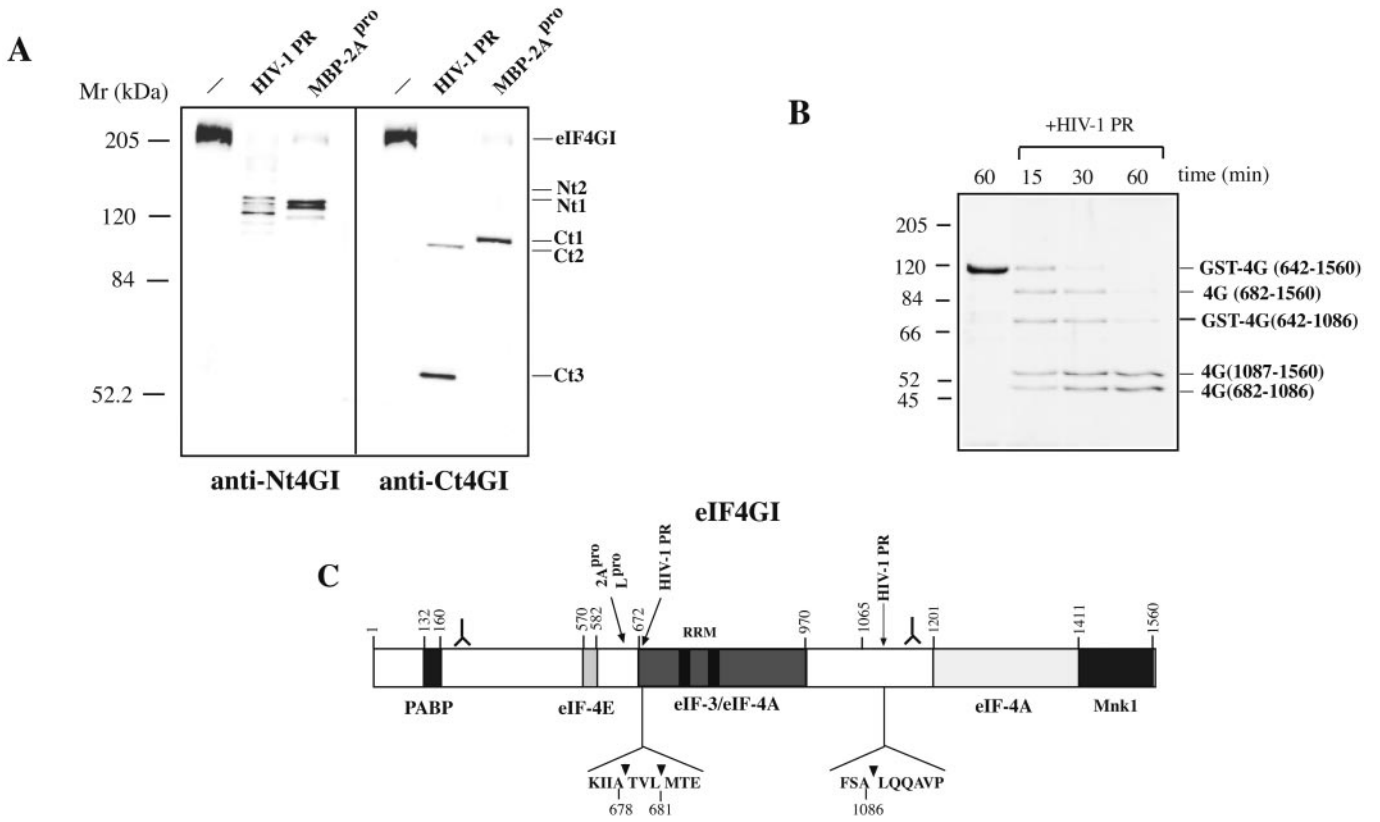

Fig. 3. Cleavage of eIF4GI by purified HIV-1 PR *in vitro* and identification of cleavage sites. (*A*) HeLa S10 extracts (≈50 μg) were incubated for 1 h with recombinant HIV-1 PR (150 nM) or MBP-2A^{pro} (2 μM) and subjected to Western blot by using antisera against N-terminal (*Left*) or C-terminal (*Right*) peptides derived from eIF4GI. Mr, molecular mass. (*B*) Cleavage of purified GST-4GI (amino acid 642–1,560) by HIV-1 PR; 5 μg of affinity-purified GST-4GI(642–1560) was digested with 50 ng of HIV-1 PR for the indicated times and analyzed by SDS-PAGE followed by Coomassie brilliant blue staining. The resulting cleavage products are indicated. (*C*) Diagram of functional domains in eIF4GI based on the available data including the position of mapped cleavage sites for HIV-1 PR. The putative double-stranded RNA binding domain (RRM) and the regions involved in binding to other translation factors are shown. The position of the peptides used to raised antibodies is also indicated. PABP, poly(A)-binding protein.

recombinant HIV-1 PR, and MBP-2A^{pro} proteases were purified from *E. coli* cells. eIF4GI integrity was analyzed by using antisera raised against N- and C-terminal regions of eIF4GI (Fig. 3*A*). The presence of as little as 150 nM HIV-1 PR was sufficient to degrade eIF4GI almost completely. Several fragments were generated, with one N-terminal product with a molecular mass of ≈140 kDa (Nt-2), slightly larger than that generated with poliovirus 2A^{pro} (≈130 kDa, Nt-1), and two C-terminal fragments with molecular masses of ≈102 kDa (Ct-2) and ≈57 kDa (Ct-3). In agreement with previous findings, there is heterogeneity in the N-terminal fragments produced (21). Ct-2 is slightly smaller than that generated by poliovirus 2A^{pro} (molecular mass ≈ 108 kDa, Ct-1). This pattern is identical to that observed in HIV PR-transfected cells.

The above data are consistent with two cleavage sites for HIV-1 PR in the eIF4GI sequence. Both sites should be located downstream from the poliovirus 2A^{pro} cleavage site (position 641). Therefore, the C-terminal fragment (amino acid 642–1,560) of eIF4GI was purified fused to GST protein (23). This purified fragment was digested with HIV-1 PR, and the resulting cleavage products of 57 (Ct-3) and 51 kDa were subjected to N-terminal sequencing (Fig. 3*B*). Analysis of amino acid derivatives of the 51-kDa protein band gave an equimolar mixture of T:M,V:T, L:E, M:D, T:I, and E:K consistent with two adjacent cleavage sites at positions 678 and 681 in eIF4GI (6). The N-terminal sequence of the 57-kDa band revealed a single peptide, LQQAVP, which corresponds to proteolysis at position 1,086. These findings support the view that HIV-1 PR hydrolyzes directly three different peptide bonds on eIF4GI: 678–679, 681–682, and 1,086–1,087. Interestingly, these cleavages divide eIF4GI into the three previously defined domains described in this initiation factor (11, 12), i.e., the N-terminal fragment containing eIF4E and poly(A)-binding protein binding regions, the middle portion that interacts with eIF3 and eIF4A, and the C-terminal regulatory domain that encompasses the second site for eIF4A binding and Mnk1 interaction (Fig. 3*C*).

Effects of eIF4GI Cleavage by HIV-1 PR on Translation. To test the impact of cleavage of eIF4G by HIV-1 PR on *in vitro* protein synthesis, rabbit reticulocyte lysates programmed with synthetic mRNAs were used. Cap-dependent translation was analyzed by using capped luciferase mRNAs. In addition, IRES-driven translation was determined by using mRNAs encoding poliovirus 2C protein under the IRES element from the encephalomyocarditis virus. Both mRNAs are cotranslated efficiently in control samples, giving rise to two major bands of 68 (luciferase) and 32 kDa (poliovirus 2C; Fig. 4*B*). Strikingly, preincubation of lysates with HIV-1 PR inhibited luciferase synthesis by almost 10-fold, whereas poliovirus 2C synthesis was only affected slightly. This inhibition was prevented by treatment of HIV-1 PR with 2 μM saquinavir, suggesting that the HIV-1 PR activity was indeed responsible for the decrease in luciferase synthesis. This agrees well with results shown in Figs. 1 and 2. Preincubation of lysates with poliovirus 2A^{pro} also caused a reduction in luciferase synthesis (2–5-fold), although to a lesser extent compared with HIV-1 PR. These data correlate with the observation that

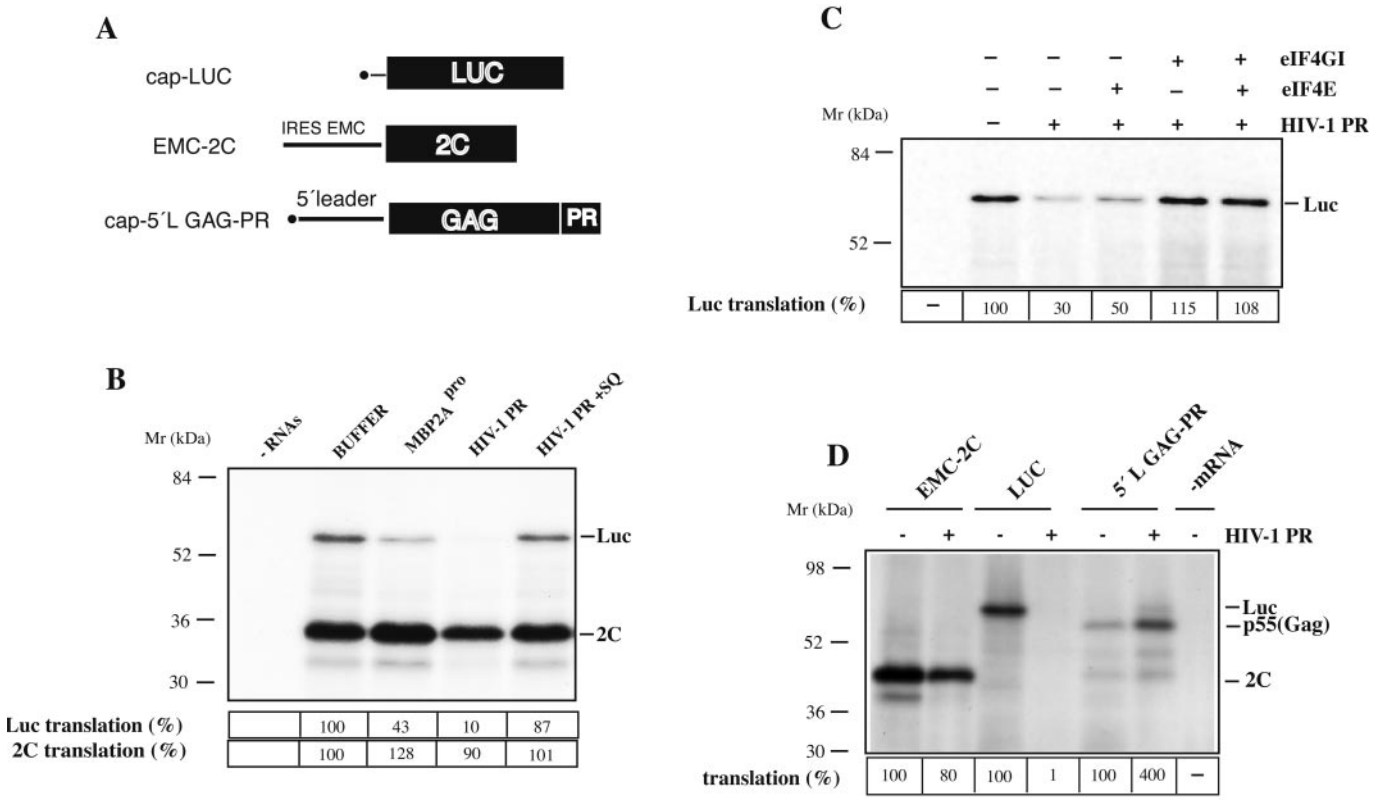

Fig. 4. Effects of eIF4G cleavage by HIV-1 PR on cap-dependent and IRES-driven translation. (*A*) Schematic diagram of synthetic mRNAs used to program *in vitro* translations. (*B*) Rabbit reticulocyte lysates (Promega) were treated with HIV-1 PR (150 nM) or MBP2A^{pro} (2 μM) for 30 min and programmed with capped luciferase (100 ng) and encephalomyocarditis virus (EMC)-polio2C (50 ng) mRNAs in a final volume of 15 μl. ^{35}S-labeled products were analyzed by SDS-PAGE and autoradiography. + SQ, HIV-1 PR preincubated with 2 μM saquinavir. Mr, molecular mass. (*C*) Restoration of HIV-1 PR-mediated inhibition of luciferase translation by the addition of recombinant eIF4GI. Lysates were preincubated with HIV-1 PR, treated with 2 μM saquinavir, and supplemented when indicated with 100 ng of flag-eIF4GI, 50 ng of eIF4E, or both. Five minutes later, translations were programmed with 100 ng of capped Luc mRNA. (*D*) Effect of HIV-1 PR on translation of HIV-1 *gag* mRNA. Lysates were incubated with buffer or HIV-1 PR as indicated. After 30 min of incubation, 2 μM saquinavir was added to all samples. The lysates were then programmed with 100 ng of 5′ L GAG-PR, encephalomyocarditis virus-2C, or Luc mRNAs. Quantitations were carried out by densitometric scanning of protein bands.

recombinant HIV-1 PR cleaves rabbit eIF4GI more efficiently than does poliovirus MBP-2A^{pro} (result not shown).

To determine whether HIV-1 PR-mediated inhibition of luciferase mRNA translation is caused by the proteolysis of eIF4GI, we tested the ability of recombinant eIF4GI to restore translation when added to extracts preincubated with HIV-1 PR. Indeed, luciferase synthesis reached control levels after the addition of intact eIF4GI, whereas eIF4E exerted only a modest stimulatory activity (20% increase in translation). The simultaneous presence of both human initiation factors did not increase luciferase mRNA translation further as compared with eIF4G alone (Fig. 4*C*).

Finally, we wanted to test the translation of HIV-1 mRNA in the presence of eIF4G cleaved. The HIV-1 mRNA assayed contained the entire 5′ leader sequence of genomic viral RNA followed by the *gag* and *pr* sequences. Previous results have shown that translation of mRNAs bearing the 5′ leader of HIV-1 is impaired severely in cell-free systems (30). Notably, translation of this mRNA not only is resistant, but it is even stimulated by 4-fold when eIF4G is cleaved by HIV-1 PR (Fig. 4*D*). As a control, cap-dependent translation was potently inhibited by HIV-1 PR under these conditions, whereas picornavirus IRES-driven translation was resistant. Perhaps cleavage of eIF4G is an event that contributes to a more efficient translation of the genomic HIV-1 mRNA. This finding is of interest to further understand the role of eIF4G and its products in the translation of viral mRNAs.

Discussion

The finding that the HIV-1 protease cleaves eIF4G is important in understanding the strategy used by this retrovirus to regulate translation in infected cells. Apart from eIF4G cleavage, the modification of another factor(s) may contribute also to this regulation of translation. This unsuspected mechanism reinforces the pivotal role that eIF4G plays during the initiation of translation in addition to serving as a key target for several animal viruses including picornaviruses, influenza virus, rotavirus, and now HIV-1 (8, 26, 31).

The proteases of animal viruses not only use viral precursor polyproteins as substrates but also frequently cleave cellular proteins (32). This is true of HIV-1 PR, in which cleavage of cytoskeleton proteins such as vimentin has been documented previously (33). The degradation of cellular proteins by these viral proteases could contribute to virus-induced cytopathogenicity (22, 34). From our data, HIV-1 PR emerges as a viral factor involved in virus-induced cytopathology, especially with acute infections in which activation of HIV-1PR is initiated at the intracellular compartment (35, 36)

A main feature of viral interference with cellular processes is the inhibition of host translation. Simultaneously, lytic viruses redirect the translational apparatus to viral protein synthesis (37). The finding that HIV-1 PR cleaves eIF4G provides additional clues to explain early data showing that acute HIV-1 infection interferes with host protein synthesis (17). Such a mechanism of inhibition of cellular protein synthesis resembles

that observed with picornaviruses. Indeed, HIV-1 PR and poliovirus $2A^{pro}$, two unrelated proteases, hydrolyze eIF4GI factor in a way that leads to an inhibition of cap-dependent translation. The observation that the retroviral protease dissects the different known domains of eIF4G is highly suggestive and could serve as a useful tool to analyze further the role of eIF4G domains in cap-dependent and IRES-driven translation.

HIV-1 mRNAs contain a cap structure at their 5′ ends. Cleavage of eIF4G by HIV-1 PR in the cytoplasm of infected cells (35, 36) raises the question of how HIV-1 late mRNAs are translated in cells lacking intact eIF4G. Our present data provide evidence that translation of HIV-1 *gag* mRNA bearing the entire 5′ leader sequence is stimulated by HIV-1 PR-mediated cleavage of eIF4G. In addition, two recent findings lend further support to our results. First, the presence of an IRES element in the *gag* gene of HIV-1 as well in the 5′ leader region of simian immunodeficiency virus has been reported (19, 38). Second, IRES-driven translation still occurs in the presence of the middle fragment of eIF4GI (57 kDa), which retains the eIF3 and eIF4A binding sites (12, 39, 40). All these evidences may provide the molecular basis for the existence of an operational internal initiation of translation for some retrovirus mRNAs.

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1. Pain, V. M. (1996) *Eur. J. Biochem*. **236,** 747–771.
2. Gingras, A. C., Raught, B. & Sonenberg, N. (1999) *Annu. Rev. Biochem.* **68,** 13–63.
3. Sachs, A. B., Sarnow, P. & Hentze, M. W. (1997) *Cell* **89,** 831–838.
4. Hentze, M. W. (1997) *Science* **275,** 500–501.
5. Wells, S. E., Hillner, P. E., Vale, R. D. & Sachs, A. B. (1998) *Mol. Cell* **2,** 135–140.
6. Imataka, H., Gradi A. & Sonenberg, N. (1998) *EMBO J.* **17,** 7480–7489.
7. Gradi, A., Imataka, H., Svitkin, Y. V., Rom, E., Raught, B., Morino, S. & Sonenberg, N. (1998) *Mol. Cell. Biol.* **18,** 334–342.
8. Etchison, D., Milburn, S. C., Edery, I., Sonenberg, N. & Hershey, J. W. B. (1982) *J. Biol. Chem*. **257,** 14806–14810.
9. Lamphear, B. J., Yan, R., Yang, F., Waters, D., Liebig, H.-D., Klump, H., Kuechler, E., Skern, T. & Rhoads, R. E. (1993) *J. Biol. Chem*. **268,** 19200–19203.
10. Kirchweger, R., Ziegler, E., Lamphear, B. J., Waters, D., Liebig, H.-D., Sommergruber, W., Sobrino, F., Hohenadl, C., Blaas, D., Rhoads, R. E. & Skern, T. (1994) *J. Virol.* **68,** 5677–5684.
11. Lamphear, B. J., Kirchweger, R., Skern, T. & Rhoads, R. E. (1995) *J. Biol. Chem*. **270,** 21975–21983.
12. Morino, S., Imataka, H., Svitkin, Y. V., Pestova, T. V. & Sonenberg, N. (2000) *Mol. Cell. Biol.* **20,** 468–477.
13. Liebig, H. D., Ziegler, E., Yan, R., Hartmuth, K., Klump, H., Kowalski, H., Blaas, D., Sommergruber, W., Frasel, L., Lamphear, B., Rhoads, R., Kuechler, E. & Skern, T. (1993) *Biochemistry* **32,** 7581–7588.
14. Ohlman, T., Rau, M., Morley, S. J. & Pain, V. M. (1995) *Nucleic Acids Res.* **23,** 334–340.
15. Borman, A. M., Kirchweger, R., Ziegler, E., Rhoads, R. E., Skern, T. & Kean, K. M. (1997) *RNA* **3,** 186–196.
16. Pelletier, J. & Sonenberg, N. (1989) *Nature (London)* **334,** 320–325.
17. Somasundaran, M. & Robinson, H. L. (1988) *Science* **242,** 1554–1557.
18. Agy, M. B., Wambach, M., Foy, K. & Katze, M. G. (1990) *Virology* **177,** 251–258.
19. Buck, C. B., Shen, X., Egan, M. A., Pierson, T. C., Walker, C. M. & Siciliano, R. F. (2001) *J. Virol*. **75,** 181–191.
20. Elroy-Stein, O., Fuerst, T. R. & Moss, B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6126–6130.
21. Aldabe, R., Feduchi, E., Novoa, I. & Carrasco, L. (1995) *Biochem. Biophys. Res. Commun.* **215,** 928–936.
22. Ventoso, I., Barco, A. & Carrasco, L. (1998) *J. Biol. Chem*. **273,** 27960–27967.
23. De Gregorio, E., Preiss, T. & Hentze, M. W. (1998) *RNA* **4,** 828–836.
24. Adachi, A., Gendelman, H. E., Koening, S., Folks, T., Willey, R., Rabson, A. & Martin, M. A. (1986) *J. Virol.* **29,** 284–291.
25. Ventoso, I., Barco, A. & Carrasco, L. (1999) *J. Virol.* **73,** 814–818.
26. Aragon, T., de la Luna, S., Novoa, I., Carrasco, L., Ortin, J. & Nieto, A. (2000) *Mol. Cell. Biol.* **20,** 6259–6268.
27. Jacobsen, H., Ahlborn-Laake, L., Gugel, R. & Mous, J. (1992) *J. Virol.* **66,** 5087–5091.
28. Marissen, W. E. & Lloyd, R. E. (1998) *Mol. Cell. Biol.* **18,** 7565–7574.
29. Molla, A., Paul, A. V. & Wimmer, E. (1991) *Science* **254,** 1647–1651.
30. Parkin, N. T., Cohen, E. A., Darveau, A., Rosen, C., Haseltine, W. & Sonenberg, N. (1988) *EMBO J.* **7,** 2831–2837.
31. Piron, M., Vende, P., Cohen, J. & Poncet, D. (1998) *EMBO J.* **17,** 5811–5821.
32. Dougherty, W. G. & Semler, B. L. (1993) *Microbiol. Rev.* **57,** 781–822.
33. Shoeman, R. L., Höner, B., Stoller, T. J., Kesselmeier, C., Miedel, M. C., Traub, P. & Graves, M. C. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6336–6340.
34. Strack, P. R., West Frey, M., Rizzo, C. J., Cordova, B., George, H. J., Meade, R., Penz Ho, S., Corman, J., Tritch, R. & Korant, B. D. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 9571–9576.
35. Kaplan, A. H. & Swanstrom, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4528–4532.
36. Kaplan, A. H., Manchester, M. & Swanstrom, R. (1994) *J. Virol*. **68,** 6782–6786.
37. Thompson, S. R. & Sarnow, P. (2000) *Curr. Opin. Microbiol*. **3,** 366–370.
38. Ohlmann T., Lopez-Lastra, M. & Darlix, J. L. (2000) *J. Biol. Chem.* **275,** 11899–11906.
39. Pestova, T. V., Shatsky, I. N. & Hellen, C. U. T. (1996) *Mol. Cell. Biol*. **16,** 6870–6878.
40. Ohlmann, T., Rau, M., Pain, V. M. & Morley, S. J. (1996) *EMBO J.* **15,** 1371–1382.