Effect of Linker Insertion Mutations in the Human Immunodeficiency Virus Type 1 *gag* Gene on Activation of Viral Protease Expressed in Bacteria

JEREMY LUBAN,¹ CATHERINE LEE,² AND STEPHEN P. GOFF^{2*}

Department of Medicine¹ and Department of Biochemistry and Molecular Biophysics,² College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, New York 10032

Received 18 December 1992/Accepted 3 March 1993

We have expressed the human immunodeficiency virus type 1 (HIV-1) protease (PR) in bacteria as a Gag-PR polyprotein (J. Luban and S. P. Goff, *J. Virol.* 65:3203–3212, 1991). The protein displays enzymatic activity, cleaving the Gag polyprotein precursor Pr55^{gag} to the expected products. The PR enzyme is only active as a dimer, and we hypothesized that PR activation might be used as an indicator of polyprotein multimerization. We constructed 25 linker insertion mutations throughout *gag* and assessed the PR activity of mutant Gag-PR polyproteins by the appearance of Gag cleavage products in bacterial lysates. All mutant constructs produced stable protein in bacteria. PR activity of the majority of the Gag-PR mutants was indistinguishable from that of the wild type. Six mutants, one with an insertion in the matrix (MA), four with insertions in the capsid (CA), and one with insertions in the nucleocapsid (NC), globally disrupted polyprotein processing. When PR was provided in *trans* on a separate plasmid, the Gag proteins were cleaved with wild-type efficiency. These results suggest that the *gag* mutations identified as disruptive of polyprotein processing did not conceal the scissile bonds of the polyprotein. Rather, the mutations prevented PR activation in the context of a Gag-PR polyprotein, perhaps by preventing polyprotein dimerization.

The three major retroviral genes, *gag*, *pol*, and *env*, are all expressed as polyproteins which are proteolytically processed during the late stages of virion particle formation (2). Genetic and biochemical evidence indicates that proteolysis of *gag* and *pol* gene products is carried out by a protease (PR) encoded by the retroviral provirus (3, 5, 9, 14–16, 20–22). Viral genomes defective in PR are capable of producing virions, but these are of immature morphology and they are not infectious. The major Gag polyprotein of human immunodeficiency virus type 1 (HIV-1), the retrovirus most commonly associated with human AIDS, is Pr55^{gag}. The cleavage products of Pr55^{gag} include the matrix protein p17 (MA), the capsid protein p24 (CA), the nucleocapsid protein p7 (NC), and the C-terminal product p6. HIV-1 *pol* is expressed as a Gag-Pol fusion protein, Pr160^{gag-pol}, via a ribosomal frameshift mechanism (13) bypassing translational termination at the *gag-pol* boundary. The *pol* region is processed to four mature products, protease p10 (PR), the two subunits of reverse transcriptase p66 and p51 (RT), and integrase p32 (IN).

The structure of the HIV-1 PR has been determined with and without bound substrate (25–27, 31, 33). PR is an aspartyl protease which differs from cellular proteases of this class in that two identical protease monomers must associate symmetrically to form the substrate binding cleft (26). This work provides a biochemical explanation for the observation that PR activation does not occur prior to virion assembly. PR is translated as part of the Gag-PR polyprotein, and activation only occurs after dimerization of the Gag-PR polyprotein at the time of virion assembly.

PR substrate recognition is highly specific; only a few unique sites in the Gag and Gag-Pol precursor proteins are cleaved. Most of the specificity is provided by four amino

acids on either side of the scissile bond that are recognized by flaps present on each monomer of PR (4, 18, 22, 26, 31). Detailed analysis of multiple PR cleavage sites has revealed no simple consensus sequence for PR substrate recognition, but certain general rules have been deduced (28). On the basis of the amino acid at position P1', there are at least two general classes of substrate recognition sequences. It has been suggested that the differential recognition of these sites may be responsible for the different rates of cleavage observed for the various recognition sites in the Gag and Gag-Pol precursor proteins (7, 10, 28).

Active HIV-1 PR has been successfully expressed in bacteria by several groups (5, 11, 23). Thus, bacterially expressed PR can dimerize when synthesized alone, without Gag. In addition, bacterially expressed PR from HIV-1 has been shown to be active when imbedded in a precursor protein (5, 6, 11, 17). Active Rous sarcoma virus PR can also be expressed as a precursor polyprotein; however, the PR activity of the polyprotein is much less than that of the excised PR (1, 19).

We previously described the PR activity of a Gag-PR fusion protein expressed in bacteria (24). We reasoned that this construct might be an interesting substrate for mutagenesis in that PR activation might be used as a readout for polyprotein multimerization. Using this expression plasmid as a substrate for mutagenesis, we have constructed a panel of linker insertion mutations distributed throughout *gag* and assayed the effect of these mutations on the ability of the expressed mutant Gag-PR polyproteins to activate PR and to cleave the substrate.

Plasmid pT7HG(Pro+) encodes native HIV-1 Pr55^{gag} and a Gag-Pol fusion protein which extends 533 nucleotides into the *pol* coding sequence (24). This plasmid contains the complete *pro* and produces active PR after induction by standard methods with isopropyl-β-D-thiogalactopyranoside (IPTG); lysates from pT7HG(Pro+)-transformed bacteria

* Corresponding author.

FIG. 1. Examples of the effect of linker insertion mutations in the HIV-1 Gag-PR polyprotein on viral PR activity in bacteria. After standard induction with IPTG (30), bacteria transformed with the indicated plasmid DNAs were pelleted and boiled in sodium dodecyl sulfate (SDS). The proteins were separated by electrophoresis on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. A Western blot was then performed with a combination of monoclonal anti-CA (p24) and monoclonal anti-MA (p17) antibodies (24). Lanes: 1, pT7HG(Pro-); 2, pT7HG(Pro+); 3, pT7HG(Pro+)-R1214; 4, pT7HG(Pro+)-A906; 5, pT7HG(Pro+)-A2315. The positions of the Gag polyprotein precursor and of the viral PR cleavage products p41, p25/24, and p17 are indicated at the right.

contain predominantly the Pr55^{gag} cleavage products, p41, p24/25, and p17, as indicated by Western immunoblotting with monoclonal anti-p24 or anti-p17 antibodies (Fig. 1, lane 2). A plasmid with a deletion in the PR coding sequence, pT7HG(Pro-), produces the full Pr55^{gag} (Fig. 1, lane 1) as well as several C-terminal cleavage products formed by bacterial proteases. The identity of these breakdown products was previously determined with a panel of monoclonal antibodies (24).

For the construction of linker insertion mutations, standard recombinant DNA technology was employed (30). pT7HG(Pro+) was partially cleaved with one of several restriction endonucleases (*AluI*, *DdeI*, *DraI*, *HaeIII*, *NlaIV*, or *RsaI*) or cut to completion with *PvuII*. The resulting linear molecules were purified by agarose gel electrophoresis. When necessary, the ends of the linear products were made blunt ended by treatment with the Klenow fragment of DNA polymerase I, and the linear DNA was then ligated with T4 DNA ligase to a nonphosphorylated oligonucleotide linker containing tandem *XhoI* sites (5'-CTCGAGCTCGAG-3'). The resulting linear molecules which possessed single-stranded linker sequences at either end were allowed to anneal and used to transform bacteria to ampicillin resistance. Clones were screened for the presence of a *XhoI* restriction site, and the position of each insertion was determined by restriction mapping.

Twenty-seven linker insertion mutations were obtained (Table 1). Two were located within *pro* coding sequence, and

TABLE 1. Insertion mutations in HIV-1 Gag-PR

Mutant ^a	Amino acid changes ^b	
	From	To
H852	R-P	R- <u>LELE</u> -P
A906	E-L	E- <u>LELE</u> -L
A975	Q-L	Q- <u>LELE</u> -L
D1009	L-R	L- <u>TRARY</u> -R
P1144	A-A	A- <u>ARAR</u> -A
R1214	V-H	V- <u>SSSR</u> -H
H1222	A-I	A- <u>RARA</u> -I
Dr1241	T-L-N	T- <u>FSSSR</u> -N
Dr1337	D-L-N	D- <u>FSSSR</u> -N
A1411	E-A	E- <u>ARAR</u> -A
H1449	G-P	G- <u>LELE</u> -P
H1463	G-Q	G- <u>SSSS</u> -Q
R1509	S-T	S- <u>LELE</u> -T
D1696	L-R	L- <u>TRARY</u> -R
A1711	A-S	A- <u>RARA</u> -S
Dr1787	I-L-K	I- <u>FSSSR</u> -K
N1801	G-P	G- <u>SSSR</u> -P
H1856	G-H	G- <u>SSSS</u> -H
A1906	A-T	A- <u>RARA</u> -T
N1941	R-N-Q	R- <u>TRARD</u> -Q
R2066	C-T	C- <u>LELE</u> -T
H2100	W-P	W- <u>LELE</u> -P
A2173	S-F	S- <u>SSSS</u> -F
N2218	Q-E-P	Q- <u>DSSSR</u> -P
D2253	L-R	L- <u>TRARY</u> -R
A2315	E-A	E- <u>ARAR</u> -A
A2462	A-I	A- <u>RARA</u> -I

^a Letters indicate the restriction site used for linker insertion: A, *AluI*; D, *DdeI*; Dr, *DraI*; H, *HaeI*; N, *NlaIV*; P, *PvuII*; R, *RsaI*. Numbers indicate the nucleotide position of the restriction site with respect to the 5' edge of the 5' long terminal repeat of the HIV-1 (HXB2C) provirus.

^b Amino acids lost or gained as a result of the mutation are underlined.

the remainder were located within *gag* coding sequence. Restriction sites used and the amino acids inserted are shown in Table 1. The net size of each insertion was either four or five amino acids.

The PR activity of wild-type or mutant Gag-PR polyproteins was assessed by examining the expression of viral proteins in JB-DE3, a *lon* mutant strain containing the T7 polymerase gene under the control of the UV5 mutant *lac* promoter. Protein induction with IPTG and Western blotting with anti-HIV-1 *gag* monoclonal antibodies was performed as described previously (24). Grossly, the level of expression of each mutant protein was comparable to that of the wild type. Precursor protein processing was revealed by the presence of the expected PR cleavage products.

The two linkers in PR coding sequence completely obliterated processing of Pr55^{gag} (Fig. 1, lane 5). Of the two, mutant A2315 contains an insertion near the active site of the enzyme. Mutant A2462 contains an insertion in a more C-terminal region, where only conservative substitutions were found in different laboratory isolates (8).

The majority of the linkers within the *gag* coding sequence had no effect on PR activity. Six of the linker insertion mutations, however, produced a substantial reduction in PR activity, globally affecting all cleavage sites in Gag (Fig. 1, lane 4). The locations of the linker mutants and the corresponding phenotypes are summarized in Fig. 2. The results show that mutations in three different areas were able to disrupt PR activation.

The fact that the six disruptive mutations blocked processing in a global fashion suggested that the effect of the linker

FIG. 2. The effect of linker insertion mutations on the PR activity of the HIV-1 Gag-PR polyprotein. (Top) HIV-1 *gag* and *pro* coding sequences. (Bottom) the position of each triangle indicates the site of a linker insertion mutation. The level of PR activity demonstrated by each mutant is indicated by the shading of the triangles: wild-type activity is white; moderate activity is grey; no activity is black.

insertions might be to disrupt protease activation. Alternatively, the mutations might prevent recognition of the altered substrate by an otherwise active PR. To distinguish between the possibilities, we chose to look at the effect of the linker insertion mutations in *gag* coding sequence when PR was provided in *trans*.

Four selected mutant constructs with linker insertion mutations in the *gag* coding sequence (A906, A975, R1509, and A1906) were modified to disrupt *pro*. DNAs were cleaved with the restriction endonuclease *Bcl*I, treated with Klenow fragment of DNA polymerase, and cyclized with T4 DNA ligase, disrupting the reading frame of *pro*. Western blots performed with protein from bacteria transformed with these plasmids confirmed that there was no longer active viral PR and that stable Pr55^{gag} was produced (data not shown). Bacterial cells transformed with these PR-defective plasmids were then cotransformed with pDPT-Pro4 (5), which expresses the HIV-1 PR from the P_L promoter. The apparent toxicity of the PR made it necessary for us to first transform the bacteria with a third plasmid, pCI857 (29), which expresses a thermosensitive mutant of the lambda *cI* repressor. Thus, *Escherichia coli* was sequentially transformed to kanamycin, ampicillin, and chloramphenicol resistance with pCI857, then with pT7HG(Pro-) or mutant derivatives, and then finally with pDPT-Pro4 (Fig. 3). All cultures were grown at 30°C until the time of protein induction. Protein was induced over a 3-h period by the

addition of IPTG at 42°C, and induction was monitored by Western blot.

Mutants A906, A975, R1509, A1906 were tested for the ability to be cleaved by PR provided in *trans* (Fig. 4). These four mutants, two of which were poorly cleaved in the previous assay (A906 and R1509), were cleaved by *trans* PR as efficiently as wild type. This demonstrates that the Gag polyproteins which contain these linker insertion mutations are capable of being cleaved normally when active viral PR is provided in *trans*. Thus, the most likely mechanism by which the mutants A906 and R1509 affect Gag cleavage in the Gag-PR setting is by disrupting PR activation, perhaps by inhibiting polyprotein dimerization. Alternatively, it is possible that these mutations disrupt PR activation by altering the conformation of the entire precursor polyprotein. Though not tested directly, we believe that the other mutations which were disruptive of protease provided in the Gag-Pr setting may block PR activation by the same mechanism as mutants A906 and R1509. To distinguish between these possible explanations it will be necessary to see whether our mutations affect virion assembly and PR function in vivo.

The three-dimensional structure of retroviral Gag polyproteins and how they self-associate to form a particle are not known. If our assay does reflect polyprotein multimerization, our results suggest that several parts of the Gag polyprotein may be involved in multimerization; the major-

FIG. 3. Plasmids used to transform bacteria for the coexpression of Pr55^{gag} and viral PR in *trans*. Bacteria were sequentially transformed with the three plasmids. The antibiotic resistance conferred by each plasmid is indicated. The bacterial origins of replication of the three plasmids are all of different incompatibility groups. (A) pT7HG(Pro-) expresses Pr55^{gag} from the T7 gene 10 promoter. (B) pCI857 expresses a temperature-sensitive mutant of the lambda *cI* repressor from its native promoter. (C) pDPT-Pro-4 expresses the HIV-1 PR from the P_L promoter. This promoter is regulated by the lambda *cI* repressor, expressed from pCI857 at the permissive temperature.

FIG. 4. Examples of the effect of linker insertion mutations in Pr55^{gag} on cleavage by viral PR expressed in *trans* in bacteria. Bacterial strain JB-DE3 was sequentially transformed with three plasmids: pC1857, then pT7HG(Pro-) with or without a linker insertion mutation in the *gag* coding sequence, and finally pDPT-Pro-4. Triple transformants were grown at 30°C until ready for protein induction. For induction, bacteria were incubated with IPTG at 42°C for 3 h. Protein expression was assayed by Western blot with a combination of anti-CA (p24) and anti-MA (p17) monoclonal antibodies (24). Lanes: 1, lysate from bacteria singly transformed with pT7HG(Pro-); 2, lysate from bacteria singly transformed with pT7HG(Pro+); 3, triple transformant with pT7HG(Pro-); 4, triple transformant with pT7HG(Pro-)-A906.

ity of our disruptive mutations map to CA, though there were single, disruptive mutations identified in MA and NC as well. This is consistent with mutational studies on virion assembly; in general, CA is particularly sensitive to disruption, though other regions in MA and at the CA-NC junction may be important as well (for a review, see reference 32). One group has reported the effect of linker insertion mutations on the ability of Gag- β -galactosidase fusion proteins to be incorporated into Moloney murine leukemia virus particles (12). Interestingly, as we found in our protease assay, linkers which disrupted incorporation of fusion proteins into particles clustered in the amino-terminal two-thirds of CA.

The authors thank Martin Rosenberg and Christine Debouck of Smith-Kline Beecham, King of Prussia, Penn., for generous assistance and for plasmids pC1857 and pDPT-Pro4.

This work was supported by grant AI 24845 from the National Institute of Allergy and Infectious Diseases to S.P.G. and by grant K11 AI 00988 from the National Institute of Allergy and Infectious Diseases and JSMF grant 91-49 from the James S. McDonnell Foundation to J.L.

REFERENCES

- Burstein, H., D. Bizub, M. Kotler, G. Schatz, V. M. Vogt, and A. M. Skalka. 1992. Processing of avian retroviral *gag* polyprotein precursors is blocked by a mutation at the NC-PR cleavage site. *J. Virol.* **66**:1781-1785.
- Coffin, J. 1984. Structure of the retroviral genome, p. 261-368. In R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), *RNA tumor viruses*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Crawford, S., and S. P. Goff. 1985. A deletion mutation in the 5' part of the *pol* gene of Moloney murine leukemia virus blocks proteolytic processing of the *gag* and *pol* polyproteins. *J. Virol.* **53**:899-907.
- Darke, P. L., R. F. Nutt, S. F. Brady, V. M. Garsky, T. M. Ciccarone, C. Leu, P. K. Lumma, R. M. Freidinger, D. F. Veber, and I. S. Sigal. 1988. HIV-1 protease specificity of peptide cleavage is sufficient for processing of *gag* and *pol* polyproteins. *Biochem. Biophys. Res. Comm.* **156**:297-303.
- Debouck, C., J. G. Gorniak, J. E. Strickler, T. D. Meek, B. W. Metcalf, and M. Rosenberg. 1987. Human immunodeficiency virus protease expressed in *Escherichia coli* exhibits autoproteolytic processing and specific maturation of the *gag* precursor. *Proc. Natl. Acad. Sci. USA* **84**:8903-8906.
- Ehrlich, L. S., H.-G. Krausslich, E. Wimmer, and C. A. Carter. 1990. Expression in *Escherichia coli* of human immunodeficiency virus type 1 capsid protein (p24). *AIDS Res. Hum. Retroviruses* **6**:1169-1175.
- Erickson-Viitanen, S., J. Manfredi, P. Viitanen, D. E. Tribe, R. Tritch, C. A. Hutchison, D. D. Loeb, and R. Swanstrom. 1989. Cleavage of HIV-1 *gag* polyprotein synthesized in vitro: sequential cleavage by the viral protease. *AIDS Res. Hum. Retroviruses* **5**:577-591.
- Fontenot, G., K. Johnston, J. C. Cohen, W. R. Gallaher, J. Robinson, and R. B. Luftig. 1992. PCR amplification of HIV-1 proteinase sequences directly from lab isolates allows determination of five conserved domains. *Virology* **190**:1-10.
- Gottlinger, H. G., J. G. Sodroski, and W. A. Haseltine. 1989. Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **86**:5781-5785.
- Gowda, S. D., B. S. Stein, and E. G. Engleman. 1989. Identification of protein intermediates in the processing of the p55 HIV-1 *gag* precursor in cells infected with recombinant vaccinia virus. *J. Biol. Chem.* **264**:8459-8462.
- Graves, M. C., J. J. Lim, E. P. Heimer, and R. A. Kramer. 1988. An 11-kDa form of human immunodeficiency protease expressed in *Escherichia coli* is sufficient for enzymatic activity. *Proc. Natl. Acad. Sci. USA* **85**:2449-2453.
- Hansen, M., L. Jelinke, S. Whiting, and E. Barklis. 1990. Transport and assembly of *gag* proteins into Moloney murine leukemia virus. *J. Virol.* **64**:5306-5316.
- Jacks, T., M. D. Power, F. R. Masiaz, P. A. Luciw, P. J. Barr, and H. E. Varmus. 1988. Characterization of ribosomal frameshifting in HIV-1 *gag-pol* expression. *Nature (London)* **331**:280-283.
- Katoh, I., Y. Ikawa, and Y. Yoshinaka. 1989. Retrovirus protease characterized as a dimeric aspartic proteinase. *J. Virol.* **63**:2226-2232.
- Katoh, I., Y. Yoshinaka, A. Rein, M. Shibuya, T. Odaka, and S. Oroszlan. 1985. Murine leukemia virus maturation: protease region required for conversion from "immature" to "mature" core form and for virus infectivity. *Virology* **145**:280-292.
- Kohl, N. E., E. A. Emini, W. A. Schleif, L. J. Davis, J. C. Heimbach, R. A. Dixon, E. M. Scolnick, and I. S. Sigal. 1988. Active human immunodeficiency virus protease is required for viral infectivity. *Proc. Natl. Acad. Sci. USA* **85**:4686-4690.
- Kotler, M., G. Arad, and S. H. Hughes. 1992. Human immunodeficiency virus type 1 *gag*-protease fusion proteins are enzymatically active. *J. Virol.* **66**:6781-6783.
- Kotler, M., R. A. Katz, W. Danho, J. Leis, and A. M. Skalka. 1988. Synthetic peptides as substrates and inhibitors of a retroviral protease. *Proc. Natl. Acad. Sci. USA* **85**:807-811.
- Kotler, M., R. A. Katz, and A. M. Skalka. 1988. Activity of avian retroviral protease expressed in *Escherichia coli*. *J. Virol.* **62**:2696-2700.
- Krausslich, H.-G., R. H. Ingraham, M. T. Skoog, E. Wimmer, P. V. Pallai, and C. A. Carter. 1989. Activity of purified biosynthetic proteinase of human immunodeficiency virus on

- natural substrates and synthetic peptides. *Proc. Natl. Acad. Sci. USA* **86**:807–811.
21. Le Grice, S. F. J., J. Mills, and J. Mous. 1988. Active site mutagenesis of the AIDS virus protease and its alleviation by trans complementation. *EMBO J.* **7**:2547–2553.
 22. Loeb, D. D., C. A. I. Hutchison, M. H. Edgell, W. G. Farmerie, and R. Swanstrom. 1989. Mutational analysis of human immunodeficiency virus type 1 protease suggests functional homology with aspartic proteinases. *J. Virol.* **63**:111–121.
 23. Louis, J. M., E. M. Wondrak, T. D. Copeland, C. A. D. Smith, P. T. Mora, and S. Oroszlan. 1989. Chemical synthesis and expression of the HIV-1 protease gene in *E. coli*. *Biochem. Biophys. Res. Commun.* **159**:87–94.
 24. Luban, J., and S. P. Goff. 1991. Binding of human immunodeficiency virus type 1 (HIV-1) RNA to recombinant HIV-1 gag polyprotein. *J. Virol.* **65**:3203–3212.
 25. Miller, M., M. Jaskolski, J. Rao, J. Leis, and A. Wlodawer. 1989. Crystal structure of a retroviral protease proves relationship to aspartic protease family. *Nature (London)* **337**:576–579.
 26. Miller, M., B. K. Sathyanarayana, M. V. Toth, G. R. Marshall, L. Clawson, L. Selk, J. Schneider, S. B. H. Kent, and A. Wlodawer. 1989. Structure of complex of synthetic HIV-1 protease with a substrate-based inhibitor at 2.3 Å resolution. *Science* **246**:1149–1152.
 27. Navia, M. A., P. M. D. Fitzgerald, B. M. McKeever, C.-T. Leu, J. C. Heimbach, W. K. Herber, I. S. Sigal, P. L. Darke, and J. P. Springer. 1989. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature (London)* **337**:615–620.
 28. Pettit, S. C., J. Simsic, D. D. Loeb, L. Everitt, C. A. Hutchison III, and R. Swanstrom. 1991. Analysis of retroviral protease cleavage sites reveals two types of cleavage sites and the structural requirements of the P1 amino acid. *J. Biol. Chem.* **266**:14539–14547.
 29. Remaut, E., H. Tsao, and W. Fiers. 1983. Improved plasmid vectors with a thermoinducible expression and temperature-regulated runaway replication. *Gene* **22**:103–113.
 30. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 31. Weber, I. T., M. Miller, M. Jaskolski, J. Leis, A. M. Skalka, and A. Wlodawer. 1989. Molecular modeling of the HIV-1 protease and its substrate binding site. *Science* **243**:928–931.
 32. Wills, J. W., and R. C. Craven. 1991. Form, function, and use of retroviral Gag proteins. *AIDS* **5**:639–654.
 33. Wlodawer, A., M. Miller, M. Jaskolski, B. K. Sathyanarayana, E. Baldwin, I. T. Weber, L. M. Selk, L. Clawson, J. Schneider, and S. B. H. Kent. 1989. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 PR. *Science* **245**:616–621.